CONGENITAL MYASTHENIC SYNDROMES: AN OVERVIEW OF THE CLINICAL PRESENTATION, DIAGNOSIS AND TREATMENT

Dimitra Veltsista[1], Zinovia Kefalopoulou[1], Kyriaki Kekou[2], Maria Svingou[2], Elisabeth Chroni[1]

[1] Department of Neurology, Medical School, University of Patras, Greece
[2] Laboratory of Medical Genetics, Medical School, National and Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece

Abstract

Congenital myasthenic syndromes (CMS) are a diverse group of rare inherited diseases caused by mutations in various genes that encode proteins related to the function or structure of the neuromuscular junction. This is a brief review of current knowledge concerning the pathophysiology, responsive genetic defect, clinical and neurophysiological features, as well as symptomatic treatment strategies of CMS.

Key words: neuromuscular junction; muscle weakness; acetylcholine; hereditary disease

Congenital myasthenic syndromes (CMS) are a group of rare inherited diseases characterized by pathological muscle fatigue and transient or permanent weakness of facial, bulbar and limb muscles, with an onset early in life. Mutations in various genes encoding proteins related to the function or structure of neuromuscular junction domains are responsible for the different subtypes of CMS [1, 2]. CMS are more rare that myasthenia gravis, although the prevalence could be underestimated due to difficulties in diagnosis and significant variations between different ethnic and racial groups [2]. CMS prevalence has been estimated at 9.2 per 1,000,000 persons under 18 years of age [3].

The aim of the presented literature review is to provide a brief update on the genetic background, clinical phenotype and treatment options for the more common CMS.

The steps for muscle membrane activation are: 1. Formation of acetylocholine (ACh) and packaging into vesicles within the nerve terminals 2. Release of ACh via exocytosis into the synaptic cleft, when an action potential reaches the nerve ending. 3. Binding of ACh to AChR on the muscle end-plate region. 4 Breakdown of ACh by the enzyme acetylcholinesterase (AChE). Disruption at any of these steps, either due to synthesis and degradation of structural proteins or their malfunction, could lead to CMS. Neuromuscular transmission requires continuous alterations of the polarized and depolarized states of nerve and muscle fibers, and is a complex procedure involving many enzymes, ion channels and structural proteins. In order for ACh to efficiently bind to the nicotinic muscular receptors a series of consecutive elements play a part interconnecting each to the next i.e Agrin → LRP4 → DOK7 → MuSK → RAPSN → clustering of AChR. These are targets for autoimmune myasthenia as well as genetically defined CMS [4-7]. Details of the physiology are beyond the scope of this short review.

CMS are classified into 3 main categories according to the site of pathology; i.e. presynaptic, synaptic and postsynaptic syndromes. The advances in genetic analysis now allow a further subdivision based on the underlying molecular defect [8]. To date, 32 CMS subtypes have been recognized. Mutations in genes encoding the subunits of AChR (CHRNA1, CHRNB1, CHRND, or CHRNE) account for approximately half of all CMS cases. In particularly, mutations in the CHRNE gene, that code for the ε subunit, are the most commonly identified, and result in AChR deficiency or kinetic abnormalities. Mutations in RAPSN gene account for 15-20% of the all CMS cases, and COLQ and DOK7 mutations for 10-15%. CHAT (4-5%) and GFPT1 (2%) gene mutations are much less common [2, 4, 8].

Summary of clinical manifestations:

There is high variability in distribution and severity of symptoms. Symptoms typically appear during infancy or early childhood (usually within the 1st year of life). However, milder cases manifesting in adulthood have been reported in many CMS subtypes [9]. Generalized fatigue is the cardinal symptom. Diurnal fluctuation is not so distinct, but long or short periods of relapses triggered by excessive exercise and

infection are more likely. Early onset typically results in hypotonia presenting as 'floppy infant syndrome' in the neonatal or early infantile period, with weak cry, stridor and feeding problems associated with apnea and respiratory insufficiency, which may lead to sudden death. When symptoms occur in early childhood, there is a delay in motor milestones, difficulty in running and climbing stairs, lifting objects (extensors are primarily involved) and fluctuating eye-lid ptosis. No cardiac involvement is reported in the majority of patients [10].

Clinical features vary depending on genetic subtype, more specifically:

- Axial muscle weakness is common. Particularly, limb-girdle weakness is typical for patients with COLQ, DOCK7, GFPT1.
- Respiratory insufficiency: COLQ, CHRNE.
- Episodic apnea: CHAT, COLQ, RAPSN.
- Facial bulbar weakness: COLQ (isolated vocal cord paralysis, facial diplegia)
- Ocular: eye-lid ptosis is very common. Other ophthalmokinetic muscles are less affected.
- Fluctuations and relapses: in all CMS. Relapses triggers by fever, excitement.
- CHAT with onset in infancy may later show improvement during childhood.
- Scoliosis: CHRNE.

The differential diagnosis mainly includes muscular dystrophies and congenital myopathies [6]. Muscle atrophy, such as tongue atrophy in DOK7, associated with needle electromyography (EMG) evidence of myopathy makes the diagnosis even more challenging. When weakness is restricted to the ocular muscles CMS can be confused with mitochondrial myopathy of chronic progressive external ophthalmoplegia.

The most common syndromes will be briefly described:

I. Pre-synaptic CMS

Eight proteins are involved in presynaptic CMS, which include SLC5A7, **CHAT**, SLC18A3, SNAP25, VAMP1, SYB1, SYT2, and MUNC13-1. Defects in these proteins cause defective choline uptake in nerve endings, abnormalities in synthesis and recycling of acetylcholine, and impairment of synaptic vesicles exocytosis [2].

Responsible gene: **CHAT** that encodes the cholin acetyl transferase, responsible for the resynthesis of ACh.

Phenotype: Eye-lid ptosis, generalized fatigability and recurrent episodic apneas which might lead to cerebral hypoxia. Onset of symptoms at birth or rarely in child- or adulthood. Possible requirement of respiratory support and permanent proximal muscle weakness

Neurophysiology: Prolonged RNS at low frequency or alternative 5-10 min of isometric exercise may unmask significant decrement [11].

Treatment: Acetylcholinesterase inhibitors (AChEIs) are mildly effective. Supplementary treatment with 3,4-DAP and salbutamol or ephedrine have been recommended as 2nd and 3rd line therapy respectively [9].

II. SYNAPTIC CMS

Four CMS are due to malfunction of synaptic proteins, including **COLQ**, LAMB2, LAMA5, and COL13A1.

Responsible gene: **COLQ** encodes a functional protein crucial for anchoring AChE to the basal lamina

Phenotype: Diverse symptomatology ranging from mild fatigue to severe weakness and loss of ambulation or respiratory failure. Proximal limb muscles are predominately affected while ocular are often spared. Usually weakness of axial muscles (limb-girdle muscular dystrophy-type) is severe and early death can occur. Relapses are reported. Isolated vocal cord paralysis, facial diplegia have been reported as sole initial symptoms [2].

Neurophysiology: Double muscle response to single nerve stimulus is seen [2].

Treatment: salbutamol or ephedrine, avoidance of pyridostigmine [5, 12].

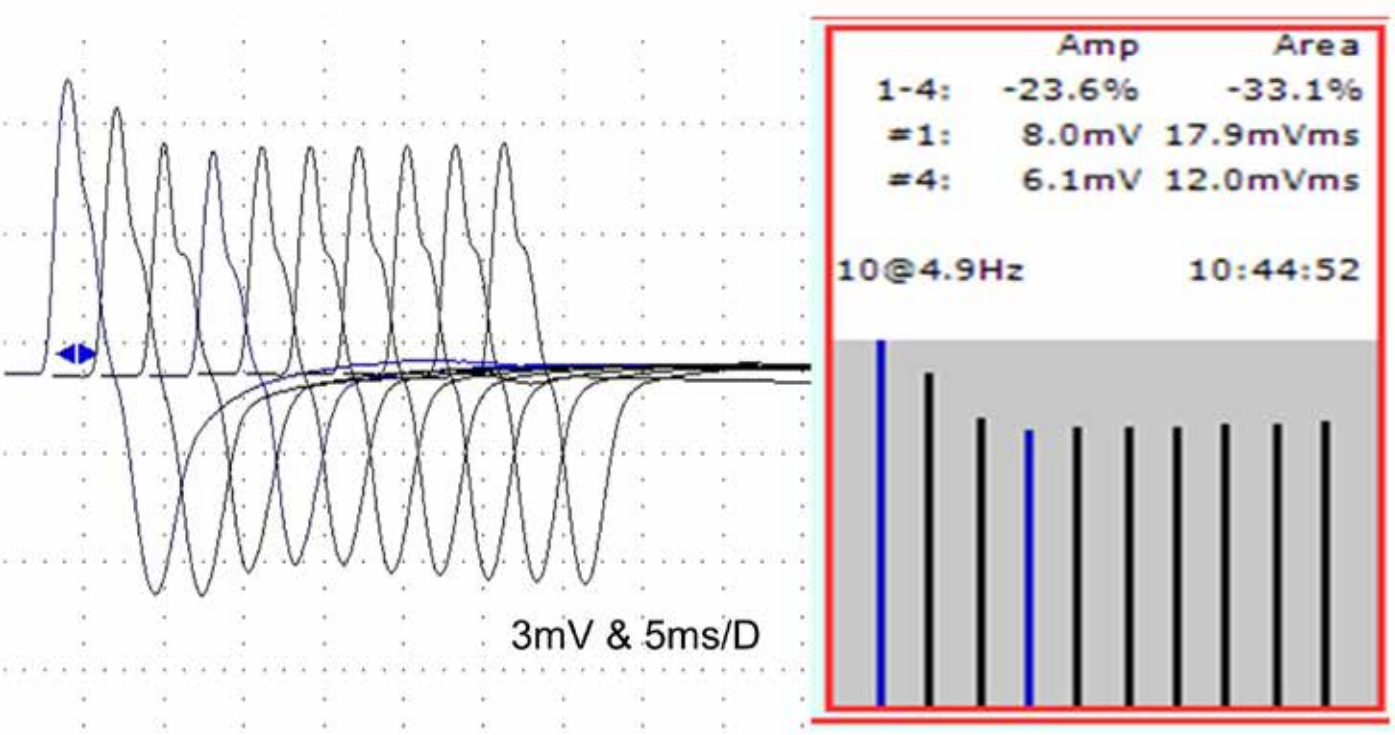
III. Post-synaptic CMS

Mutations in genes encoding post-synaptic proteins are responsible for 15 CMS, including CHRNA1, CHRNB1, CHRND, **CHRNE**, CHRNG**,DOK7**, MUSK, MYO9A, AGRN, LRP4, PREP1, SCN4A,**RAPSN**, PLEC, and SLC25A1 [2].

The majority of CMS belong to this category. These mutations are associated with primary deficiencies of the AChR, kinetic abnormalities of the AChR, or defects within the AchR-clustering pathway.

Responsible gene: **CHRNE** encodes the ε subunit of the AChR. Various recessive mutations i.e missense, nonsense, or splice site and promoter region mutations in all five AChR subunits have been identified. CHRNE variants affecting AChRε subunit are estimated to account for over 50% of CMSs related to AChR deficiency in humans [13]. The most common variant, CHRNE:c.1267delG (also known as ε1267delG), has been detected in many populations whereby resulting to a frequency of ~0.000128 in GnomAD. This frame shift alteration abolishes the normal stop codon in the last exon and gives rise to a different and extended nonfunctional protein, where the last 51 amino acids are replaced and 12 more are added (ClinVar entry ID: > 243031). The variant is mostly detected in European Gypsy patients presenting with symptoms of a myasthenic syndrome [14]. A survey of CHRNEc.1267delG in a large cohort of patients from the Roma populations within the Greek territory (unpublished data) revealed homozygosity of

Figure 1. Repetitive stimulation at 10Hz of the ulnar nerve and recording from abductor digiti minimi muscle: reduction >20% of compound muscle action potential amplitude and area



the variant in 25 (11 females, 14 males) patients of 0-49 years. Given that Roma population extents to ~175.000 subjects in Greece a prevalence of about 1/7000 Roma habitants is indicated. All patients presented with symptoms within the first years of life, of which the most prominent were blepharoptosis and swallowing difficulties.

***Phenotype**:* Variety in distribution and severity of symptoms

Weakness of ocular muscles present at birth or generalized fatigue and respiratory failure, with some patients experiencing delays or inability to achieve ambulation.

Neurophysiology: RNS could be abnormal and S-F EMG shows increased jitter. Due to long existing manifestations, classical EMG reveals myopathic changes of motor unit potentials (MUPs). Some patients may show repetitive CMAPs

Treatment: Initiation with AChEI, but in some cases it might fail or worsen the symptoms. Salbutamol or ephedrine can be effective. Combination of salbutamol with fluoxetine has been reported to be beneficial [2, 9].

Responsible gene: **RAPSN** encodes rapsyn a postsynaptic membrane protein that anchors the nicotinic AchR to the motor endplate and also binds to β-dystroglycan. It is necessary for clustering AchR

Phenotype: Fluctuating ptosis, bulbar symptoms and mild axial weakness. Relapses can occur, particularly precipitated by infections. Additional features are arthrogryposis multiplex congenita, contractures and hyperlordosis [2, 10].

Treatment: AchEI is favorable but outcomes can be improved by adding 3,4 DAP. General anesthesia can worsen the weakness.

Responsible gene: **DOK7** encodes protein responsible for activation of MuSK. Abnormal protein causes a default in AChR clustering

Phenotype: LGMD like pattern of muscle weakness or gait disturbance, occasionally mechanical ventilation. Ptosis but rarely ophathalmoparesis, severe relapses, vocal paralysis, tongue atrophy and feeding difficulties which may require PEG [2, 10].

Treatment: AchEI are usually ineffective and may even worsen clinical manifestations Ephedrine (initially 25 mg/dand increased to 75-100 mg/d) seems to be an effective. Alternatively, salbutamol may be successfully given.

Two well-described syndromes related to kinetics of AChR are: 1. Fast-channel (FCCMS) caused by unusual short time of AchR subunits able to interact with AChE. Depending on the mutation for AChR subunits, which is often loss-of function, increase rate of channel closing or reduced rate of channel opening responsible for this syndrome. Symptoms typically appear in infancy or early childhood.

Treatment: FCCMS responds to pyridostigmine or the addition of 3,4-DAP.

2. Slow-channel (SCCMS) is characterized by prolonged channel opening and hyperexcitability of the muscle fibers, which is usually caused by a gain-of function mutation in gene of AChR subunits. In terms of inheritance, unlike the majority of AChR deficiency syndromes, which had a autosomal recessive type, SCCMS follow an autosomal dominant inheritance. The clinical onset of SCCS is variable with the patients usually presenting symptoms after adolescent, although cases with severe symptoms in early life and leading consecutively to permanent disability might occur. Typically, weakness affects the cervical, scapular, wrist, and finger extensor zones [2]. Additionally symptoms involve the ocular, pharyngeal, proximal limb and respiratory muscles [15].

DEFECT IN GLYCOSYLATION OF POST-SYNAPTIC PROTEINS

Currently, mutations in five genes are known that are involved in protein glycosylation and may be associated with CMS. These genes include ALG2, ALG14, DPAGT1, **GFPT1**, and GMPPB.

Responsible gene: **GFPT1** encodes the enzyme that controls the flux of glucose for the glycosylation of proteins and lipids

Phenotype: LGMD like weakness fatigability and milder involvement of facial and bulbar muscles.

Treatment: Most patients respond beneficially to AChEI and in some patients the effect is significant [2].

Neurophysiology

Tests for neuromuscular junction have similar findings with those of the auto-immune pre and post synaptic disorders:

1. Standard procedure of repetitive nerve stimulation might show amplitude decrement, but normal findings does not exclude the diagnosis. Prolonged exercise or long stimulation at slow frequency (5 trains of 1 min at 3Hz separated by 5sec rest) might be necessary to reveal amplitude reduction. The recovery period in acetylcholinesterase deficiency (CHAT mutations) is long from 5 to 15 minutes [11]. In SCCMS and COLQ, a rate-dependent response is expected where the amplitude decrement is enhanced with high stimulation frequency [6]. Occasionally, such a pattern was also recorded in RAPSN syndrome [3]. A response similar to that seen in L-E syndrome i.e. more than 60% increase after 10 sec of exercise is indicative of presynaptic CMS.

2. Single-fiber EMG required prolonged axonal stimulation or voluntary contraction in order to demonstrate increased jitter and blocking [16].

3. Double muscle response to single nerve stimulus is seen in CMS caused by synaptic or post-synaptic disorders i.e. CHRNE, COLQ, SCCMS [17]. After discharges occurred by single stimulus, in small hand and foot muscles, and are abolished by fast (>0.5 Hz) repetitive stimulation.

4. Concentric needle EMG, particularly in chronic cases, demonstrates short duration, polyphasic MUAPs (similar to myopathic), described with the term "endplate myopathy" which can be reversible. For this reason mild CK increase is seen [3].

Treatment

Although CMS are genetic diseases and corrections of the underlying gene defects are not yet possible, most CMS subtypes are susceptible to pharmacotherapy [2, 4, 8, 10].

Available medication:

AChEI (pyridostigmine) inhibits degradation of ACh and is the most frequently used medication, although it can cause deterioration particularly in CMS presenting with excessive amount of ACh. In adults the daily dosage can reach 500mg in 4-6 divided doses. Gastrointestinal symptoms are the most common reported side-effects. Cholinergic crisis due to depolarized block, that may occur with high doses of pyridostigmine in patients with auto-immune myasthenia gravis, has not been reported in CMS [8]. Prophylactic administration or increased dose of pyridostigmine is recommended in cases of infection together with antibiotics to prevent the occurrence of episodic apnea and respiratory insufficiency [2].

3,4-Diaminopyridine (3,4-DAP) is the next most common medication after AChEIs. It is administrated as monotherapy or in combination with AChEI. 3,4-DAP acts as a potassium channel blocker that prolongs the opening of calcium channels and thus the duration of the presynaptic action potential resulting in enhancement of Ach quantal release from nerve terminal. It has also been proposed that 3,4-DAP has an effect on postsynaptic potassium channels but the mechanism is less clear [4]. For adults, initiation with tablets 5mg twice dayly, titrated at weekly intervals by 5mg up to a dosage between 15 and 80mg daily divided in 3 to 4 doses [8]. A limited number of case reports appeared in the literature regarding the administration of 3,4-DAP in CMS and several in patients with Lambert-Eaton syndrome. No interaction was reported with this drug when given together with pyridostigmine. Serum half-life is 20 min to 2 hours [18]. The most serious side-effect is epileptic seizures particularly at high dose (90-100mg/day). Tendency for epilepsy or electroencephalographic abnormalities should be excluded in children prior to drug administration. Other common side-effects are paresthesias in distal limbs and perioral, myoclonia, supraventricular tachycardia, epigastric distress [8, 18].

Salbutamol, known as albuterol in the United States, and ephedrine are β2 adrenergic receptor

agonists with a beneficial effect as a first-line treatment in some CMS, while in other has been used supplementary to pyridostigmine and 3,4-DAP [4]. Their mechanism of action is poorly understood; it has been hypothesized that these drugs backup agrin complex, stabilizing endplate structure and stimulate intracellular potassium uptake [4, 9]. There may be a significant delay of several months prior to achievement of clinical benefit. Salbutamol, which is more commonly prescribed nowadays, is given at a daily dosage of 8-12 mg in 2-3 divided doses. It is necessary to monitor patients for side-effects including restlessness and insomnia, tachyarrhythmia, hypertension and hypokalemia [8].

Two drugs with different indications are reported to provide clinical and neurophysiological improvement in some patients with SCCMS. Fluoxetine is a selective serotonin reuptake inhibitor and quinidine an antiarrhythmic agent. Both are long-lived drugs that blocks nicotinic AchRs in nerve and muscle at the open state and thus shorten the duration of the pathologically prolonged synaptic current and suppress the depolarization block [4, 8]. Fluoxetine is more oftenly prescribed compared to quinidine and their side effects during chronic administration should be considered. Fluoxetine is started at 10 mg/d and titrated up to 80 mg/d. Beneficial effect in CHRNE but deterioration in RAPSN [2]. Quinidine sulfate was administered per os. at a dose of 200 mg three times daily with possible increase to 900mg/day. Attention should be focused on severe cases to exclude respiratory insufficiency as a side effect, possibly due to excessive blockage of neuromuscular transmission in respiratory muscles [19].

Treatment depends on the subtype. Certain drugs which have a favorable response in some CMS type may have no effect or more over a negative effect in others [9].

There are two main groups in terms of treatment approach:

The era of rapidly evolution of neurogenetics leads the way for the discovery of CMS related new genes and their role in neuromuscular junction as well as the muscle fibers and the nervous system as a whole, justifying the variability of manifestations in these patients. The optimum treatment would require genetic diagnosis and the conduction of well-designed, randomized control, clinical trials. The latter is goal that is far from being achieved due to the underdiagnosis and rarity of the disease. For the time, the awareness of CMS as a potential diagnosis of cases with early onset weakness is the first step. The referral to specialized center with a multidiscipline approach in patients' monitoring is the second step.

In brief, one should be aware that CMS:

1. Typically manifest very early in life, but may do so in adulthood
2. Present no characteristic or pathognomonic symptoms
3. Fluctuate in severity, showing stability or even improvement during motor development in childhood and periods of remission
4. In most cases have usually a negative family history, since the majority are autosomal recessive diseases
5. Require high degree of suspicion due to difficult to diagnosis, mainly via specific neurophysiological testing

References

[1] Engel AG, Shen XM, Selcen D, et al. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. *Lancet Neurol.* 2015;14:420-434.
[2] Finsterer J. Congenital myasthenic syndromes. *Orphanet J Rare Dis.* 2019;14:57.
[3] Iyadurai SJP. Congenital Myasthenic Syndromes. *Neurologic Clinics.* 2020;38:541-552.
[4] Iyadurai SJP. Congenital Myasthenic Syndrome

I. Patients who benefit from increase of acetylcholine levels

Treatment line	CHAT	AChR deficiency	FCCMS	RAPSN*	GFPT1
1st: pyridostigmine	+	+	+	+	+
2nd: add 3,4 DAP	+	+	+	+	+
3rd: add salbutamol or ephedrine		+ (CHRNE)	+	+	+

* fluoxetine reported to cause symptoms worsening in some patients

II. Patients who do not improve or worsen following pyridostigmine and 3, 4 DAP administration, since they further increase the already prolonged action of Ach on the receptors

Treatment line	COLQ	DOC7	SCCMS
1st:	salbutamol or ephedrine		Fluoxetine
2nd:	add 3,4 DAP		Quinidine

Treatment Heterogeneity makes diagnosis and treatment challenging. *Practical Neurology* 2019.
[5] Lee M, Beeson D, Palace J. Therapeutic strategies for congenital myasthenic syndromes. *Ann N Y Acad Sci.* 2018;1412:129-136.
[6] Finlayson S, Beeson D, Palace J. Congenital myasthenic syndromes: an update. *Pract Neurol.* 2013;13:80-91.
[7] Rodríguez Cruz PM, Cossins J, Beeson D, et al. The Neuromuscular Junction in Health and Disease: Molecular Mechanisms Governing Synaptic Formation and Homeostasis. *Front Mol Neurosci.* 2020;13:610964.
[8] Farmakidis C, Pasnoor M, Barohn RJ, et al. Congenital Myasthenic Syndromes: a Clinical and Treatment Approach. *Curr Treat Options Neurol.* 2018;20:36.
[9] Thompson R, Bonne G, Missier P, et al. Targeted therapies for congenital myasthenic syndromes: systematic review and steps towards a treatabolome. *Emerg Top Life Sci.* 2019;3:19-37.
[10] Ciafaloni E. Myasthenia Gravis and Congenital Myasthenic Syndromes. *Continuum (Minneap Minn).* 2019;25:1767-1784.
[11] Juel VC. Evaluation of neuromuscular junction disorders in the electromyography laboratory. *Neurol Clin.* 2012;30:621-639.
[12] Laforgia N, De Cosmo L, Palumbo O, et al. The First Case of Congenital Myasthenic Syndrome Caused by a Large Homozygous Deletion in the C-Terminal Region of COLQ (Collagen Like Tail Subunit of Asymmetric Acetylcholinesterase) Protein. *Genes (Basel).* 2020;11.
[13] Abicht A, Dusl M, Gallenmüller C, et al. Congenital myasthenic syndromes: achievements and limitations of phenotype-guided gene-after-gene sequencing in diagnostic practice: a study of 680 patients. *Hum Mutat.* 2012;33:1474-1484.
[14] Morar B, Gresham D, Angelicheva D, et al. Mutation history of the roma/gypsies. *American journal of human genetics.* 2004;75:596-609.
[15] Otero-Cruz JD, Báez-Pagán CA, Dorna-Pérez L, et al. Decoding pathogenesis of slow-channel congenital myasthenic syndromes using recombinant expression and mice models. *P R Health Sci J.* 2010;29:4-17.
[16] Stålberg EV, Trontelj J, Sanders DB. *Single Fiber EMG*, third ed., Edshagen Publishing House, Fiskebäkskil, Sweden, 2010.
[17] Howard JF, Jr. Electrodiagnosis of disorders of neuromuscular transmission. *Phys Med Rehabil Clin N Am.* 2013;24:169-192.
[18] Lindquist S, Stangel M. Update on treatment options for Lambert-Eaton myasthenic syndrome: focus on use of amifampridine. *Neuropsychiatr Dis Treat.* 2011;7:341-349.
[19] Harper CM, Engel AG. Quinidine sulfate therapy for the slow-channel congenital myasthenic syndrome. *Ann Neurol.* 1998;43:480-484.