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# Congenital myasthenic syndromes in adults: clinical features, diagnosis and long-term prognosis

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## Abstract

Congenital myasthenic syndromes (CMS) are clinically and genetically heterogeneous diseases caused by mutations affecting neuromuscular transmission. Even if the first symptoms mainly occur during childhood, adult neurologists must confront this challenging diagnosis and manage these patients throughout their adulthood. However, long-term follow-up data from large cohorts of CMS patients are lacking and the long-term prognosis of these patients is largely unknown.

We report the clinical features, diagnostic difficulties, and long-term prognosis of a French nationwide cohort of 235 adult patients with genetically confirmed CMS followed in 23 specialized neuromuscular centres. Data were retrospectively analysed.

Of the 235 patients, 123 were female (52.3%). The diagnosis was made in adulthood in 139 patients, 110 of whom presented their first symptoms before the age of 18. Mean follow-up time between first symptoms and last visit was 34 years (SD=15.1). Pathogenic variants were found in 19 disease-related genes. *CHRNE*-low expressor variants were the most common (23.8%), followed by variants in *DOK7* (18.7%) and *RAPSN* (14%). Genotypes were clustered into four groups according to the initial presentation: ocular group (*CHRNE*-LE, *CHRND*, FCCMS), distal group (SCCMS), limb-girdle group (*RAPSN*, *COLQ*, *DOK7*, *GMPPB*, *GFPT1*), and a variable-phenotype group (*MUSK*, *AGRN*). The phenotypical features of CMS did not change throughout life. Only four genotypes had a proportion of patients requiring intensive care unit (ICU) admission that exceeded 20%: *RAPSN* (54.8%), *MUSK* (50%), *DOK7* (38.6%) and *AGRN* (25.0%). In *RAPSN* and *MUSK* patients most ICU admissions occurred before age 18 years and in *DOK7* and *AGRN* patients at or after 18 years of age. Different patterns of disease course (stability, improvement and progressive worsening) may succeed one another in the same patient throughout life, particularly in *AGRN*, *DOK7* and *COLQ*. At the last visit, 55% of SCCMS and 36.3% of *DOK7* patients required ventilation; 36.3% of *DOK7* patients, 25% of GMPPB patients and 20% of GFPT1 patients were wheelchair-bound; most of the patients who were both wheelchair-bound and ventilated were *DOK7* patients. Six patients died in this cohort. The positive impact of therapy was striking, even in severely affected patients.

In conclusion, even if motor and/or respiratory deterioration could occur in patients with initially moderate disease, particularly in *DOK7*, SCCMS and *GFPT1* patients, the long-term prognosis for most CMS patients was favourable, with neither ventilation nor wheelchair needed at last visit. *CHRNE* patients did not worsen during adulthood and *RAPSN* patients, often severely affected in early childhood, subsequently improved.

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**Running title**: Follow-up and long-term prognosis in CMS

**Keywords:** genetic; neuromuscular junction; myasthenia; CMS

# Introduction

Congenital myasthenic syndromes (CMS) are clinically and genetically heterogeneous diseases characterized by a neuromuscular transmission defect caused by mutations affecting the synaptic structure or function.[1] In the past decades, the molecular bases 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, the first symptoms can occur in adulthood.[9] The reported prevalence of CMS, around 2.8-14.8/1,000,000, is likely 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] Indeed, the diagnosis can be particularly challenging in adult patients, in whom autoimmune myasthenia gravis (MG) is frequently considered in the first place due to its higher prevalence.[9] Moreover, some CMS patients presenting with marked proximal weakness can easily be misdiagnosed with limb-girdle dystrophy or congenital myopathy.[9] Clinical symptoms and severity range from mild ocular or bulbar symptoms, such as ptosis, diplopia or swallowing disturbances, to severe limb weakness leading to loss of ambulation.[5] Decrement or increment evidenced on repetitive nerve stimulation (RNS) supports the neuromuscular transmission defect in these diseases. Moreover, electrophysiological features can help to orient the genetic diagnosis and validate the pathogenicity of variants.[15] Like the phenotype, the response to treatment is also heterogeneous and depends on the underlying molecular mechanism and thus the precise genetic defect.[1] For example, acetylcholinesterase (AChE) inhibitors can worsen the symptoms of patients with *COLQ* or slow-channel variants.[16,17] Although the clinical spectrum of the different CMS subtypes keeps expanding,[5–8] large cohorts of adult CMS patients with long-term follow-up and detailed clinical characteristics are lacking. In previous studies, the median follow-up time was frequently short (maximum 12.8 years) and/or the cohort size was small, without precise evaluation criteria.[9,16,18,19] Yet these data are of the utmost importance to better define the long-term prognosis of CMS according to the genetic background and help neurologists to improve their management of adult patients.

By retrospectively analysing the clinical data of 235 adult patients with genetically confirmed CMS followed in 23 French specialized neuromuscular centres, we aimed to better define the long-term prognosis of these patients. We also aimed to determine the most common misdiagnoses to help clinicians better recognize this condition and to give straightforward treatment recommendations.

# Materials and methods

## Study design and population

This retrospective, observational, multicentre study included all adult patients followed for genetically confirmed CMS until July 2023 in the specialized neuromuscular centres of 23 University Hospitals in France (Amiens, Angers, Bordeaux, Brest, Caen, Clermont, Créteil,

Grenoble, Limoges, Lille, Lyon, Marseille, Montpellier, Nancy, Nantes, Nice, Nîmes, Paris, Rennes, Rouen, Saint-Etienne, Strasbourg and Toulouse) within the French neuromuscular network FILNEMUS. 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 Assistance Publique des Hôpitaux de Paris (APHP ethics approval #20230524134437) and with the 1964 Helsinki declaration.

## Clinical, laboratory and electrophysiological data

The demographic data collected included sex, ethnic origin, family history of CMS, mode of inheritance, consanguinity, age at first symptoms, age at clinical and molecular diagnoses and age at last follow-up visit. The patients were further classified according to age at onset of their symptoms in six subgroups: neonatal period, infancy (1-3 years), childhood (4-9 years), teenage (10-17 years), adulthood (18-40 years) and late onset (more than 40 years). Clinical data of interest included the presence of limb weakness, either proximal or distal, axial muscle deficit, facial weakness, fatigability, bulbar symptoms (including dysphonia and swallowing disturbances), ptosis, oculomotor disturbances, arthrogryposis, intellectual disability, delayed motor milestones, scoliosis, dyspnoea, need for ventilation, need for tube feeding, need for a wheelchair and need for admission to an intensive care unit (ICU). The 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. Electroneuromyography (ENMG) examinations were performed in each specialized neuromuscular centre by trained neurologists. We collected the presence of an RNS decrement or increment, post-effort increment and repetitive compound muscle action potential (R-CMAP). Creatine kinase (CK) values were also recorded and were considered elevated if above 200 UI/L. Lastly, we collected the type of treatment (AChE inhibitors, 3,4-diaminopyridine [3,4-DAP] quinidine, fluoxetine, ephedrine and salbutamol) and its efficacy according to the clinician in charge of the patients, based on patients' feedback and clinical examination.

## 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 patients' genomic DNA and sequenced using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems®, Life TechnologiesTM). From 2016, next-generation sequencing (NGS) of CMS gene panels was used. Three panels (v2 2016-2017, v3 2017-2021 and v4 since 2021) 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 five additional genes (*COL13A1*, *GMPPB*, *LAMA5*, *MYO9A*, *UNC13A*, *VAMP1*) and v4 one additional gene (*TOR1AIP1*). 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.

## Statistical analyses

All data were analysed with R.4.0. To visualize the relationship between neuromuscular symptoms and implicated genes, a heatmap was generated using the library ComplexHeatmap.[41] Only the genotypes with at least four patients were included in the heatmap. The remaining genotypes, with fewer than four patients, were described separately. 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 colour-coded to represent the range of proportions of symptoms, and an accompanying metadata panel was incorporated to display the mean age at onset of the first symptom. To identify symptoms that exhibited significant patterns in the heatmap, Z-scores were calculated to facilitate the interpretation of symptoms in relation to the involved gene, normalizing the data around a mean of zero and a standard deviation of one. Associations between prognostic outcomes 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.

# Results

## Demographic, genetic and diagnostic characteristics

A total of 235 patients belonging to 195 unrelated families were included in the study; 123 were female (52.3%). A positive family history was reported in 107 patients (45.6%), and consanguinity was found in 55 cases (23.4%). In terms of ethnicity, 177 patients were Caucasian (75.3%), 32 originated from North Africa (13.6%), 10 from the Middle East (4.2%), seven from Sub-Saharan Africa (3.0%) and five came from Romani families (2.1%); the remaining four (1.7%) had diverse other origins (Asia and South America). Causative variants (Supplementary Table 1) were found in 19 disease-related genes (*AGRN*, *CHAT*, *CHRNA1*, *CHRNB1*, *CHRND*, *CHRNE*, *COL13A1*, *COLQ*, *DOK7*, *DPAGT1*, *GFPT1*, *GMPPB*, *LRP4*, *MUSK*, *RAPSN*, *SCNA4*, *SLC5A7*, *SLC18A3*, *TOR1AIP1*). These 180 variants were either described as likely pathogenic or pathogenic in the literature or were novel and retained as probably or certainly disease-causing. All patients had pathogenic mutations linked to CMS: 56 in *CHRNE* (23.8%), characterized as low-expressor (*CHRNE*-LE) variants responsible for a low expression of the encoded protein, 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 already described *CHRNE* variants (1.7%),[20,21] and 16 (6.8%) in other genes (*CHAT*, *CHRNA1* low-expressor, *CHRNB1*, *COL13A1*, *LRP4*, *SCNA4*, *SLC5A7*, *SLC18A3*, *TOR1AIP1*) (Fig. 1). 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. Symptom 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%). Twenty-five patients had their first symptoms between age 18 and 40 (10.6%). These patients belonged to the *DOK7* (6/25), *AGRN* (4/25), SCCMS (4/25), *RAPSN* (3/25), *COLQ* (2/25), *GMPPB* (2/25), *CHRND*

(1/25), *GFPT1* (1/25), *LRP4* (1/25) and *TOR1AIP1* (1/25) groups. Only five patients had their first symptoms after age 40 (2.1%). In these five patients, the genetic analysis disclosed SCCMS, *DOK7, MUSK* genes for one patient each and *RAPSN* gene for two. The age at first symptoms could not be clearly determined in seven patients (3.0%). A total of 138 patients were previously misdiagnosed (58.7%). Among them, the main misdiagnoses were congenital myopathy (50%), autoimmune MG (29.0%), muscular dystrophy (15.9%) and mitochondrial myopathy (8.7%) (Table 1, Supplementary Fig. 1). Some patients were misdiagnosed with several different pathologies during their disease course. The mean delay between first symptoms and clinical diagnosis was 17.2 years (SD=15.3), while the mean delay until molecular diagnosis was 22.0 years (SD=15.2). The clinical diagnosis was made before 18 years in 82 patients (35.0%). Among the 139 patients in whom the diagnosis was made in adulthood (59.1%), 110 presented symptoms before the age of 18 (46.8%) and 29 had their first symptoms at or after the age of 18 (12.3%, Table 1). This categorization was not possible in 14 patients (5.9%) as age at first symptoms was not available in seven patients and age at clinical diagnosis could not be determined in seven others. The mean follow-up time between first symptoms and last visit was 34 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 genotype ($P$=0.11).

# Genotype-phenotype correlations

## Initial and final phenotype

The proportion of symptoms per genotype at initial presentation is shown in Fig. 2A. Genes were clustered using the Ward.D2 method according to the initial clinical presentation in 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 patients, with a high proportion having upper distal weakness at initial presentation (16/20 patients, 80%). The third group was composed of *AGRN*- and *MUSK*-mutated patients. In this group, the phenotype was variable. Some *AGRN* patients could also have a distal weakness (4/12, 33%), while others had a proximal weakness (6/12, 50%). These patients could also have facial weakness (4/12, 33.3%), respiratory symptoms (5/12, 41.7%) or bulbar symptoms (25%).

*MUSK* patients also presented with variable symptoms, such as proximal weakness (4/8, 50%), respiratory symptoms (5/8, 62.5%), bulbar symptoms (5/8, 62.5%) and ophthalmoparesis (4/8, 50%). The last group included *RAPSN*, *COLQ*, *DOK7*, *GMPPB* and *GFPT1*. Proximal weakness was the hallmark of this last group, as observed in the following mutated patients: *GMPPB* (4/4, 100%), *GFPT1* (15/15, 100%), *COLQ* (18/19, 94.7%) and *DOK7* (40/44, 90.9%). Interestingly, axial muscle weakness was always found in all *GFPT1* patients (10/10, 100%) and *GMPPB* patients could have intellectual disability (1/4, 25.0%). Then, 23/44 *DOK7* patients (52.2%) had scoliosis, while 7/32 (21.9%) and 9/32 (28.2%) *RAPSN* patients had arthrogryposis and contractures, respectively. Only four genotypes had a mean age at first symptoms of over 10 years: *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 shown in Fig. 2B. There were no significant differences in any proportion of symptoms per genotype between the initial presentation and the last follow-up visit. The genotypes that were associated with symptoms at initial presentation with a Z-score > 0.85 are shown in Fig. 3.

## Adult onset

Regarding the 30 patients with onset of symptoms in adulthood, 5/7 *DOK7* (71%) patients presented with upper limb proximal weakness as the first symptom. The remaining two patients developed acute respiratory insufficiency as the initial manifestation of the disease. Interestingly, two adult-onset *AGRN* patients initially manifested respiratory symptoms and the remaining two had fatigability and proximal muscle weakness. All five SCCMS patients with an onset of symptoms in adulthood presented finger extension weakness as the first manifestation of the disease. The two *COLQ* patients, two *GMPPB* patients and one *GFPT1* patient who had their first symptoms in adulthood had proximal muscle weakness as the initial manifestation of the disease. The first symptoms of the five *RAPSN* patients with disease onset at or after 18 years were not specific and included ptosis, proximal muscle weakness and bulbar symptoms. The only *CHRND* patient with disease onset after 18 years presented with ptosis, ophthalmoparesis and bulbar symptoms. Finally, the *MUSK* patient who developed her first symptoms in adulthood presented with fatigability, bulbar symptoms, proximal muscle weakness and respiratory symptoms. The *TOR1AIP1* and *LRP4* adult-onset patients have previously been reported.[22,23]

## Paraclinical investigations

Regarding electrophysiological features, 213/220 patients (96.8%) with available ENMG data had a decrement superior to 10% on RNS at 3 Hz in at least one nerve-muscle pair. Four patients had an increment on post-exercise CMAP: three *AGRN* patients 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 genotypes compared to the others ($P<0.01$).

CK levels were available in 134 patients and were elevated (>200 UI/L) in 34 of them (25.4%). The proportion of patients with raised 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 genotype associated with elevated CK was the *GFPT1* genotype, with 7/12 patients with available CK having raised levels (mean of 311.5 UI/L, SD=238.0). All three *MUSK* patients with available CK data had elevated values (339, 57 and 4558 UI/L, respectively). Regarding the two most frequently represented genes, 3/30 *CHRNE* patients (10%) and 7/28 *DOK7* patients (25%) had elevated CK values.

Muscle biopsy was performed in 117 patients (49.8%). The results were available in 104 patients. The biopsy was considered normal in 16 patients (15.4%). The main abnormality was type 1 fibre predominance (n=44, 42.3%), type 2 fibre atrophy (n=30, 28.9%) and fibre size disproportion (n=21, 20.2%). Other features included: lipid surcharge (n=14, 13.4%), nuclear internalizations (n=10, 9.6%), mitochondrial abnormalities (n=5, 4.8%) and core-like lesions (n=4, 3.8%). Tubular aggregates were seen in eight patients (7.7%), including seven *GFPT1* patients and one *DGAPGT1* patient. Necrotic/regenerating fibres were observed in six patients (5.8%), including the four *GMPPB* patients. Abnormal neuromuscular junctions on electron microscopy were reported in 11 patients (10.6%). All clinical and paraclinical findings are detailed according to genotype in Supplementary Tables 2 to 12.

# Long-term prognosis

## Disease course

The type of disease course according to the genotype is shown in Fig. 4A. Most *CHRNE*-LE (40/56, 71.4%), *CHRND* (4/4, 100%) and FCCMS (3/4, 75.0%) patients had a stable disease course. A progressive improvement was reported in 16/33 *RAPSN* (48.5%) and in 3/8 *MUSK*

patients (37.5%). Conversely, 6/20 SCCMS (30%), 12/44 *DOK7* (27%), 2/4 *GMPPB* (50.0%) and 6/15 *GFPT1* (40.0%) patients had a progressively worsening course. A proportion of *DOK7* (11/44, 25.0%), *COLQ* (5/19, 26.3%) and *AGRN* (3/12, 25.0%) patients had a multiphasic disease course, combining successive periods of improvement, stability and progressive worsening. An example of multiphasic disease, with late-onset deterioration, is given in Supplementary Fig. 2. Intrafamilial variability was also a hallmark in some *CHRNE*-LE and SCCMS families.

## Exacerbations and ICU admissions

The proportion of patients having acute disease exacerbations reached 20% in most of the genotypes (Fig. 4B). The duration of exacerbations could be very long in some cases, as noted in a *COLQ* patient who lost her ambulation within a week for three years before regaining it completely in one month. *RAPSN* patients required significantly more ICU admissions 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. Moreover, four *RAPSN* patients required two ICU admissions in their disease course. Three other genotypes had a proportion of patients requiring ICU admission that exceeded 20% (Fig. 4C): *MUSK* (4/8, 50%), *DOK7* (17/44, 38.6%) and *AGRN* (3/12, 25.0%). Four *DOK7* patients and one *AGRN* patient required two ICU admissions in their disease course. In *RAPSN* and *MUSK* patients, 19/21 (90.4%) and 4/4 (100%) ICU admissions, respectively, occurred before age 18; in *DOK7* and *AGRN* patients, 11/21 (52.3%) and 4/4 (100%) ICU admissions, respectively, occurred at or after age 18.

## Pregnancy and other triggers

Of the 74 female patients who had a pregnancy, 24 (32.4%) reported a worsening of symptoms during pregnancy; 20/123 female patients (16.2%) reported a worsening of symptoms during menstruation. In the cohort of 235 patients, other triggers for symptom worsening were infection in 35 patients (14.9%), warm temperatures in 19 patients (8%), cold temperatures in 15 patients (6.3%), anaesthesia in nine patients (3.8%) and psychological stress in five patients (2.1%).

## Disability and ventilation

Among the different genotypes, the proportion of patients requiring ventilation at the last follow-up was significantly elevated in SCCMS (11/20, 55%, $P<0.01$) and *DOK7* patients (16/44, 36.3%, $P=0.04$) (Fig. 4D). This proportion did not exceed 25% in the other genotypes (Fig. 4D). Six patients were tracheotomized, with invasive ventilation, at last follow-up: one SCCMS patient, one *CHRNE* patient, three *DOK7* patients and one *SLC5A7* patient. Only two patients (one *CHRND* and one SCCMS) required a feeding tube at last visit. Regarding the motor long-term prognosis, the proportion of *DOK7* patients who were wheelchair-bound was significantly higher compared to the other genotypes (16/44, 36.3%, $P<0.001$). One *GMPPB* patient (25.0%) and 3/15 *GFPT1* patients (20%) were wheelchair-bound at last visit (Fig. 4E). The proportion of patients per MGFA category according to the genotype is shown in Fig. 4F. The highest proportion of MGFA category 4 patients was found in the *DOK7* patients (12/44, 27.2%) (Fig. 4F). The highest proportion of patients both wheelchair-bound and ventilated was in the *DOK7* patients (9/44, 20.5%). This proportion did not exceed 10% in the other genotypes (SCCMS: 2/20, 10%, *AGRN*: 1/12, 8.3%, *GFPT1*: 1/15, 6.7%, *RAPSN*: 2/33, 6.1%, *COLQ*: 1/19, 5.2%, *CHRNE*: 1/56, 1.8%).

## Death

Only six patients died in our cohort (2.6%). An *AGRN* patient died at 50 years of age from respiratory insufficiency. She was tetraplegic with severe bulbar involvement after three decades of progressive worsening (Patient 1 in Jacquier *et al.*[24]). One *COLQ* patient died at 52 years of age from cancer. Two patients with *DOK7* variants died. The first one was misdiagnosed with seronegative autoimmune MG and died from acute vocal cord palsy at 41 years of age, possibly favoured by AChE inhibitors. The second died at 56 years of age after an accidental fall in the stairs (Supplementary Fig. 2). A *DPAGT1* patient died at 36 years from aspiration pneumonia secondary to swallowing disorders. Finally, a patient with *RAPSN* variants died at 86 years, but the cause of 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 each for *CHRNE*-LE, *MUSK*, *GMPPB*, *GFPT1*, *COL13A1*, *SCNA4*, *SCL5A7*).

# Treatment

Twenty-five patients (10.6% of the cohort), misdiagnosed as seronegative autoimmune MG, received immunomodulatory treatments before the diagnosis of CMS. These treatments included corticosteroids, intravenous immunoglobulin, plasma exchange and immunosuppressive treatments (azathioprine, mycophenolate mofetil). Moreover, eight of them had a thymectomy. None of these 25 patients reported a long-term improvement with these therapies.

A total of 224 patients received non-immunomodulatory CMS treatments (95.3%). These treatments included AChE inhibitors, 3,4-DAP, salbutamol, ephedrine, fluoxetine and quinidine; 138 patients received more than one of these treatments (58.8%). Responses to non-immunomodulatory therapies are summarized in Fig. 5.

All SCCMS (5/5), *COLQ* (7/7) and *DOK7* (23/23) patients reported either no effect or worsening with AChE inhibitors. Only 1/5 *MUSK* patients (20.0%) and 1/10 *AGRN* patients (10.0%) claimed symptom improvement with these treatments, the remaining patients reporting no effect or symptom worsening. AChE inhibitors were effective in 75% or more of the patients in the other genotypes: *CHRNE*-LE (46/53), FCCMS (4/4), *CHRND* (3/4), *RAPSN* (28/30), *GFPT1* (13/14) and *GMPPB* (3/4). 3,4-DAP was reported as effective in more than half of the *CHRNE*-LE (23/33), *CHRND* (3/3), *RAPSN* (11/15), *DOK7* (11/16) and *GFPT1* (10/11) patients. Half of FCCMS (1/2), SCCMS (1/2) and *COLQ* (3/6) patients had their symptoms improved with 3,4-DAP, while this treatment was ineffective in 3/4 *MUSK* patients (75.0%) and 6/7 *AGRN* patients (85.7%) and even led to symptom worsening in 1/7 *AGRN* patients (14.3%). Salbutamol improved the symptoms of 75% or more of *CHRNE*-LE (12/16), *CHRND* (1/1), *MUSK* (4/5), *AGRN* (5/6), *RAPSN* (4/5), *COLQ* (8/10), *DOK7* (20/22), *GFPT1* (6/7) and *GMPPB* (1/1) patients. One of the two FCCMS patients (50.0%) treated with salbutamol reported symptom improvement, while the other reported no effect. Two of the five SCCMS patients (40.0%) treated with salbutamol reported treatment efficacy, while the three others reported no effect. Ephedrine was reported to improve symptoms in the only FCCMS patient treated, and 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 one *CHRND* patient and one *RAPSN* patient treated, and in 2/7 *AGRN* (28.6%), 1/8 *COLQ* (12.5%) and 1/23 *DOK7* (4.3%) patients. Four previously wheelchair-bound *DOK7* patients were able to walk unaided, three after being

treated with both salbutamol and ephedrine and one after ephedrine alone. One previously wheelchair-bound *COLQ* patient became ambulant after being treated with salbutamol, as did another *COLQ* patient thanks to ephedrine. Fluoxetine was found to be effective in 7/13 SCCMS patients (53.8%), the remaining 6/13 patients (46.1%) reporting no benefit. In the other genotypes, one FCCMS patient, one *AGRN* patient and three *RAPSN* patients were treated with fluoxetine but reported no effect. Finally, all five SCCMS patients treated with quinidine reported an improvement of motor weakness.

## Clinical summary for genes with a small number of patients (n ≤ 3)

### *TOR1AIP1* gene

Three patients with *TOR1AIP1* variants were included in our cohort (Table 2). One of them (Patient 1) had previously been published (main proband of Malfatti *et al.*[22]). We report herein two other patients, who are brothers, harbouring the same c.63dupC (p.Arg22Glnfs*88) found in Patient 1, and the c.72dupC (p.Ile25Hisfs*85) heterozygous variants (Patients 2 and 3). They were born to Algerian healthy parents. The first variant was inherited from the mother and the second variant from the father. Their motor milestones were normal, but they had difficulties in sports activities during their teenage years. Patient 2 developed progressive walking and respiratory difficulties at age 35 years, leading to his needing a banister to climb stairs at age 40. At age 47, the patient underwent a coronary angiography for an acute coronary syndrome. Because he did not tolerate the supine position during the examination, pulmonary investigations including blood-gas analysis and pulmonary functional tests were performed and showed an alveolar hypoventilation requiring non-invasive ventilation. Neurological examination found proximal muscle and finger extensor muscle weakness in the upper limbs, associated with distal muscle weakness in the lower limbs, and cervical spine, finger, wrist and Achilles tendon contractures. Repetitive nerve stimulation at 3 Hz performed at age 48 showed a 48% decrement in tibialis anterior and anconeus muscles and a 39% decrement in trapezius muscle. Serum CK was mildly elevated at 227 UI/L (N<200 U/L). He was mildly improved by AChE inhibitors with an increased walking distance. At last follow-up (age 51), he was still ambulant without walking aid but required nocturnal non-invasive ventilation. Patient 3 was admitted to the ICU at age 48 for an acute respiratory insufficiency revealing an alveolar hypoventilation and requiring

intubation. At discharge, he walked unaided and had non-invasive ventilation. His neurological examination showed elbow and finger contractures and mild deltoid muscle weakness. His CK levels were normal. RNS at 3 Hz showed a 19% decrement on tibialis anterior and 15% on trapezius muscle.

### *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 (Patients 5 and 6) 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 in one of them. AChE inhibitors were reported to improve their symptoms in two of the three patients and were considered ineffective in the other. At last follow-up, Patient 4 (age 49) was still ambulant but required a banister to climb stairs, while Patients 5 and 6 were wheelchair-bound (age 34 and 21, respectively). None required respiratory assistance. Patient 5 died at age 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 admission. One of them (Patient 8) had arthrogryposis (equinovarus) and developed epileptic seizures. Mild efficacy was reported for both with AChE inhibitors. However, their final prognosis was different. While Patient 7 improved and was asymptomatic at age 20, Patient 8 progressively became wheelchair-bound and required nocturnal non-invasive ventilation at last visit (age 22).

The remaining patients are described in Supplementary Table 13. The paraclinical findings associated with genes with a small number of mutated patients ($n \leq 3$) are available in Supplementary Table 14. Of note, the *LRP4* and *SCN4A* patients have previously been reported.[23,25]

## Discussion

This study of a French nationwide multicentre cohort of 235 adult patients has enabled us to better describe CMS patients' phenotype and long-term prognosis, according to their genotype.

*CHRNE*-LE variants were the most common and are considered as the main cause of CMS worldwide.[6,26] As described in an Austrian cohort, *DOK7* was herein the second most commonly involved gene.[27] *RAPSN* variants were also frequent, as in previously published cohorts.[6,8,28] *COLQ* was only the fifth most frequently involved gene in our cohort, whereas it was one of the three main genes in several previous studies in different populations.[7,8,16]

Adult neurologists can encounter CMS patients in three different situations. In the first and most straightforward scenario, the diagnosis has already been made by a paediatric neurologist, and the adult neurologist assumes responsibility for 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 reached due to mild and/or short-duration symptoms insufficient to initiate a diagnostic investigation or due to misdiagnosis (mainly congenital myopathy). This was the most common situation in our study (46.8% of patients), as previously observed.[9] In the third, more rarely encountered situation, symptom onset occurs in adulthood (12.3% of patients in our cohort). These last two situations are particularly challenging, especially for adult neurologists non-specialized in the neuromuscular field. In these cases, finger extension weakness or proximal muscle weakness, even if non-specific, are suggestive of the diagnosis. Clinicians should also keep in mind that acute respiratory insufficiency can be the first manifestation of the disease in adult patients.

Misdiagnoses were frequent in our cohort (58.7%) and the diagnostic delay was long, in line with previously published cohorts.[9,26] Congenital myopathy was the most common misdiagnosis as there are overlapping clinical and histological features. Autoimmune MG can easily be suspected in late-onset cases, and this was the second most frequent misdiagnosis in our cohort, leading to an immunosuppressive treatment in 25 patients. With the development of new immunosuppressive treatments in the past decade, we recommend considering the diagnosis of CMS in patients with seronegative MG before starting such treatments which could cause serious adverse events.[29]

We clustered patients' genotypes according to their initial phenotypes. The clustering method we applied led to the formation of four groups of phenotypes. The first group was composed of *CHRNE*, *CHRND* and FCCMS patients and was characterized by predominantly ocular symptoms, such as ptosis and ophthalmoparesis. Acetylcholine receptor endplate deficiencies are

known to cause predominantly ocular symptoms, and FCCMS patients have essentially the same phenotype.[4,8,28] The second group was represented solely by SCCMS patients, who presented a particular phenotype with predominantly upper limb distal weakness, especially affecting finger extensors, frequently associated with neck extensor muscle weakness, as previously found.[30] The third group was composed of *AGRN* and *MUSK* patients. They developed variable symptoms such as ocular symptoms, bulbar symptoms, respiratory involvement and muscle weakness, which led us to consider this group as a variable-phenotype group. These genes have already been associated with such diverse symptoms.[31,32] Interestingly, *AGRN* patients frequently had distal weakness but, contrary to SCCMS patients, they rarely had axial muscle weakness. The fourth group was composed of *GMPPB*, *GFPT1*, *DOK7*, *COLQ* and *RAPSN* patients, and could be categorized as having a limb-girdle muscle dystrophy (LGMD)-like phenotype, associated with some additional characteristic features for some of these genes. *GMPPB* and *GFPT1* are essential for N- and O-glycosylation and N- and O-mannosylation.[4] As previously described, *GMPPB* and *GFPT1* patients presented with relatively pure proximal weakness.[33,34] However, *GMPPB* patients could also have CNS involvement with delayed motor milestones and intellectual disability. *DOK7* and *COLQ* patients also presented with a proximal weakness but were more prone to have associated symptoms, such as bulbar or ocular symptoms.[35,36] *DOK7* was associated with a high rate of scoliosis, as previously observed.[35] *RAPSN* was included by the clustering method in this group due to frequent proximal and axial muscle weakness. However, *RAPSN* patients developed more ocular, bulbar and respiratory symptoms than the other patients of this group. *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. Taken together, these hallmarks are evocative of *RAPSN*-related CMS.[37] Regarding the electrophysiological data, we confirmed that an R-CMAP is a hallmark of *COLQ* and *SCCMS* patients, present in around three-quarters of these cases, resulting from a neuromuscular junction gain-of-function.[15] Highly elevated CK levels are suggestive of *GMPPB* gene mutation, as this gene has also been reported in LGMD or overlapping LGMD-CMS phenotype.[38] We confirmed that tubular aggregates point towards the diagnosis of *GFPT1*- and *DPAGT1*-associated CMS, and that features of muscular dystrophies can be observed in *GMPPB* patients.[34,39,40]

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. We acknowledge that this finding may have been influenced by the different treatments that patients received throughout their lives. *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 symptom exacerbations, these later exacerbations were relatively moderate since patients rarely required ICU admission. This relatively good prognosis is supported by the findings reported in previous cohorts.[16,18] SCCMS patients were frequently stable regarding their disease course but could worsen in approximately one-third of cases. Although they remained 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 course during their lives. Regarding the latter finding, clinicians should be aware that phases of worsening and improvement can succeed one other, and caution is needed when informing a particular patient about the long-term prognosis. Patients with *RAPSN*, *DOK7*, *MUSK* and *AGRN* mutations were more prone to have severe exacerbations requiring ICU admissions. While most ICU admissions in *RAPSN* and *MUSK* patients occurred in childhood, most of them in *DOK7* and *AGRN* patients occurred in adulthood. Thus, adult neurologists should be aware that severe exacerbations can be expected 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.[19] Our previous study on *COLQ* patients, which included some of the patients reported here, had already shown that most of these patients remained ambulant without respiratory assistance at last follow-up.[41] 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 was quite favourable. Finally, *GMPPB* and *GFPT1* patients were prone to have

worsening disease courses and around 20% of them were wheelchair-bound at last follow-up. However, they remained ventilation free. In these patients with glycosylation defects, myopathic changes can be observed in muscle biopsies and MRI, which could partly explain the worsening course.[34] 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 pregnancy. This frequency was lower than that previously reported.[9,42] This apparent discrepancy could be explained by the retrospective nature of our study, which was not specifically designed to address this question. Only six patients died in our adult cohort. Thus, the overall vital prognosis of adult CMS patients appears quite favourable. However, we found a family history of early death in infancy in 15 patients, with most of them bearing *RAPSN* and *COLQ* gene mutations, suggesting a possible life-threatening condition for these genes, in some cases during childhood. Importantly, future clinical trials aiming to evaluate the efficacy of treatments already available or in development will need to consider the different clinical courses. Outcomes and effect sizes need to be conceived and chosen according to the disease course of each genotype. For example, investigators will have to consider the improving course of *RAPSN* patients, the progressive worsening of *GMPPB* and *GFPT1* patients and the multiphasic course of *DOK7*, *COLQ* and *AGRN* patients. Moreover, further large-scale prospective studies will help to better define the natural history of CMS according to the genotype.

The small number of patients per gene prevented us from drawing conclusions regarding the long-term prognosis of patients with rare CMS genes. However, patients with mutations in pre-synaptic genes implicated in acetylcholine production and transport (*CHAT*, *SLC5A7*, *SLC18A3*) seem to have a favourable long-term motor and respiratory prognosis despite severe symptoms in infancy, such as hypotonia, feeding difficulties and episodic apnoea, even if one *SLC5A7* patient was wheelchair-bound and ventilated at last visit. This favourable prognosis contrasting with a severe onset was suggested in a previous study in *SLC5A7* patients.[43] *DPAGT1* patients were prone to develop CNS signs such as intellectual disability, as previously reported;[40] they seem to have a poor motor prognosis with the need for a wheelchair.

This cohort also provides important and valuable information regarding CMS treatment. We confirm that AChE inhibitors should be avoided in SCCMS, *COLQ* and *DOK7* patients, in whom their use could lead to symptoms worsening.[16,17,44] This treatment was often ineffective in *AGRN* patients.[45] Furthermore, 3,4-DAP was frequently ineffective in *AGRN* and *MUSK* patients, raising

the question of early treatment with salbutamol. SCCMS patients' symptoms were difficult to improve because 3,4-DAP and salbutamol were not effective in about half of treated patients. Fluoxetine, a selective serotonin reuptake inhibitor that acts as a channel blocker therapy, can be useful in some patients, and a previous study suggests that treatment is more effective the sooner it is started after the onset of symptoms.[46] Finally, quinidine could be an interesting option in these patients.

Apart from these cases, most patients responded favourably to AChE inhibitors and other treatments regularly administrated as second-line therapies. Salbutamol and ephedrine were particularly effective in *DOK7* and *COLQ* patients, with a high proportion of patients reporting a durable and significant improvement. Interestingly, the few patients in whom the treatment was considered ineffective had particularly severe disease with marked muscle weakness.

We also report 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) having already been published.[22,47] This frameshift variant is localized between the first two 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), not previously reported, is also present between the two first start codons and, like the first variant, is predicted to selectively impact LAP1B. It was absent from the gnomAD database. Each variant was 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.[22,48] However, contrary to previous cases, our patients developed severe acute respiratory insufficiency requiring admission to ICU, and they required non-invasive ventilation at discharge. Thus, our data indicate that respiratory involvement can be a major feature of *TOR1AIP1*-related CMS. Nevertheless, the motor prognosis seems favourable because all published patients were still ambulant at last visit.[22,48]

Our study has several limitations. Due to its retrospective design some clinical data on the initial phenotype could have been missed. The number of patients per genotype was not equal between genes, due to the variable prevalence of the different CMS genotypes, and this could lead to

difficulties in comparing them. Spirometry data were not available, but a recent study reported a progressive worsening of forced vital capacity in *DOK7* and *COLQ* patients.[19] Finally, treatment efficacy was determined retrospectively, based on clinicians' reports in the medical file and not on objective and repeated validated scales. However, the large size of this cohort and the mean follow-up of 34 years allowed us to obtain reliable data regarding prognosis and follow-up.

In conclusion, even if the phenotypical features of CMS do not change during the patient's life, the long-term prognosis is more complex and difficult to foresee due to various patterns of evolution from worsening to improvement, which can be multiphasic in some patients. However, knowing which gene is involved is informative: no long-term worsening was observed in *CHRNE* patients. *RAPSN* patients, even if severely affected in infancy, improved later. The situation is more critical for SCCMS, *DOK7* and *GFPT1* genotypes, with a significant proportion of patients requiring, at last visit, ventilation (SCCMS and *DOK7*), a wheelchair (*DOK7* and *GFPT1*), or both (*DOK7*). The positive impact of therapy was striking even in severely affected patients, some of them regaining walking capacity. Most patients of this cohort did not require ventilation and/or a wheelchair at last follow-up. Our results inform clinical practice, hopefully improving the diagnosis and management of these rare conditions.

# Data availability

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

# Acknowledgements

The authors thank Fatou Diop, Floriane Llorens, Marina Dupre, Karine Ferraud, Mathilde Ferey, Maud Pirotte and Emilie Laheranne-Martinez for their help in data acquisition. They thank Prof. Michel Fardeau, Prof. Jeanine Koenig, Dr. Daniel Hantaï and Dr. Arnaud Isapof for their key role in the initiation and development of the CMS project in France. The thumbnail was generated using BioRender.com and an illustration (https://commons.wikimedia.org/wiki/File:CPAP.png) licensed under the Creative Commons Attribution 3.0 unported license.

# Funding

No financial assistance was received in support of the study.

# Competing interests

The authors report no competing interests.

# Supplementary material

Supplementary material is available at *Brain* online.

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# Figure Legends

**Figure 1 Genetic and diagnostic characteristics of the cohort.** (**A**) Proportion of genotypes 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.

**Figure 2 Genotype-phenotype correlations** Heatmap and clustering of genotypes according to symptoms at initial presentation (**A**) and at last follow-up (**B**), and age at first symptoms. LE = low-expressor.

**Figure 3 Characteristic symptoms at diagnosis, by genotype.** Illustration of the human body showing genotypes with high prevalence of symptoms (Z-score > 0.85) in specific categories of symptoms. Created with BioRender.com. LE = low-expressor.

**Figure 4 Long-term data of CMS patients, by genotype.** (**A**) Disease course category. (**B**) Proportion of patients with exacerbations. Proportion of patients requiring (**C**) ICU admission during their disease course, (**D**) ventilation at last follow-up, (**E**) wheelchair at last follow-up. (**F**) MGFA category at last follow-up. CMS = congenital myasthenic syndrome; ICU = intensive care unit; LE = low-expressor; MGFA = Myasthenia Gravis Foundation of America. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$.

**Figure 5 Treatment efficacy according to the genotype.** Blue = improvement; Grey = no effect; Magenta = worsening; AChE = acetylcholinesterase; LE = low-expressor.

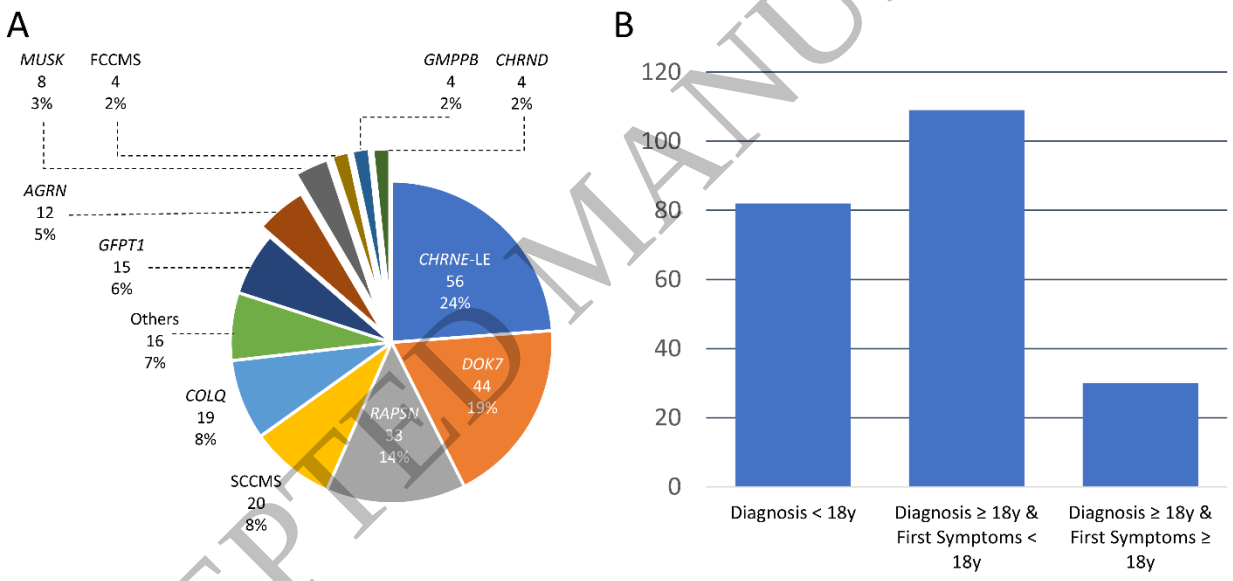


*Figure 1*
*199x93 mm ( x DPI)*

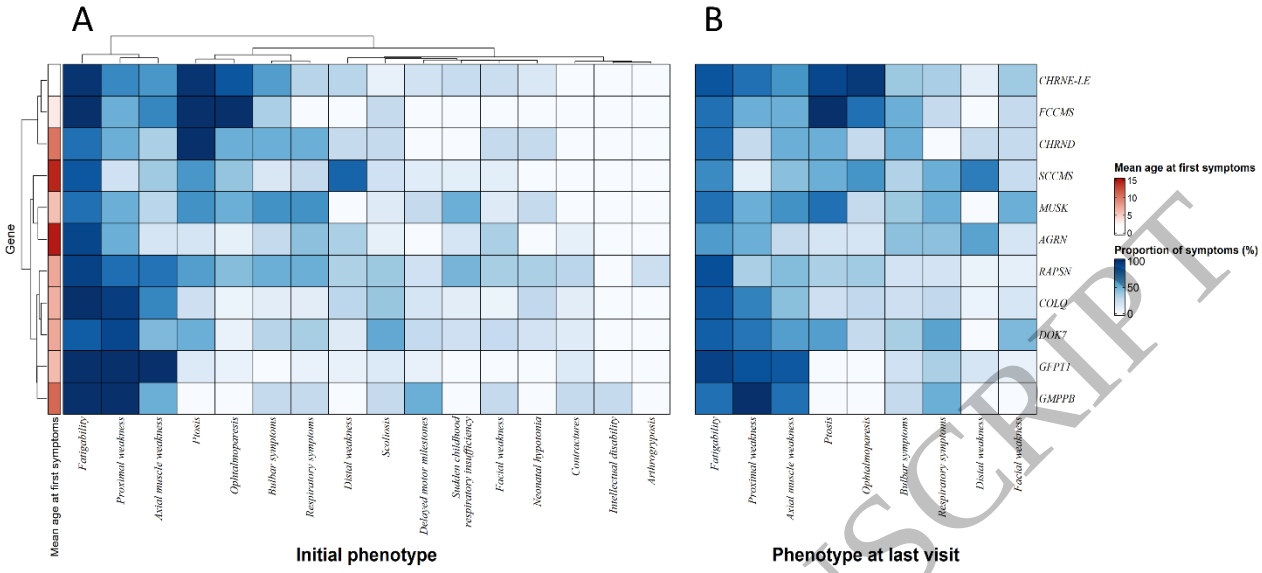


*Figure 2*
*209x95 mm ( x DPI)*

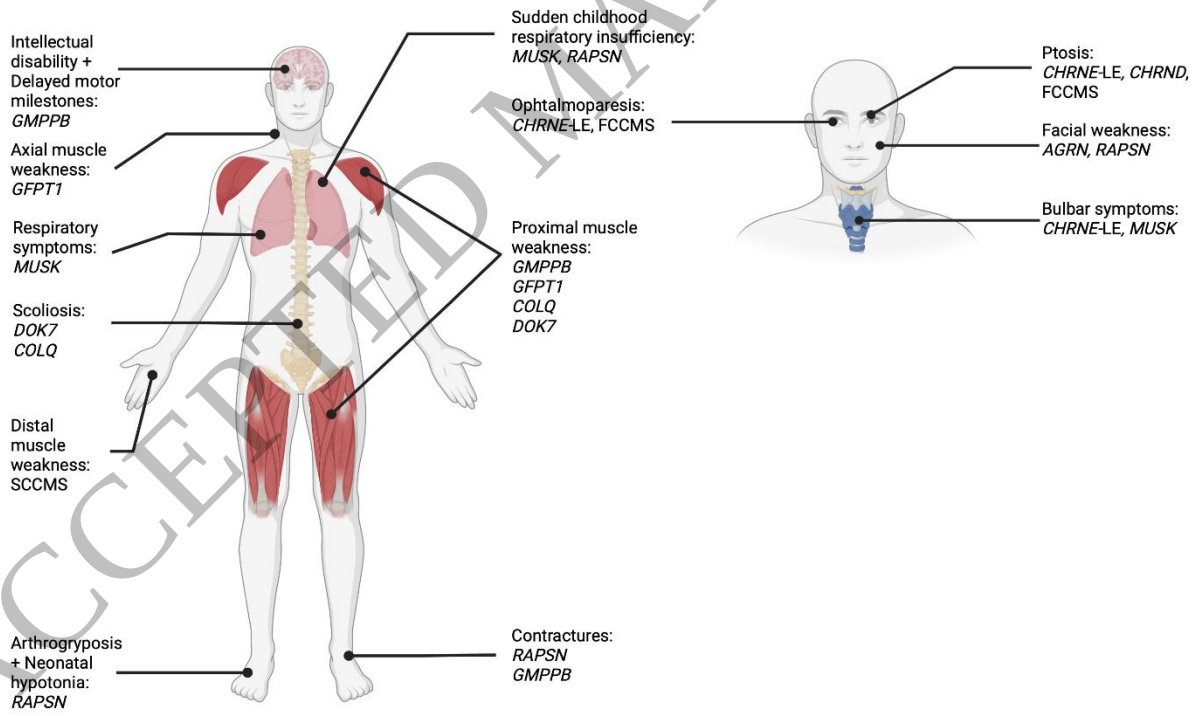


*Figure 3*
*254x147 mm ( x DPI)*

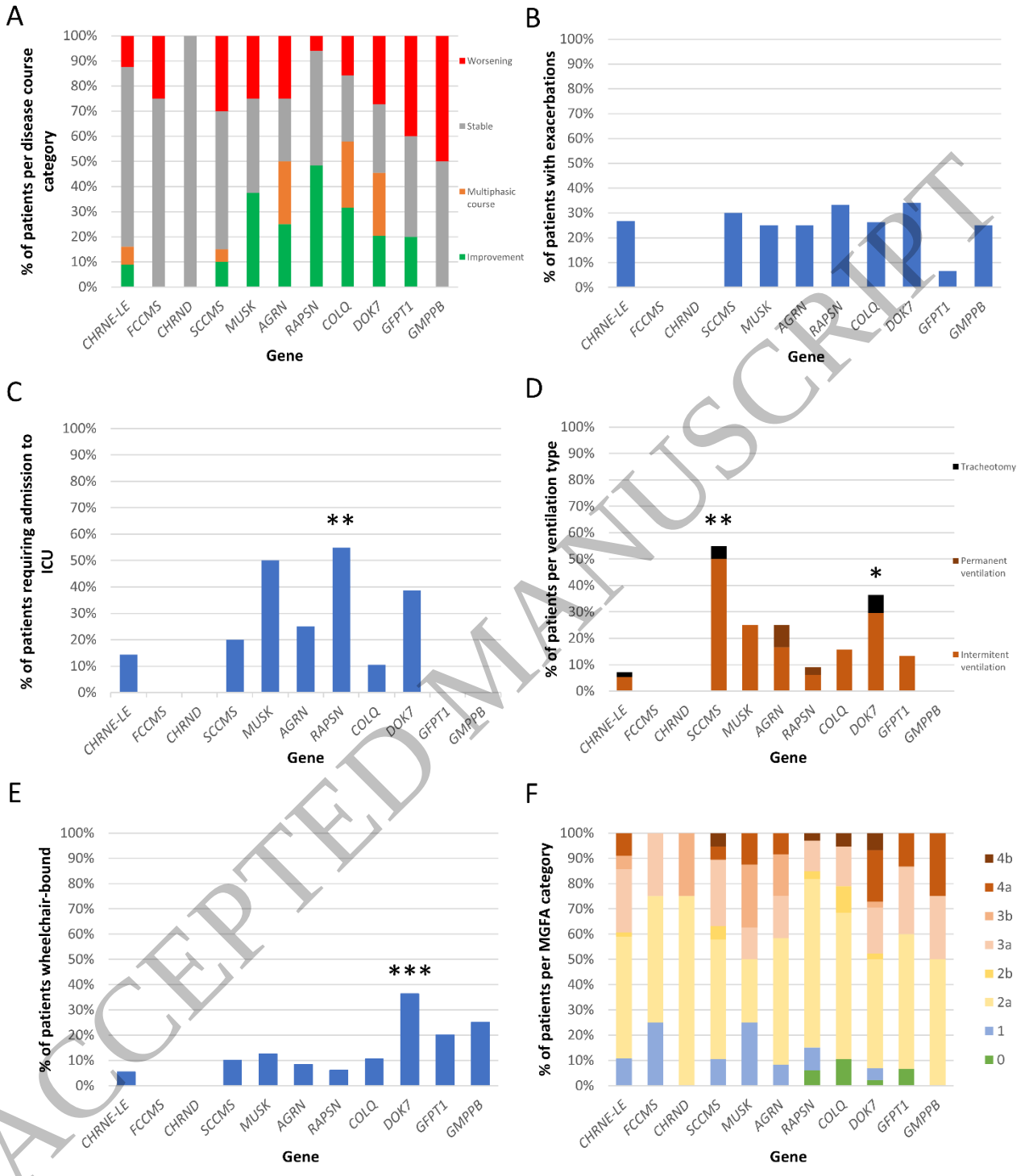


*Figure 4*
*209x245 mm ( x DPI)*

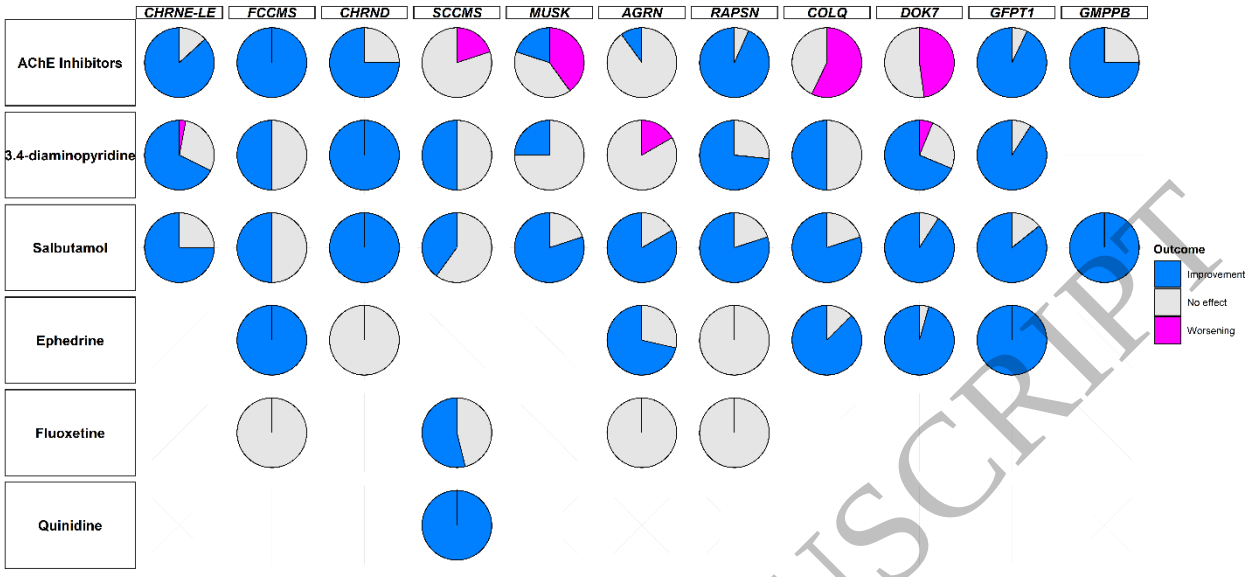


*Figure 5*
*446x210 mm ( x DPI)*

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

| **Misdiagnosis** | **Number of patients (% of patients with misdiagnosis / % of all patients)[a]** |
|---|---|
| Congenital myopathy | 69 (50 / 29.4) (*DOK7/RAPSN/CHRNE*) |
| Myasthenia gravis | 40 (29.0 / 17.0) (*DOK7/RAPSN/CHRNE*) |
| Muscular dystrophy | 22 (15.9 / 9.4) (*DOK7/RAPSN/GMPPB*) |
| Mitochondrial myopathy | 12 (8.7 / 5.1) (*CHRNE/MUSK/COLQ*) |
| Distal myopathy | 4 (2.9 / 1.7) |
| Spinal muscular atrophy | 4 (2.9 / 1.7) |
| Metabolic myopathy | 3 (2.2 / 1.3) |
| Channelopathies and 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
[a]For the four main misdiagnoses, the three genes that are mostly involved in terms of the number of patients are shown in parentheses.

**Table 2 Clinical characteristics and long-term prognosis of patients with rare genotypes (n ≤ 3)**

| Patient | Gene | Sex | Age at first symptoms | First symptoms | Disease course | ICU admission (age in years) | 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 stature |
| 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 respiratory insufficiency, sudden apnoea | 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 |

Treatment response: (+) = improvement; (/) = no effect; (-) = worsening. F = female; M = male; ICU = intensive care unit; AChE = acetylcholinesterase; 3,4-DAP = 3, 4-diaminopyridine; NIV = non-invasive ventilation; NA = not available.