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ORIGINAL ARTICLE

# Congenital myasthenic syndrome: Correlation between clinical features and molecular diagnosis

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## Abstract

**Objectives:** To present phenotype features of a large cohort of congenital myasthenic syndromes (CMS) and correlate them with their molecular diagnosis.

**Methods:** Suspected CMS patients were divided into three groups: group A (limb, bulbar or axial weakness, with or without ocular impairment, and all the following: clinical fatigability, electrophysiology compatible with neuromuscular junction involvement and anticholinesterase agents response), group B (limb, bulbar or axial weakness, with or without ocular impairment, and at least one of additional characteristics noted in group A) and group C (pure ocular syndrome). Individual clinical findings and the clinical groups were compared between the group with a confirmed molecular diagnosis of CMS and the group without molecular diagnosis or with a non-CMS molecular diagnosis.

**Results:** Seventy-nine patients (68 families) were included in the cohort: 48 in group A, 23 in group B and 8 in group C. Fifty-one were considered confirmed CMS (30 *CHRNE*, 5 *RAPSN*, 4 *COL13A1*, 3 *DOK7*, 3 *COLQ*, 2 *GFPT1*, 1 *CHAT*, 1 *SCN4A*, 1 *GMPPB*, 1 *CHRNA1*), 7 probable CMS, 5 non-CMS and 16 unsolved. The chance of a confirmed molecular diagnosis of CMS was significantly higher for group A and lower for group C. Some individual clinical features, alterations on biopsy and electrophysiology enhanced specificity for CMS. Muscle imaging showed at least mild alterations in the majority of confirmed cases, with preferential involvement of soleus, especially in *CHRNE* CMS.

  

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**Conclusions:** Stricter clinical criteria increase the chance of confirming a CMS diagnosis, but may lose sensitivity, especially for some specific genes.

**KEYWORDS**
congenital myasthenic syndromes, muscle MRI, neuromuscular disorders, neuromuscular junction, phenotype/genotype correlation

## INTRODUCTION

Congenital myasthenic syndromes (CMS) are a heterogeneous group of rare inherited diseases in which neuromuscular transmission is compromised [1,2]. For the clinical suspicion of CMS, a clinical tetrad has been suggested comprising fatigable weakness most pronounced in ocular and cranial muscles, paediatric onset, negative myasthenia gravis autoantibody testing, and supportive electrophysiological data [3]. However, some CMS types may not present with ocular or cranial weakness and/or may have clinical onset in adulthood [4–6]. In addition, electrophysiological testing is not easily performed in very young children, and may not present typical decrement after low-frequency repetitive nerve stimulation in some CMS patients [1,2]. CMS diagnosis can be rather difficult in neonates, in whom the only clinical features may be unspecific, such as feeding difficulties, weak cry, hypotonia and arthrogryposis [1,2].

Phenotypic overlap between CMS and other neuromuscular diseases is possible, especially with congenital myopathies and mitochondrial myopathies, making the final diagnosis even more difficult. These similarities are not only regarding clinical features but also neuromuscular transmission dysfunction, and occasionally some response to pyridostigmine [7–16].

This phenotypic variability and overlap mean that identifying the most likely molecular defect from clinical observation alone remains challenging. More precise mapping of clinical features to final diagnosis is valuable to improve this situation. Here, we present clinical, histological and electrophysiological features of a large cohort of CMS patients and correlate the observed features with the final molecular diagnosis to improve the genotype/phenotype correlation. In addition, we determine the relative frequency of mutations in different genes among this cohort, and depict alterations of muscle magnetic resonance image (MRI) found in some CMS patients.

## PATIENTS AND METHODS

Patients with suspected CMS evaluated at Hospital das Clínicas (HC-FMUSP) between May 2014 and May 2020 were included in the study. Suspected CMS was characterized by ocular, limb, bulbar or axial weakness (verified by clinical examination) and at least one of the following indications of neuromuscular junction involvement: electrophysiological myasthenic changes, clinical fatigability (reported or verified), or clinical improvement with anticholinesterase agents.

The exclusion criteria were: electrophysiological findings showing demyelination or axonal degeneration when no electrophysiological evidence of neuromuscular junction defect was present; elevation of serum creatine kinase higher than 50 times the upper normal limit; mitochondrial or evident neurogenic alterations on muscle biopsy; the presence of anti-acetylcholine receptor or anti-MuSK antibodies; and current use of drugs that trigger myasthenic syndrome.

Patients, or parents, were invited to participate in the study after informed consent was obtained according to the Declaration of Helsinki. The study was approved by the local ethics committee (CAAE#: 42612515.2.0000.0068).

All patients were evaluated by the first author (E.P.E.). Clinical data were collected, and general physical and neurological examination was performed on all subjects. Motor strength was graded using the Medical Research Council scale before fatigability tests. Eye movements were accessed by clinical observation only, and eyelid fatigability was tested by 2 min of sustained upgaze. To test fatigability, patients were asked to undertake the following tests, when strength allowed: upgaze sustained for 2 min, arms extended for 1 min, to move arms extended up and down 10 times, and sit and rise from a chair 10 times. Subjective fatigability reported by the patient or parent was also noted.

**TABLE 1** Congenital myasthenic syndrome: correlation between clinical features and molecular diagnosis in a large case series

| Phenotype | Group A ($n = 48$) | Group B ($n = 23$) | Group C($n = 8$) |
|---|---|---|---|
| Ocular weakness: eyelid drop, ophthalmoparesis | Facultative presence | Facultative presence | Obligatory presence |
| Facial weakness | Facultative presence | Facultative presence | Facultative presence |
| Limb, bulbar or axial weakness | Obligatory presence | Obligatory presence | Obligatory absence |
| All present: abnormal decrement, abnormal jitter, clinical fatigability, improvement with pirydostigmine | Obligatory presence | Obligatory absence | Obligatory absence |
| At least one present: abnormal decrement, abnormal jitter, clinical fatigability, clinical improvement with pyridostigmine | Obligatory absence | Obligatory presence | Obligatory presence |

*Note:* Phenotype features of clinical groups.

Based on clinical scenarios commonly seen in practice, patients were allocated to one of three clinical groups (Table 1; Figure 1):

Group A: Patients with limb, bulbar or axial weakness (verified by clinical examination), with or without ocular impairment (blepharoptosis and/or ophthalmoparesis) associated with all of the following indications of neuromuscular junction involvement: electrophysiological myasthenic changes, clinical fatigability (reported or verified), and clinical improvement with anticholinesterase agents.

Group B: Patients with limb, bulbar or axial weakness with or without ocular impairment and at least one of the following indications of neuromuscular junction involvement: electrophysiological myasthenic changes, clinical fatigability (reported or verified), or clinical improvement with anticholinesterase agents.

Group C: Patients with ocular and/or facial symptoms without a limb, bulbar or axial weakness detectable on physical examination (even after clinical fatigability tests); and at least one of the following indications of neuromuscular junction involvement: electrophysiological myasthenic changes, clinical fatigability (reported or verified), or clinical improvement with anticholinesterase agents.

Ancillary examinations such as muscle biopsy, nerve conduction studies and electromyography (NCS/EMG) and serum creatine kinase levels were noted. Repetitive stimulation of distal, proximal and facial muscles at a rate of 3 Hz was carried out in all patients. When available, concentric needle single fibre electromyography (SFEMG) was also recorded. All SFEMG tests were done on orbicularis oculi muscles [16].

Muscle biopsy slides were reviewed by a physician with expertise in muscle pathology (E.Z.).

Data from muscle MRI done with at least T1, T2 and short TI inversion recovery images were collected when available. Two experienced neurologists and an experienced radiologist independently evaluated all the examinations to reach a consensus. In the absence of consensus, the less intense score was used. Muscle fatty degeneration was assessed according to the distribution of abnormal muscle signal intensity on T1-weighted sequences. The grade of involvement was ranked based on the Mercuri scale [17].

All patients were screened for common CMS mutations: c.130dupG (*CHRNE*) [18] p.N88K (c.264C>A) (*RAPSN*) [19] and c.1124_1127dup (*DOK7*) [20]. After this step, unsolved cases underwent whole exome sequencing (WES) or a customized sequencing panel (3 patients; Table S1).

For the majority of cases, WES was performed by the Genomics Platform at the Broad Institute of MIT and Harvard, Cambridge, USA. Libraries were created with an Illumina exome capture kit (38 Mb target) and sequenced with a mean target coverage of >80×. Exome sequencing data were processed and analysed on the RD-Connect Genome-Phenome Analysis Platform (https://platform.rd-connect.eu/genomics – identifiers listed in Table S1). Likely pathogenic variants, affecting the gene's function and potentially causing disease, were identified by applying standard filtering criteria: minor allele frequency <1%, and high to moderate variant effect predictor (i.e., nonsense, splice site, frameshift, in-frame and non-synonymous variants). Shortlisted variants were interrogated for their predicted in silico deleteriousness and previously known association with human disease.

Seven cases were submitted to commercial molecular testing without any prior screening. Of these, four underwent WES on an Illumina platform (NovaSeq 6000), BWA (Burrows-Wheeler Aligner) for alignment to the reference genome and call of variants with bioinformatics script validated according to GATK recommendations (Broad Institute), with coverage of 95% with 10 or more reads. Three patients underwent sequencing with a customized panel for 90 neuromuscular disorder genes, including 16 CMS genes (Table S1).

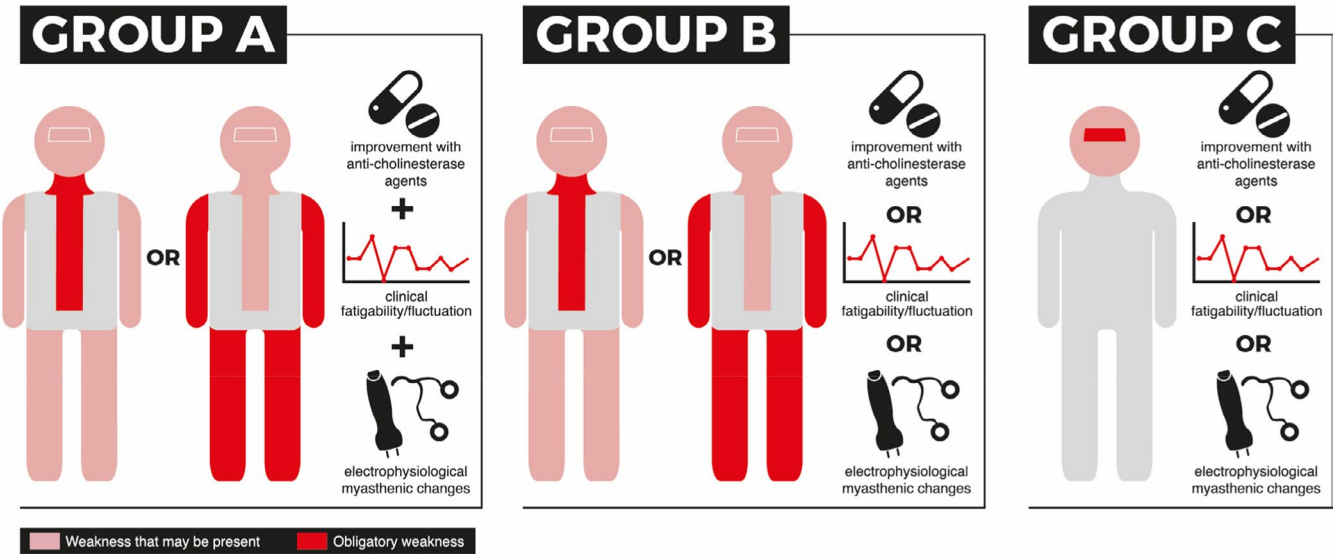


**FIGURE 1** Schematic bodies representing patterns of affected muscles and clinical indications of neuromuscular junction involvement in each clinical group

Following complete analysis of the WES data, cases were stratified into four categories: confirmed molecular diagnosis of CMS, confirmed molecular diagnosis of a condition other than CMS, probable molecular diagnosis of CMS, and unsolved. Probable cases were those where a highly plausible single pathogenic variant was found in a typically autosomal recessive condition, and those where a novel likely pathogenic variant was found with an atypical corresponding syndrome.

### Statistical analysis

Individual clinical findings and the categorized clinical groups A, B and C were compared between the group with a confirmed molecular diagnosis of CMS and the group without molecular diagnosis or with a non-CMS molecular diagnosis. Patients classified as probable molecular diagnosis were excluded from the comparison. Two-tailed Fisher's exact test was used to calculate exact $p$ values from $2 \times 2$ contingency tables (http://www.graphpad.com/quickcalcs/contingency2.cfm).

## RESULTS

Of 81 patients initially evaluated, two were excluded; one diagnosed as autoimmune myasthenia gravis and another diagnosed as mitochondrial myopathy. Seventy-nine patients (45 females), from 68 unrelated families, aged from 1 to 68 years at last clinical evaluation, were included in the final cohort.

Based on the clinical and electrophysiological findings, 48 patients were allocated to group A, 23 to group B and 8 to group C (Table 2).

After molecular tests, 51 of the 79 cases were considered to have a confirmed molecular diagnosis of CMS, 5 to have a confirmed diagnosis of a condition other than CMS, 7 to have a probable molecular diagnosis of CMS and 16 to be unsolved. *CHRNE* was the disease-causing gene in 22 of 68 families, with c.130dupG present in 19 families. It was followed by *RAPSN* ($n = 5$ families), *COL13A1* ($n = 3$), *DOK7* ($n = 3$), *COLQ* ($n = 2$), *GFPT1* ($n = 2$), *SCN4A* ($n = 1$), *CHAT* ($n = 1$), *CHRNA1* ($n = 1$) and *GMPPB* ($n = 1$). All *RAPSN*, *DOK7* and *COLQ* confirmed cases shared at least one recurring variant (c.264C>A for *RAPSN* patients, c.1124_1127dupTGCC for *DOK7* and c.219+1G>C for *COLQ*).

In three families diagnosed as probable *CHRNE* with receptor deficiency syndrome (families 2, 5 and 23) and in one as probable *RAPSN* (family 60) only a single heterozygous variant was found. In these patients, the clinical and electrophysiological findings were highly compatible with the molecular findings, and the diagnosis was considered probable on the assumption that a second variant could not be identified by WES.

One patient (family 31) harboured novel biallelic likely pathogenic variants (classified as depicted in the Methods section) in *SCN4A* and another (family 50) in *CHRND* (with receptor deficiency syndrome). Considering that the phenotype was not wholly compatible, they were therefore considered probable diagnoses. Probable cases were always excluded from the comparative analysis.

The *CHRNA1* patient and some of the *CHRNE*, *COL13A1* and *RAPSN* patients were previously reported [18,21–23]. In the end, 16 families remained unsolved, and 5 were diagnosed as non-CMS syndromes (*PTPN11*, *VWA3B*, *LDB3*, *DHCR7*, *SCN8A*). CMS had originally been considered a possible clinical diagnosis because of their ocular symptoms (mainly eyelid ptosis) and subjective fluctuation (Table S2).

In group A, 40 of 48 patients reached a confirmed molecular diagnosis of CMS (83%), while 4 (8.5%) were considered probable and 4 remained unsolved. In group B, 11 (47.8%) reached a confirmed diagnosis, 2 (8.5%) a probable diagnosis and 7 (30.5%) were unsolved. In group C only 1 patient (12.5%) had a probable diagnosis and 5 (62.5%) were unsolved (Table 2). The non-CMS cases were 3 (13%) in group B (*SCN8A*, *PTPN11*, *LDB3*) and 2 (25%) in group C (*VWA3B*, *DHCR7*).

Some clinical characteristics were particularly frequent in patients with confirmed CMS (Table 3). Particularly, eye movements were impaired in 82.3% (42 cases); 74.5% (38 cases) of which had only partial impairment. Downgaze was spared in 70.5% (36 cases), as the only movement spared or with other eye movement spared. *CHRNE* patients were the main contributor to these numbers: 100% of 30 confirmed cases had impairment of eye movement, of which 90% were partial ($n = 27$), and 83.5% sparing downgaze ($n = 24$). Moreover, 100% of confirmed *COLQ* (3 cases), 80% of confirmed *RAPSN* (4 of 5 cases), 50% of *COL13A* (2 of 4 cases) and the cases of confirmed *SCN4A* and *CHAT* presented with eye movement impairment; all of them sparing downgaze (Figure 2). Other frequent clinical findings in confirmed cases were facial weakness (66.6%), bulbar symptoms (62.7%), delayed motor milestones (60.7%) and masticatory weakness (57.8%). Ninety-six per cent of confirmed cases were without major disabilities or progressive symptoms, considering them after treatment. All patients were tried on pyridostigmine; 23 were also tried on salbutamol and 16 on ephedrine. Good response to pyridostigmine, ephedrine and salbutamol was seen in 80.3%, 100% and 81.2% of confirmed cases, respectively.

NCS/EMG data were available in all 79 cases. In 38 patients, SFEMG of the orbicularis oculi had been performed. More than 10% decrement on 3 Hz repetitive nerve stimulation was seen in 85.7% of 49 confirmed cases. Myopathic changes on electromyography were seen in 12 patients with confirmed molecular diagnosis (24.4%). Of these, SFEMG examination revealed increased jitter in 95.2%.

Muscle biopsy data were collected from 41 patients. Muscle biopsy of 24 confirmed CMS patients was available and 91.6% was altered. The most frequent findings were type II fibres atrophy (37.5%) and type I fibres predominance (24.4%). The two patients with *GFPT1* CMS presented muscle biopsy with tubular aggregates.

The main clinical features of all patients are detailed in Table S3.

Nineteen patients (*CHRNE = 12*, *COLQ = 2*, *COL13A1 = 2*, *RAPSN = 2*, *DOK7 = 1*) had whole-body MRI data available, 6 had thighs and legs MRI (*CHRNE = 5*, *GFPT1 = 1*), 2 had only thigh

**TABLE 2** Congenital myasthenic syndrome: correlation between clinical features and molecular diagnosis in a large case series

| Molecular outcome | Group A ($n = 48$) | Group B ($n = 23$) | Group C($n = 8$) |
|---|---|---|---|
| Confirmed molecular diagnosis | 83%*** | 48% | 0%* |
| *CHRNE* | 60.5%*** | 4.5% | 0% |
| *RAPSN* | 8.5% | 4.5% | 0% |
| *COL13A* | 0% | 17.5%* | 0% |
| *DOK7* | 0% | 13%* | 0% |
| *COLQ* | 2% | 8.5% | 0% |
| *GFPT1* | 4% | 0% | 0% |
| *GMPPB* | 2% | 0% | 0% |
| *SCN4A* | 2% | 0% | 0% |
| *CHAT* | 2% | 0% | 0% |
| *CHRNA1* | 2% | 0% | 0% |
| Probable molecular diagnosis | 8.5% | 9% | 12% |
| *CHRNE* | 8.5% | 0% | 0% |
| *RAPSN* | 0% | 4.5% | 0% |
| *SCN4A* | 0% | 4.5% | 0% |
| *CHRND* | 0% | 0% | 12% |
| Unsolved | 8.5% | 30% | 63% |
| Molecular diagnosis other than CMS | 0%** | 13% | 25% |

*Note:* Molecular diagnoses of suspected congenital myasthenic syndrome (CMS) cases among three different groups. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, comparing each category of one group against two other groups.

muscles MRI (*CHRNE* and *GMPPB*) and 1 *CHRNE* patient had only leg muscles MRI.

Of the *CHRNE* patients, 18 had an MRI of the legs, and in 10 (55%) the examination was considered altered (Figure 3). All 10 patients had the soleus muscle affected that was the most affected muscle in 4 patients (22%), and the only affected muscle in 2 patients (11%) (one with whole-body MRI and one with MRI of the thigh and leg muscles). Thigh muscles were mildly and homogeneously impaired, and from 18 *CHRNE* patients with thigh images, muscles were altered in 9 (50%). Considering the 12 *CHRNE* patients with whole-body MRI, 8 (66%) had an abnormal examination; all of them with the soleus muscle affected. In general, muscle impairment was mild and symmetrical.

Of the two *RAPSN* patients, one was normal and the other presented diffuse and mild impairment of all leg muscles. The *DOK7* patient had diffuse and mild impairment of obliquus externus abdominis muscle and all lower limb muscles, but the soleus was the most affected muscle. The *GMPPB* patient showed diffuse and mild impairment of thigh muscles, and the *GFPT1* patient showed diffuse and mild impairment of thigh and leg muscles. In the two *COL13A1* patients, paravertebral and abdominal muscles were markedly affected, and all other muscles were considered normal. All muscles evaluated of all patients are depicted in Tables S4–S6.

The chance of a confirmed molecular diagnosis of CMS was significantly higher for clinical group A in comparison with group B ($p < 0.001$), group C ($p < 0.0001$) and both groups together ($p < 0.0001$) (Table 2). It was the case even when probable cases were considered as confirmed CMS diagnosis ($p < 0.001$ for A × B; $p < 0.0001$ for A × C; $p < 0.0001$ for A × [B + C]). In group B, confirmed molecular diagnosis of CMS was significantly higher than in group C, both when probable cases were considered as confirmed CMS diagnosis ($p < 0.05$) and when they were excluded ($p < 0.05$). Finally, comparing group C with the other two groups together confirmed that molecular diagnosis of CMS was statistically significantly lower, including when probable cases were considered diagnosed ($p < 0.001$) and when they were excluded ($p < 0.001$). No cases from group A were diagnosed as non-CMS and this diagnosis was significantly less frequent compared with group B ($p < 0.05$), group C ($p < 0.05$) and with both together ($p < 0.01$). Conversely, patients from group A were proportionally more frequent among confirmed cases ($p < 0.0001$) and less frequent among unsolved and non-CMS cases ($p < 0.05$). *CHRNE* confirmed cases were more frequent in group A ($p < 0.0001$), while *COL13A1* and *DOK7* cases were more frequent in group B ($p < 0.001$ and $p < 0.05$) (Table 2).

Presence of family history of CMS and early infancy clinical features such as neonatal hypotonia, breastfeeding difficulties and delayed motor milestones were significantly more frequent in CMS confirmed cases ($p < 0.05$), considering each separately. This was also the case with presence of limb, facial and masticatory weakness ($p < 0.001$, $p < 0.01$, $p < 0.05$). A considerable period with frequent myasthenic crisis (more than once a year) was also more frequent in confirmed cases ($p < 0.05$), although this feature was present in only 33% of these cases.

Among non-CMS cases, intellectual disability was significantly more frequent ($p < 0.001$) and electrophysiology alterations less frequent ($p < 0.01$ for decrement and $p < 0.05$ for increased jitter). In this cohort, the probability of a non-CMS diagnosis was 100% when these two clinical features were present together (intellectual disability and absence of neurophysiological changes typical for neuromuscular transmission).

When electrophysiological evidence of neuromuscular junction impairment was present, the probability of a confirmed molecular diagnosis was 87%. This probability was higher if another typical CMS feature was present: 91% with a non-pure ocular syndrome, 91% with a positive response to pyridostigmine, 92% with early infancy clinical features and 95% with familial history of similar phenotype. A summary of all comparisons can be found in Tables 3 and 4.

## DISCUSSION

Our study showed that when most rigorous criteria are used for the diagnosis of CMS, the confirmed cases were far more frequent, and no non-CMS case was found. In group A that has more restricted criteria, the chance of confirmed diagnosis was as high as 83%, and only 8.5% remained unsolved. In contrast, a confirmed molecular

**TABLE 3** Congenital myasthenic syndrome: correlation between clinical features and molecular diagnosis in a large case series

| Features | Confirmed ($n = 51$) | Probable ($n = 7$) | Unsolved ($n = 16$) | Non-CMS($n = 5$) |
|---|---|---|---|---|
| Group A ($n = 48$) | 78%*** | 57% | 25%* | 0%* |
| Group B ($n = 23$) | 22% | 29% | 44% | 60% |
| Group C ($n = 8$) | 0 | 14% | 31% | 40% |
| Early onset of symptoms | 90%* | 86% | 50% | 80% |
| Fluctuation of symptoms | 96% | 100% | 94% | 100% |
| Family cases | 49%* | 28% | 18% | 0% |
| Eyelid drop | 92% | 86% | 94% | 62% |
| Ocular muscle impairment | 82% | 71% | 69% | 25% |
| Ocular muscle impairment: partial | 74% | 71% | 56% | 25% |
| Ocular muscle impairment: sparing downgaze | 70% | 57% | 56% | 25% |
| Limb weakness | 90%** | 86% | 50% | 25% |
| Axial weakness | 41% | 14% | 19% | 12% |
| Bulbar symptoms | 63% | 43% | 56% | 37% |
| Scoliosis | 23% | 14% | 12% | 20% |
| Masticatory weakness | 57%* | 43% | 25% | 25% |
| Facial weakness | 67%* | 57% | 31% | 12% |
| Mild functional restriction | 92% | 100% | 94% | 100% |
| Severe respiratory impairment | 16% | 0% | 6% | 0% |
| Cognitive impairment | 10% | 0% | 0% | 80%* |
| Exacerbations crisis: at least one in life | 49% | 57% | 37% | 0% |
| Exacerbation crisis: more than one per year | 33%* | 28% | 12% | 0% |
| Delayed motor milestones | 90%* | 85% | 50% | 80% |
| Neonatal hypotonia | 72%* | 43% | 25% | 100% |
| Breastfeeding difficulty | 43%* | 14% | 12% | 20% |
| High-arched palate | 76% | 28% | 62% | 60% |
| Congenital contractures | 14% | 0% | 12% | 40% |
| Response to pyridostigmine: positive | 78% | 86% | 62% | 40% |
| Response to pyridostigmine: negative | 10% | 0% | 6% | 0% |
| Response to pyridostigmine: none | 12% | 14% | 32% | 60% |
| Response to β2-agonists: ephedrine ($n = 15$) | 100% ($n = 13$) | 100% ($n = 1$) | 50% ($n = 1$) | Not tested |
| Response to β2-agonists: salbutamol ($n = 23$) | 81% ($n = 13$) | 100% ($n = 3$) | 33% ($n = 1$) | 0% |
| Muscle biopsy ($n = 41$): any alteration | 91%* ($n = 22$) | 100% ($n = 3$) | 40% ($n = 4$) | 50% ($n = 2$) |
| Muscle biopsy ($n = 41$): type 2 atrophy | 37% ($n = 9$) | 66% ($n = 2$) | 10% ($n = 1$) | 25% ($n = 1$) |
| Muscle biopsy ($n = 41$): type 2 predominance | 29%* ($n = 7$) | 0% | 0% | 0% |
| Abnormal decrement | 86%*** | 57% | 31% | 0%* |
| EMG with myopathic features | 23% | 14% | 25% | 40% |
| Increased jitter | 95%*** ($n = 21$) | 75% ($n = 4$) | 30% ($n = 10$) | 0%* ($n = 3$) |

*Note:* Main clinical features of confirmed, probable, unsolved and non-congenital myasthenic syndrome cases.

*$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$; $p$ value = comparing each category of one group against two other groups, excluding probable cases.

Abbreviations: CMS, congenital myasthenic syndrome; EMG, electromyography.

diagnosis of CMS was significantly lower in group C (group with pure ocular syndrome) compared with the other two groups (together and individually). In fact, none of the group C patients reached a confirmed molecular diagnosis despite extensive analysis. One patient in this group, with ocular symptoms associated with facial weakness, was classified as probable *CHRND* case (receptor deficiency). This strengthens the notion that a pure ocular syndrome is not a usual phenotype of CMS, as was previously suggested [24]. Although it is known that congenital myasthenia patients do not usually have pure ocular symptoms, this phenotype has sometimes been reported [25]. Actually, our patient 7 was previously reported as a pure ocular syndrome at 19 years old [18], and now, aged 23 years, it was possible

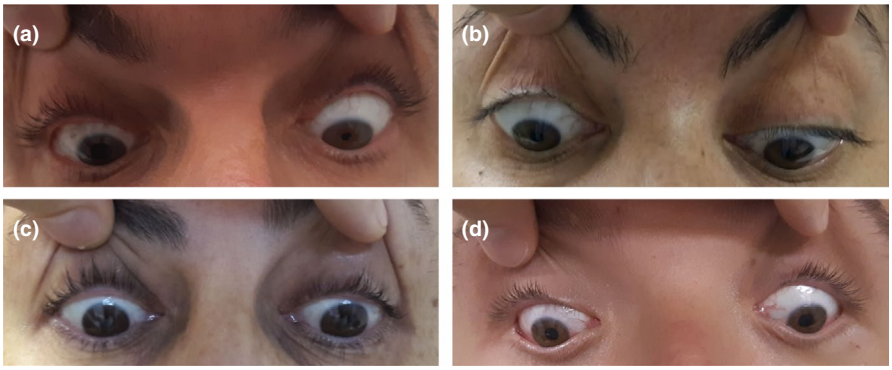


**FIGURE 2** Relatively sparing of downgaze among patients with partial ophthalmoparesis. Patients with distinct causative congenital myasthenic syndrome genes presented different grades of asymmetrical impairment of the ocular movements. (a) Patient 61 (*COLQ*). (b) Patient 29 (*COLQ*). (c) Patient 59 (probable *SCN4A*). (d) Patient 27 (*CHRNE*)

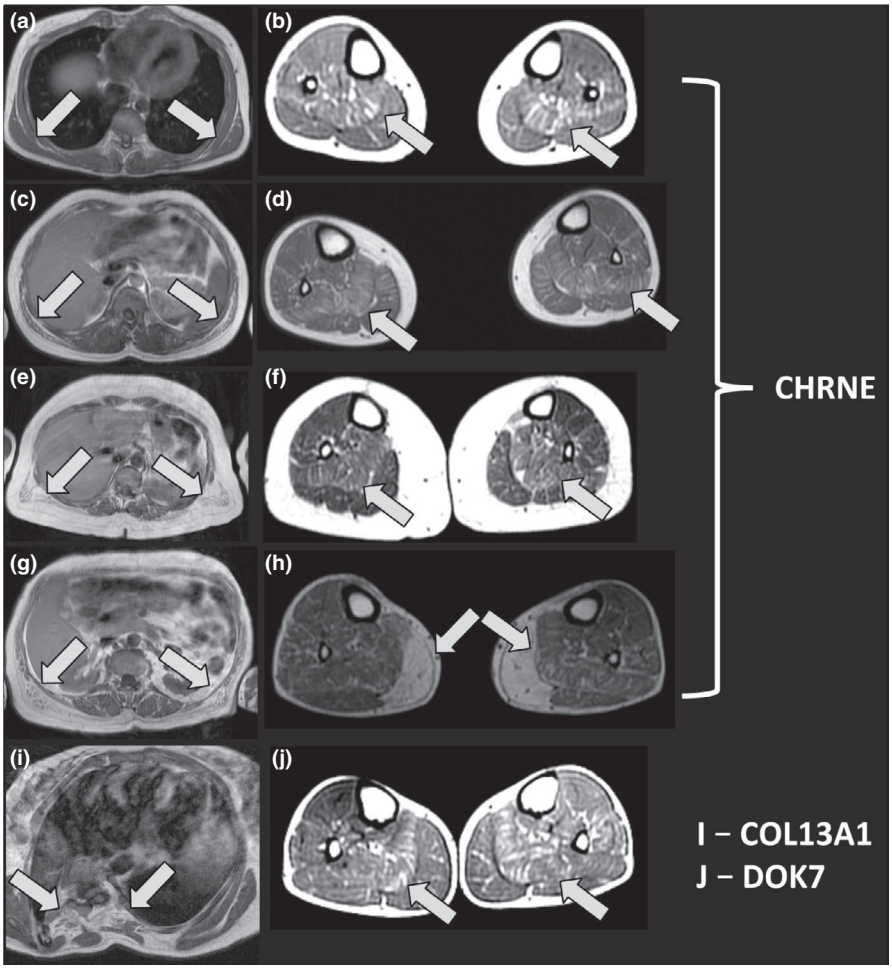


**FIGURE 3** Magnetic resonance imaging (axial T1-weighted images) of six congenital myasthenic syndrome patients. (a), (c), (e) and (g) depict the latissimus dorsi muscle (arrows): normal in (a), mild atrophy (grade 1) in (c) and severe atrophy (grade 3) in (e) and (g). (b), (d), (f), (h) and (j) show moderate atrophy (grade 2) of the soleus muscles [arrows in (b), (d), (f), (j)] and end-stage atrophy of the medial gastrocnemius [arrows in (h)]. (i) depicts severe atrophy (grade 3) of the paravertebral muscles (arrows) and deformity of the spine and thorax. (a) and (b) = patient 4, (c) and (d) = patient 17, (e) and (f) = patient 40, (g) and (h) = patient 39, (i) = patient 38, (j) = patient 47

to see limb weakness. However, all *DOK7* and *COL13A1* patients were found in group B, as these cases do not respond to pyridostigmine, and *COL13A1* patients often do not present fluctuations of symptoms [22,26], both characteristics included in group A criteria. One of three *COLQ* cases was in group A, because initial symptoms improved with pyridostigmine, although after a few weeks the effect was the opposite. The incidence of unsolved cases is in part explained by the broad criteria of groups B and C. In addition, mutations in unknown regulatory regions, deep intronic mutations, large deletions or duplications might have escaped our screening strategy. Finally, yet unidentified CMS genes might also contribute to the lack of confirmed diagnosis.

The diagnosis of some non-CMS cases was also expected considering the broad inclusion criteria, especially in groups B and C, where these cases had been allocated. A total of five cases were diagnosed with a non-CMS molecular diagnosis other than the typical phenocopies of CMS (congenital and mitochondrial myopathies). The absence of those can be explained by the high rate of muscle biopsies, which probably allowed the identification of such myopathies. The five non-CMS cases were excluded from group A by its restrictive criteria, and most of them presented some unusual symptoms for CMS: ataxia, deafness, dysmorphisms and intellectual disability. Intellectual disability was statistically less frequent among confirmed cases. Although some CMS due to *GMPPB* [27,28],

**TABLE 4** Congenital myasthenic syndrome: correlation between clinical features and molecular diagnosis in a large case series

| Clinical picture | Confirmed CMS ($n = 51$) | Unsolved ($n = 16$) | Non-CMS ($n = 5$) |
|---|---|---|---|
| Pure ocular syndrome ($n = 7$) | 0%*** | 71% | 29% |
| Cognitive impairment ($n = 9$) | 55% | 0% | 45% |
| No NMJ dysfunction, cognitive impairment ($n = 4$) | 0% | 0% | 100%*** |
| NMJ dysfunction ($n = 55$) | 87%*** | 13% | 0% |
| NMJ dyfunction, family cases ($n = 22$) | 95%* | 5% | 0% |

*Note:* Percentage of main clinical pictures among CMS cases, unsolved cases and non-CMS cases. Probable cases were excluded for comparison. *$p < 0.05$, ***$p < 0.0001$, comparing each category of one group against two other groups.

Abbreviations: CMS, congenital myasthenic syndrome; NMJ, neuromuscular junction.

*DPAGT1* [29,30] and *SNAP25* [31] frequently present with associated cognitive symptoms, this feature is known to be rare among CMS in general.

Dividing patients based on clinical aspects before genetic testing was not done with the intent to propose a classification. We rather aimed to demonstrate the relevance of some clinical scenarios commonly seen in clinical practice, and provide insights for managing patients before the genetic confirmation. In the real world, notably in low-income countries, we have to make decisions about treatment before genetic diagnosis. In the confirmed *SCN4A* patient (case 16), for example, invasive ventilation was possible to be removed after treatment was started, 3 years before we had genetic results. We could verify that although electrophysiology and response to piridostigmine are highly indicative of CMS, only suspecting cases with both features (group A) would lead to a loss of a considerable proportion of cases. The confirmed cases in group B, which lack one of those features, illustrate this point. Conversely, it was also verified that pure ocular syndrome is a very uncommon presentation of CMS, once none of the group C cases was confirmed as CMS. Indeed, seronegative was a possibility for group C cases, especially case 53 in which onset of symptoms was relatively late, at the age of 9 years. Palpebral ptosis was symmetrical in all patients of this group, but none of them were tested for clustered antibodies.

All non-CMS cases were included in the cohort because of fluctuating ocular symptoms, with or without extra-ocular weakness. With stricter phenotype criteria (only including patients with confirmed decrement or jitter, disregarding clinical weakness pattern), no non-CMS cases would have been included. Only three confirmed patients would be lost (*CHRNE = 1*, *1 RAPSN = 1*, *DOK7 = 1*). These three cases had no decrement on low-frequency repetitive stimulation, but SFEMG was not performed. In the other three cases without decrement, SFEMG was performed and increased jitter was present. In fact, decrement on electromyography and increased jitter on SFEMG were significantly more frequent in confirmed cases, and the presence of any of them predicted an 87% probability of CMS molecular diagnosis.

Ocular movement impairment with relative sparing of downgaze was a frequent finding among confirmed cases, not only in *CHRNE* and *RAPSN* patients as was already described in previous studies [18,21], but also in *COLQ*, *COLA13A1*, *SCN4A* and *CHAT*. The absence of significance of ocular alterations, combined with the presence of significance regarding extra-ocular symptoms, suggests that ocular symptoms are less specific for CMS phenotype than extra-ocular symptoms. This strengthens the notion that a pure ocular syndrome may not be indicative of CMS.

Regarding muscle MRI, the majority of confirmed cases showed at least mild alterations. Unlike as previously reported by Finlayson and colleagues [32], whereas patients with receptor deficiency presented normal muscle MRI, the soleus muscle seemed to be preferentially affected in *CHRNE* patients. In our study, muscle changes were apparently more present in older patients, and the mean age was slightly higher (31.2 × 24) compared to the previous study, and we included six patients older than the oldest case in the other study. In this way, age differences could be an explanation for the different results. In *COL13A1* patients the axial muscles were preferentially affected. These findings suggest that muscle MRI may play a role in diagnosing CMS and differentiating subtypes. In the end, muscle alterations were a frequent CMS feature, not only depicted by MRI findings, but also by myopathic changes in EMG, as seen in 24% of confirmed cases, and significantly higher rate of altered muscle biopsy. However, the histological findings observed in our patients such as type II fibres atrophy and type I fibres predominance are usually described as unspecific. Tubular aggregates, as found in two *GFPT1* patients, are fairly characteristic of CMS due to a glycosylation defect [33,34]. It is not the case that we would routinely recommend muscle MRI or biopsy in suspected CMS. But in non-obvious cases of CMS, in which a biopsy or muscle resonance is performed to search for also other hypotheses, the myopathic changes that we have shown to be frequent in CMS might be in favour of CMS. Furthermore, the findings described allow for a more specific phenotypic characterization, which can be useful when interpreting variants not yet described, which are increasingly common with next-generation sequencing.

The relative frequency of mutations in the different genes in our cohort roughly corresponds to other published frequencies [6], with some commonly reported variants in European cohorts (*CHRNE* c.130dupG, *RAPSN* p.N88K, *DOK7* c.1124_1127dupTGCC, and *COLQ* c.219+1G>C). In fact, 45% of cases were of European descent, 22% from Portugal, 14% from Italy and 7% from Spain. Native

Indians were present in 9% of the patients' antecedents, and in 40% of the cases the ethnicity data were not known by the patients. Interestingly, mutations in *RAPSN* were found at a relatively high frequency and were not found in the previously reported Brazilian cohort [35], which can be explained by differences in population, the methodology of genetic tests and the number of families included. Furthermore, here we found *COL13A1* to be an unexpected important causative gene for CMS.

In conclusion, as next-generation sequencing is becoming increasingly available, it is essential to have data from the features of genetic syndromes that can help to make the phenotypic profile of the disease more specific. This helps to select genes to prioritize for variant searches and to interpret potentially causal variants of unknown significance. Our findings enhance the specificity of phenotypic profiling for CMS. In particular, we show that a pure ocular syndrome is not a usual phenotype of CMS, electrophysiology plays an important role in diagnosis, and the absence of fluctuating symptoms and/or good response to anticholinesterase treatment should not rule out CMS. Ocular movement impairment with relative sparing of downgaze may be a frequent feature of CMS, as well as myopathic alterations, notably preferential affectation of soleus muscle on MRI. Finally, clinical diagnosis of CMS cannot always be confirmed, even by extended genetic testing, and additional CMS genes likely remain to be discovered.

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## CONFLICT OF INTEREST

The authors report no competing interests.

## AUTHOR CONTRIBUTIONS

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## DATA AVAILABILITY STATEMENT

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

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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