A Missense Mutation in Epsilon-subunit of Acetylcholine Receptor Causing Autosomal Dominant Slow-channel Congenital Myasthenic Syndrome in a Chinese Family

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Abstract

Background: Congenital myasthenic syndromes are a group of rare disorders that are clinically and genetically heterogeneous and caused by mutations in the genes encoding proteins of the neuromuscular junction. Here, we described a Chinese family that presented with phenotypes of classic slow-channel congenital myasthenic syndrome (SCCMS).
Methods: Clinical characteristics and electrophysiological features of three patients from a Chinese family were examined, and next-generation sequencing followed by direct sequencing was carried out.
Results: The patients revealed variability in clinical and electrophysiological features. However, weakness, scoliosis, and repetitive-compound muscle action potential were found in all affected members in the family. A heterozygous C>T missense mutation at nucleotide 865 in acetylcholine receptor epsilon-subunit (*CHRNE*) gene that causes a leucine-to-phenylalanine substitution at position 289 (L289F) was found.
Conclusions: We reported a SCCMS family of Chinese origin. In the family, classical clinical phenotype with phenotypic variability among different members was found. Genetic testing could help diagnose this rare disease.

Key words: Acetylcholine Receptor Epsilon-subunit Gene; Repetitive-compound Muscle Action Potential; Repetitive Nerve Stimulation; Slow-channel Congenital Myasthenic Syndrome

INTRODUCTION

Congenital myasthenic syndromes (CMSs) are a group of rare genetic disorders affecting neuromuscular junction transmission. The subtype of CMS depends on whether the defect is presynaptic, synaptic, or postsynaptic. In the past 33 years, at least 23 genes encoding proteins of the neuromuscular junction have been identified containing causative mutations.[1] CMSs are clinically heterogeneous and characterized by fatigable weakness of skeletal muscle that occurs between infancy and adulthood. Ptosis and extraocular muscle, facial, bulbar, and generalized weakness are the common presentations. Subtypes exist with onset later in childhood that exhibit morbid muscle fatigability with difficulty in running or climbing stairs. Clinical manifestation, severity, and course of CMS vary, even between patients from the same family. Although myasthenic symptoms may be mild, respiratory insufficiency may occur in patients with CMS if respiratory muscles are severely affected.[1]

It has been reported that 75% of CMS cases are postsynaptic subtype, and a genetic deficiency of acetylcholine receptor (AChR) tends to be the most frequent etiological basis.[2] Certain mutations in AChR subunit genes may alter the kinetic electrophysiological properties of AChR.[3,4] CMSs that show decreased synaptic response to acetylcholine are referred to as fast channel; conversely, those with an increased acetylcholine response are referred to as slow channel. In patients with slow-channel CMS (SCCMS), electrophysiological examination has revealed that prolonged opening activity of the AChR channel causes

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depolarization block, which contributes to muscle weakness and fatigability.[1] SCCMS is usually inherited in an autosomal dominant pattern. Onset in milder cases occurs during childhood or early adulthood. Most patients show cervical, paraspinal, and wrist and finger extensor muscle weakness.[5] Progressive spinal deformities or scoliosis result from paraspinal muscle weakness and are usually found in adult SCCMS patients.[6]

Patients with CMS have been reported worldwide, including in East Asia,[7,8] but there have been only a few published cases of CMS in China.[9] Herein, we identified three patients with SCCMS from a Chinese family who presented with muscle weakness during early or late childhood. In these patients, we found autosomal dominant inheritance of a heterozygous mutation in acetylcholine receptor epsilon-subunit (CHRNE) gene.

METHODS

Patients

The proband was referred to the Department of Neurology, The First Affiliated Hospital of Chongqing Medical University in March 2014. Detailed medical history was obtained from each family member, and the affected family members underwent laboratory tests and physical and electrophysiological examination, including repetitive nerve stimulation (RNS) and nerve conduction velocity studies. For low-frequency stimuli, decrements were compared between 1st and the 4th stimuli. For high-frequency stimuli, 75 stimuli were given and decrements were compared between the first and last stimuli. Laboratory tests on the proband consisted of assaying for blood biochemicals, endocrine hormones, antibodies against nuclear antigens, and antibodies against AChR. Patients included in this study provided written informed consent, and the study was approved by Medical Ethics Committee of The First Affiliated Hospital of Chongqing Medical University. The pedigree of this family is shown in Figure 1a.

Mutational analysis

Genomic DNA was extracted from peripheral leukocytes of fresh blood samples from the proband (III-3), elder sister of proband (III-2), and mother of proband (II-2), using standard methods of proteinase K digestion and phenol-chloroform extraction. We used next-generation gene sequencing to screen the proband DNA for genes associated with neuromuscular diseases, including genes that have been reported to encode factors involved in myasthenic syndromes. The identity of the mutated CHRNE gene detected by this screen was verified by Sanger sequencing.

All exons of the CHRNE gene were amplified by polymerase chain reaction (PCR) using the GeneAmp PCR System 9700 thermal cycler (Perkin Elmer, Shelton, CT, USA). The promoter region and the entire coding sequence of the CHRNE gene (GenBank accession number AF105999/gi4580858) were determined as described.[10] Each 25 µl PCR reaction contained 50 ng genomic DNA, 10 pmol of each forward and reverse primers, 5 mmol dNTPs, and 2.5 U of Taq polymerase in Taq reaction buffer (Takara Biotechnology, Dalian, China). Thermal cycling consisted of a denaturation step at 94°C for 5 min, and then 35 cycles at 94°C for 30–40 s, 57–63°C for 30 s, and 72°C for 30 s, followed by a final elongation step at 72°C for 10 min. The PCR products were purified by 1.5% agarose gel electrophoresis and directly sequenced with an ABI PRISM 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The data were analyzed with Chromas 2.22 chromatogram file editor software (Technelysium Pty Ltd., Tewantin, Queensland, Australia). The base mutations in CHRNE gene were described and numbered according to criteria provided by the Ensembl genome browser (http://www.ensembl.org).

RESULTS

Clinical findings

The proband (III-3), 21-year-old man, had generalized muscle weakness at 4 years of age. He was born healthy and walked at 18 months. His ability to run and jump was lower compared with his same-age peers. He had experienced fatigue while walking since 4 years old. Symptoms of weakness fluctuated in severity, and exacerbated transiently by physical activity, but slightly relieved during rest breaks. The symptoms progressed gradually, and the upper limbs became affected at the age of 9. Symptoms exacerbated several times each year, with each episode lasting a few days to a month, especially during winter. The symptoms gradually progressed in the last 12 months and he experienced difficulty in climbing stairs; however, he did not experience diplopia, dysphagia, respiratory insufficiency, or muscle twitching. Neurological examination revealed severe bilaterally restricted eye movements without ptosis. Bilateral facial weakness was observed, and the bulbar muscles were slightly involved, manifesting as nasal speech; however, swallowing difficulties were not present, and chewing problems were not marked. Mild atrophy was found in bilateral forearm and interosseous muscles. Examination showed mild weakness of the neck flexor muscles, bilateral muscles of the tibialis anterior and gastrocnemius, and finger extensor muscles. Gowers' sign was negative, and there was marked scoliosis.
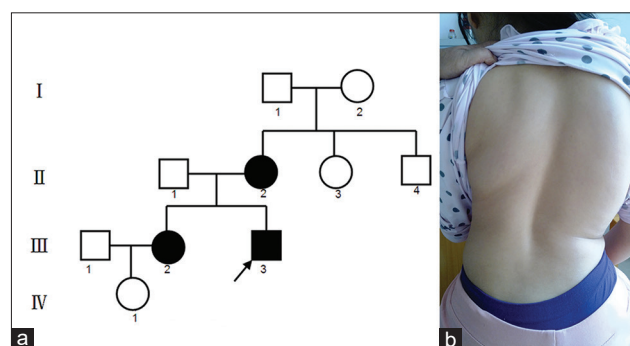
Figure 1: (a) Pedigree of this affected family. (b) Image of severe scoliosis in the elder sister (III-2).

The mother of the proband (II-2), a 47-year-old woman, had difficulties in grasping and finger extension at 10 years of age. At age 12, she presented marked muscular weakness and fatigability, especially in the lower limbs. Mild dyspnea variably presented when symptoms exacerbated. Interestingly, symptoms stopped progressing and she began to recover gradually, though not to normal levels, but she was now able to complete daily housework and perform mildly taxing farm work. She had bilaterally limited eye movements without ptosis, symmetric bilateral facial weakness, and mild weakness of neck flexor and bilateral muscles of the limbs (grade 4/5 on the Medical Research Council scale for the proximal muscles and 4/5 for the distal muscles of the limbs). Gowers' sign was negative, and there was marked scoliosis.

The affected elder sister (III-2) was 24 years old and had symptoms similar to the proband. She began to have difficulty in walking and extending fingers at 11 years of age. The weakness progressed and she was unable to jump. Mild bucking sometimes occurred when she drank water. Facial weakness, muscle wasting in bilateral forearm and interosseous muscles, and bilateral ophthalmoplegia without ptosis were also found. Muscle strength on the Medical Research Council scale was 3/5 for the neck flexor muscles and 4/5 for the proximal muscles. She was neither able to extend her fingers nor able to walk using either the toe or heel. Scoliosis was even more serious than the proband, as shown in Figure 1b. The daughter of the elder sister (IV-1), a 4-year-old girl, however, had no symptoms or complaints. Her development milestones were normal.

The laboratory tests for proband showed that the levels of creatine kinase and thyroid and sex hormones were normal,

and antibodies to AChR were not present. A neostigmine test showed no recovery for muscle weakness.

With the proband's consent, we prescribed fluoxetine at a dosage of 20 mg/d as treatment. After no improvement was observed in his condition for 2 weeks, we increased the dosage to 40 mg daily; 10 days later, fatigue and weakness were mildly improved. When the elder sister underwent the same fluoxetine treatment, symptoms also were relieved; however, after 6 months of therapy, neurological examination showed no changes for either patient.

Electrophysiological examination

Motor nerve conduction studies revealed a repetitive-compound muscle action potential (R-CMAP) after a single stimulation in the median, ulnar, and peroneal nerves in affected patients from the pedigree [Figure 2a–2c], which even included the asymptomatic 4-year-old girl although she did not complain of weakness or fatigue [Figure 2d]. However, compared with the recordings from the proband [III-3; Figure 2a] and his elder sister [III-2; Figure 2b], the R-CMAP of the peroneal nerve of their mother [II-2; Figure 2c] was more noticeably affected. After fluoxetine therapy, R-CMAP was still present in the proband (III-3) and his elder sister (III-2).

RNS induced CMAP decrements in several nerves of the affected patients in response to low- or high-frequency RNS, as summarized in Table 1 and shown in Figure 3. For the proband's mother (II-2), no decrements were detected in response to low-frequency RNS. Decrements of the proband (III-3) were more obvious than those of the other family members, despite similar degrees of illness.
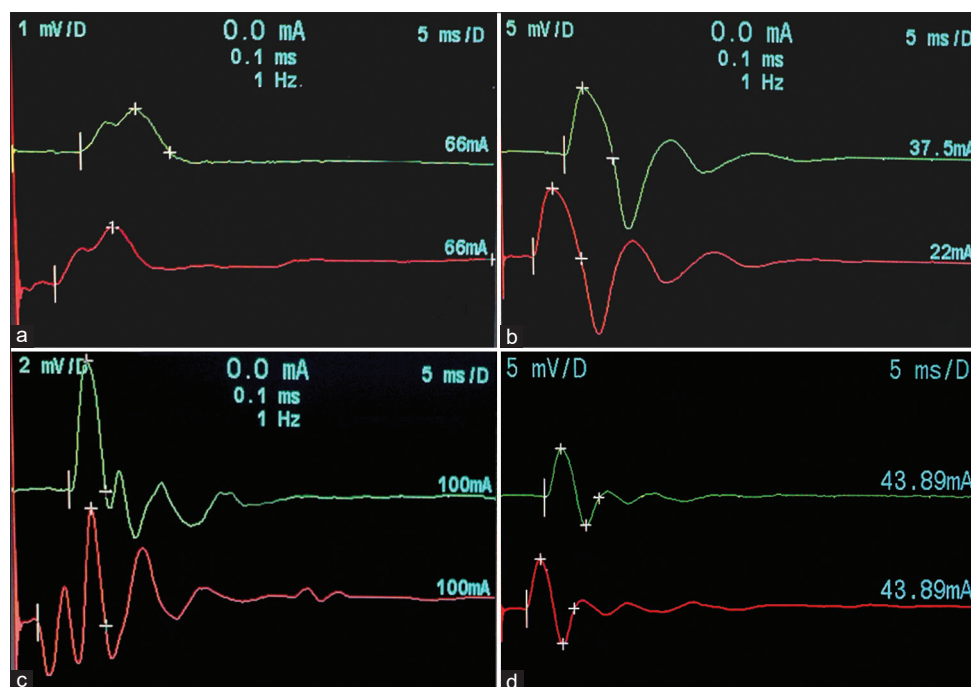

Figure 2: Repetitive compound muscle action potentials after a single stimulation of the median nerve. (a) III-3: Two peaks, the second peak of the second wave overlaps the first peak of the first wave. (b) III-2: Three peaks. (c) II-2: Four peaks. (d) IV-1: Three peaks.

Table 1: Repetitive nerve stimulation tests in the affected patients of the pedigree (% amplitude)

Patients	Decrement 1 Hz		Decrement 3 Hz		Decrement 30 Hz	
	Before treatment with fluoxetine	After treatment with fluoxetine	Before treatment with fluoxetine	After treatment with fluoxetine	Before treatment with fluoxetine	After treatment with fluoxetine
III-3						
Right facial nerve	12	19	34	12	ND	ND
Right median nerve	66	60	77	66	68	66
Right ulnar nerve	ND	4	ND	25	ND	74
Right peroneal nerve	40	15	67	18	72	69
III-2						
Right facial nerve	1	6	8	8	ND	ND
Right median nerve	ND	3	ND	3	ND	28
Right ulnar nerve	2	2	11	10	54	42
Right peroneal nerve	2	1	25	23	70	67
II-2						
Right facial nerve	–	NA	–	NA	ND	NA
Right median nerve	7	NA	–	NA	19	NA
Right ulnar nerve	–	NA	–	NA	9	NA
Right peroneal nerve	–	NA	–	NA	8	NA
IV-1						
Right ulnar nerve	21		27		ND	

NA: Not available because II-2 did not receive fluoxetine treatment; ND: Decrement was not detected; –: Signal not detected.
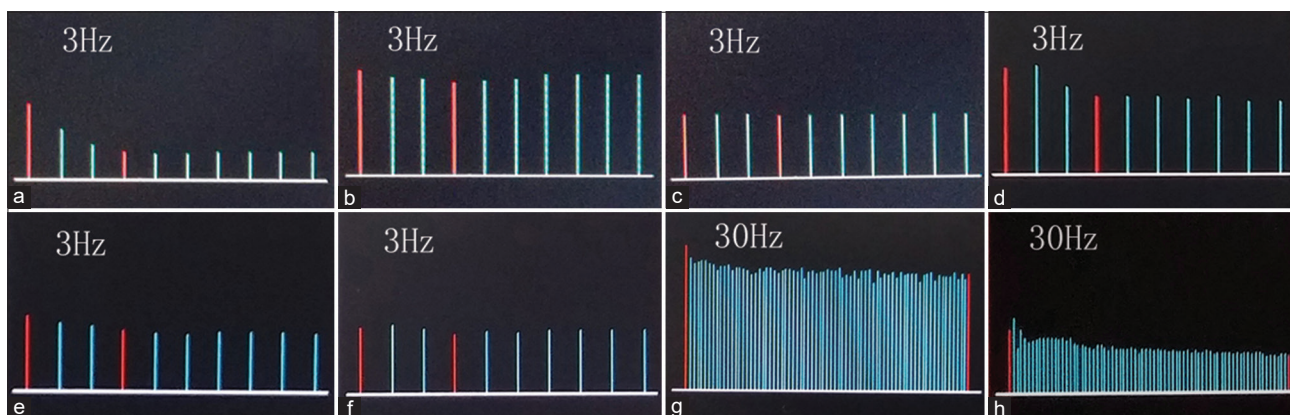

Figure 3: Representative diagrams of specific nerve responses in repetitive nerve stimulation tests of family members. (a–d) All the four patients were tested at 3-Hz stimulation. Proband (III-3; e) and his elder sister (III-2; f) monitored for the effects of fluoxetine treatment at low frequency. The proband's mother (II-2; g) and elder sister (III-2; h) tested at higher 30-Hz stimulation.

Decrements in response to high-frequency RNS were more significant than those to low-frequency RNS for all affected members except the 4-year-old girl (IV-1), who did not receive high-frequency RNS to avoid inflicting unnecessary pain. For the proband (III-3), fluoxetine treatment partially restored the decrease in CMAP for the facial and peroneal nerves in response to low-frequency RNS [Figure 3a and 3e, peroneal nerve]. However, significant improvements in the decrements were not observed for any of the tested nerves for the elder sister (III-2), especially in response to low-frequency RNS [Figure 3b and 3f, ulnar nerve]. For the 4-year-old girl (IV-1; daughter of III-2), although she did not have symptoms at the time, we measured a decrease in CMAP for the ulnar nerve [Figure 3d, ulnar nerve]. In the elder sister (III-2), a significant decrease in the median nerve response at higher frequency RNS was not found [Figure 3g]; however, a significant decrease in the ulnar nerve response at higher frequency RNS was observed even after fluoxetine treatment [Figure 3h].

Genetic studies

Next-generation sequencing revealed a mutation in the *CHRNE* gene of the proband (III-3). Proband's DNA was amplified and sequenced. A heterozygous missense mutation c.865C>T in exon 8 was found [Figure 4], resulting in a substitution from leucine to phenylalanine at position 289 (L289F). The mother (II-2) and elder sister (III-2) [Figure 4] carried the same heterozygous mutation. DNA of the 4-year-old girl (IV-1) was unavailable because her mother refused it. The mutation c.865C>T appears to have been initially reported as c.805C>T, leading to the changed L269F (i.e., a different residue number).[4,11,12] This variant is located in chromosome 17 (focus: 4804140 G>A). The Ensembl transcript ID is ENST00000293780,
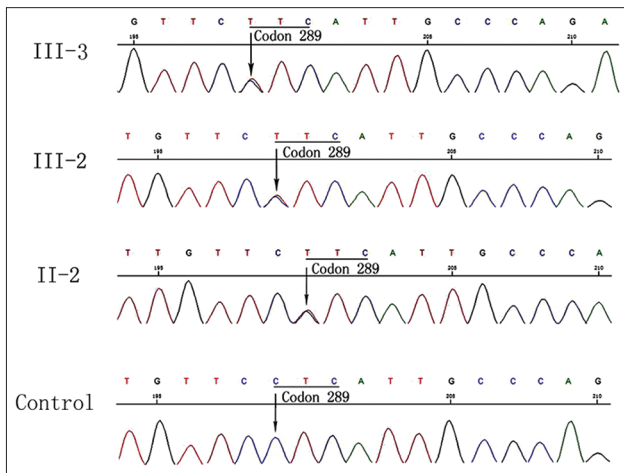
Figure 4: DNA sequencing chromatograms of exon 8 of the acetylcholine receptor epsilon-subunit gene. The heterozygous missense mutation (arrows), c.865C>T, was identified in the proband (III-3), his elder sister (III-2), and his mother (II-2).

leading to a cDNA.865C>T change.[13] According to the latest mRNA sequence of GenBank (mRNA Sequence NM_000080), these two residue positions are in fact identical, and the current accepted numbering is L289; accordingly, the mutation site has been updated as c.865C>T. In the Human Gene Mutation Database (HGMD), this mutation c.865C>T has been reported as a known disease mutation (HGMD ID CM960300).

The potential functional impacts of mutations within *CHRNE* gene were predicted using Polymorphism Phenotyping 2 (PolyPhen-2) software (http:// geneics.bwh. harvard.edu/pph2/), Sorting Intolerant from Tolerant (SIFT) software (http://sift.jcvi.org/) and MutationTaster (http:// www.mutationtaster.org/). The p.Leu289Phe substitution was predicted by the PolyPhen-2 software to be "probably damaging" and disruptive of the function of CHRNE. MutationTaster and SIFT predicted that the p.Leu289Phe mutation is functionally "disease causing" and "damaging", respectively. With MutationTaster software, this mutation is predicted to result in splice site changes and that protein features might be affected. It has been reported that the mutation can lead to an unusually high rate of spontaneous AChR channel openings and a 9-fold increase in affinity for acetylcholine.[4] This mutation has been found to be absent in the 100 healthy controls (50% male).

Discussion

SCCMS is a progressive disorder and may present with ophthalmoplegia, ptosis, facial paralysis, weakness, fatigue of trunk and limb muscles, and spinal deformity such as scoliosis. Once respiratory muscles be severely affected, respiratory failure follows. SCCMS is transmitted through autosomal dominant inheritance. A pathological gain of function of AChR located in the postsynaptic membrane has been observed in SCCMS,[4] which was caused by mutations in the AChR alpha-subunit (*CHRNA*) gene and *CHRNE*

gene.[6] However, mutations of the *CHRNE* gene can also lead to the fast-channel variation of CSM.[14] The clinical phenotype can range from mild to severe and vary among SCCMS patients with different mutations and even among patients with the same mutation.[15,16]

The mutation c.865C>T/L289F of *CHRNE* gene identified in our study was first identified in 1995.[11] The mutation identified in 1995 came from a family that was initially described in 1982.[17] At least seven patients with this mutation in *CHRNE* gene have been reported;[6,12] however, no patient with this mutation has been found in Asia until now.

Compared with other patients reported to have the same mutation, certain characteristics were different in our Chinese family. For example, all members in the family experienced childhood onset and none presented ptosis. Respiratory failure did not occur, hence ventilator-assisted breathing was not used. However, severe symptoms have been reported in other patients carrying this identical mutation, including onset in infancy and severe effects on respiratory muscles that caused respiratory failure requiring mechanical ventilation in two cases.[4,12] Interestingly, the proband in our family experienced episodes of weakness and deterioration in winter for unknown reasons, the influence of climate on the disease has not been reported previously. We also discovered that, in the mother of the proband, the weakness improved gradually with an increase in age, which suggested that even without medications, this disease might gradually improve with age during adulthood.

The role of the mutation c.865C>T in the pathogenesis of CMS has been investigated in several studies.[3,4] This mutation was definitively demonstrated to be a causative factor in the development of SCCMS[5] and was localized to CHRNE and more precisely to the pore, within the M2 domain, which forms a part of the AChR channel. Mutations in this M2 domain have more severe phenotypic consequences than those in the extracellular domain.[6] This mutation has been reported to change the kinetic properties of the AChR channel.[4] Indeed, patch clamp studies revealed an unusually high rate of spontaneous AChR channel openings and a 9-fold increase in affinity for acetylcholine resulting from this mutation,[4] leading to pathological gain of function. In addition, ultrastructural studies showed that endplate myopathy occurs in the postsynaptic muscle fiber.[4,18]

After fluoxetine treatment, our patients reported mild improvement of muscular weakness although neurological signs did not change. Electrophysiological studies showed that R-CMAP remained after fluoxetine treatment in both the proband (III-3) and his elder sister (III-2), which were consistent with a previous study.[19] Nevertheless, R-CMAP has been reported to disappear after quinidine treatment.[12] Varying degrees of decrements in response to RNS were found in the affected family members in our study, especially

in response to high-frequency stimulation, which indicated that high-frequency RNS is a more sensitive indicator than low-frequency RNS for this disease. In contrast, decrements were not observed in the mother (II-2) in response to low-frequency RNS. Heterogeneities in the family's electrophysiological data were also found, but we could not fully address the issue, at least not yet, as to whether the magnitude of the CMAP decrease observed in the RNS test positively correlated with the severity of illness. The 4-year-old girl (IV-1) did not have any symptoms at present; however, decrements were observed when she received low-frequency RNS, especially with 3 Hz, which indicated that she would likely suffer from this disorder in the future. Furthermore, decrements in response to low-frequency RNS of the ulnar nerve of this girl (IV-1) were more pronounced than those in her mother (III-2). In addition, although the decrease in RNS was greater in the proband (III-3) than the elder sister (III-2), the severity of the clinical symptoms was nearly same. Therefore, we hypothesized that the extents of decrements in response to RNS did not necessarily correlate with the severity of illness. After fluoxetine treatment, decrements in response to RNS were observed to improve in a subset of the nerves of one patient but not in the others receiving the same treatment, although they both reported clinical relief of symptoms.

Electrophysiological heterogeneities in CMS due to *CHRNE* gene mutations have been reported. A patient with c.855C>T mutation in *CHRNE* gene showed a mild but significant decrement in all the muscles except for the tibialis anterior. The greatest decrement was observed in the anconeus muscle (−15% in amplitude).[20] A mild decrement (−10%) was found in a patient due to the mutation (epsilon1369delG) of *CHRNE* gene, but another patient with the same mutation had no decrement.[21] Similarly, among patients with *CHRNE* 1293insG mutation, only a fraction displayed decrements.[22] Patients due to duplication mutations 123_127dupCTCAC in exon 2 of the *CHRNE* gene showed decrements.[23] For SCCMS patients whose illness can be attributed to the same mutation as in this study, a decremented electromyographic response was observed on 2 to 3 Hz of stimulation; however, R-CMAP was not found.[11,17] RNS test revealed 30%–70% decrements in different muscles of a Spanish boy with the same mutation L269F (L289F) in *CHRNE* gene observed in our patients.[12]

Accurate and differential diagnosis of CMS is very important. CMS may be misdiagnosed as myasthenia gravis or congenital muscular dystrophy or myopathy, such as Ulrich congenital muscular dystrophy, which could lead to incorrect or delayed treatment. For example, pyridostigmine treatment for SCCMS patients can induce endplate myopathy, and it is thus contraindicated.[5] The presence of R-CMAP indicates a diagnosis of either SCCMS or acetylcholine esterase deficiency, which is caused by mutations in the acetylcholinesterase-associated collagen (*COLQ*) gene;[1] hence, the identification of gene mutations underlying SCCMS will be necessary for further differential diagnosis.

Conflicts of interest

There are no conflicts of interest.

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