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Wide heterogeneity of congenital myasthenic syndromes: analysis of clinical experience in a tertiary center

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Purpose: Congenital myasthenic syndrome (CMS) is a clinically and genetically heterogeneous group of disorders characterized by impaired neuromuscular transmission. This study aims to provide the clue for early diagnosis and improved therapeutic strategies in CMS.

Materials and Methods: Through the targeted panel sequencing including twenty CMS causative genes, eleven patients were genetically confirmed and enrolled in this study. A retrospective medical record review was carried out for the clinical and laboratory data analysis.

Results: The age of patients ranged from 5 to 23 years, with the median age of 16 years. The peak age at onset of symptoms was the neonatal period. Seven out of the eleven patients were symptomatic at birth. The most commonly reported initial finding was generalized hypotonia with poor sucking and crying. Mean time to accurate diagnosis was 9.3±5.0 years. Total fifteen different variants in seven genes associated with CMS (*DOK7, AGRN, RAPSN, CHRNE, COLQ, SLC5A7,* and *GFPT1*) were identified.

Conclusion: We describe the clinical and genetic characteristics of CMS patients and treatment outcome in a single tertiary center. High clinical suspicion and timely molecular diagnosis is particularly important for the tailored therapy to maximize clinical improvement in CMS.

Key words: Congenital myasthenic syndrome, Therapeutic effect, Next generation sequencing

Introduction

Congenital myasthenic syndrome (CMS) is a clinically and genetically heterogeneous group of disorders characterized by impaired neuromuscular transmission [1-3]. It is a very rare hereditary muscle disease with the prevalence of individual diseases reported as 0.1-1/100,000 [4-7]. Clinically, it shows various symptoms such as motor weakness, ptosis, respiratory failure, dysphagia, scoliosis, and joint contractures. It shows a broad spectrum of severity from the case of floppy infant syndrome that develops in the neonatal stage to the case with only mild ptosis in adulthood. Often, it is difficult to distinguish CMS from

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other neuromuscular disorders including congenital myopathy, congenital muscular dystrophy, limbs girdle muscular dystrophy, and seronegative myasthenia gravis [8,9].

The causative genes for congenital myasthenia syndrome are very diverse. More than 30 causative genes of CMS have been reported to date and new genes are constantly being discovered [10,11]. Commonly identified causative genes of CMS include *CHRNE, RAPSN, COLQ, DOK7, GFPT1*, and *CHAT* with variations of ethnicity [4,6,12-14]. Since next generation sequencing has been increasingly used for the diagnosis of hereditary myopathies, a number of patients with CMS have been genetically diagnosed and receiving drug treatment with remarkable clinical improvement. The importance of genetic diagnosis is particularly emphasized because the response to medical treatment varies depending on the molecular subtypes in CMS [2,3,10,15].

Here, we describe the clinical and genetic characteristics of CMS patients diagnosed and treated in a single tertiary center. Through this, we aim to provide the clue for early diagnosis and improved therapeutic strategies in CMS.

Materials and Methods

1. Patients

This study was approved by Seoul National University Hospital Institutional Review Board (IRB No. H-2009-070-1157). All cases were ascertained from the neuromuscular disorders database in the Seoul National University Children's Hospital. We performed next generation sequencing (NGS) in total 230 patients with genetically unclassified neuromuscular disorders (onset age ≤10 years). Before the NGS testing, genetic tests including spinal muscular atrophy, Duchenne muscular dystrophy, and myotonic dystrophy were performed in some patients, based on the clinical and pathologic findings, but were negative. Eleven patients from nine unrelated Korean families and one Emirati family were included in this study. All cases were genetically confirmed as CMS. A retrospective medical record review was carried out for the clinical data analysis.Diurnal variation and ptosis were subjectively classified to mild, moderate, and severe symptoms. The severity of scoliosis was scaled by Cobb angles (mild: 10-30 degree, moderate: 30-45 degree, and severe: >45 degree). Respiratory dysfunction was described as severe when the patient was dependent on permanent ventilator care or underwent therapeutic tracheostomy. Laboratory data including serum creatine kinase, electrophygiologic studies, and muscle pathology were also reviewed.

2. Genetic analysis

DNA was extracted from peripheral blood leukocytes. Informed consent was obtained from all families for the extraction of DNA to perform mutation analysis. We performed targeted panel sequencing of 432 selected genes known to cause monogenic neuromuscular disorders including 20 CMS causative genes (*CHRNA1, CHRNB1, CHRND, CHRNE, RAPSN, CHAT, COLQ MUSK, DOK7, AGRN, GFPT1, DPAGT1, LAMB2, SCN4A, CHRNG, PLEC, ALG2 ALG14, SYT2 PREPL*) [16]. The genetic variants identified were validated using Sanger sequencing and segregation studies were performed using parental DNA samples when needed. The variants identified were considered to be pathogenic or likely pathogenic according to the 2015 American College of Medical Genetics and Genomics guidelines [17].

Results

We found eleven CMS patients from ten unrelated families (Table 1). The age of patients ranged from 5 to 23 years, with the median age of 16 years. The peak age at onset of symptoms was the neonatal period. Seven out of the eleven patients were symptomatic at birth. The most commonly reported initial finding was generalized hypotonia with poor suck and cry. Diurnal variation in seven cases and mild ptosis in six cases were observed. Respiratory distress at birth was noticed in seven patients, four of them suffered transiently and recovered after the neonatal period. Eight patients showed variable degrees of scoliosis. Mean time to accurate diagnosis was 9.3±5.0 years. Total fifteen different variants in seven genes associated with CMS (DOK7, AGRN, RAPSN, CHRNE, COLQ, SLC5A7, and GFPT1) were identified.

1. Postsynaptic endplate development and maintenance defects (*DOK7, AGRN, RAPSN*)

The homozygous c.1185C>G mutations and compound heterozygous c.467C>A and c.1502c>T mutations of *DOK7* genes were identified in two patients (case 1 and 2). The homozygous c.5023G>A mutations of *AGRN* were identified in two brothers in one family (case 3-1 and case 3-2). One case of the compound heterozygous c.133G>A and c.690G> mutations of *RAPSN* were found (case 4). All five patients were symptomatic at birth. Four patients with *DOK7* and *AGRN* mutations were initially presenting as floppy infant syndrome with a clinical diagnosis of congenital myopathy. One patient (case 1) was able to walk with assist in early childhood, but weakness worsened to the state where she could not stand up on her own at 10 years old. The

Table 1. Clinical and genetic characteristics of the eleven patients with congenital myasthenic syndromes

	Case 1	Case 2	Case 3-1	Case 3-2	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
	F/16	M/5	M/10	M/8	M/14	M/19	M/19	M/10	M/21	M/19	M/23
Classifications	Postsynaptic (endplate development and maintenance defects)					AChR def	AChE def	Presynaptic	Protein glycosylation defects		
Genotypes	DOK7	DOK7	AGRN	AGRN	RAPSN	CHRNE	COLQ	SLC5A7	GFPT1	GFPT1	GFPT1
	c.1185C>G homo	c.476C>A c.1502C>T	c.5023G>A homo	c.5023G>A homo	c.133G>A c.690G>A	c.850A>C hetero	c.107-2A>G c.1354C>T	c.886G>A homo	c.520G>A c.766G>C	c.706A>T c.1549A>C	c.128A>T c.706A>T
Clinical findings											
Onset	At birth	At birth	At birth	At birth	At birth	At birth	3 mon	At birth	6 yr	7 yr	6 mon
Presentations	FIS	FIS	FIS	FIS	Ptosis	FIS	Motor DD	FIS	Weakness	Weakness	Weakness
Clinical diagnosis	CMP	CMP	CMP	CMP	MG	MG	CMS	CMS	LGMD	LGMD	CMD
Diurnal variation	+	–	–	–	++	+	+	–	+	++	++
Ptosis	+	–	–	–	+	+	–	+	–	–	+
Scoliosis	+++	++	++	++	–	+	+++	+	–	–	+++
Respiratory dysfunction	Transient NICU 2w	+++	+++	+++	–	Transient NICU 1w	–	Transient NICU 2m	–	Transient NICU 3w	–
Laboratory findings											
Serum CK (IU/L)	46	106	26	36	102	123	55	NA	162	257	376
Jolly test	NA	Negative	NA	NA	NA	Positive	Positive	NA	Negative	Positive	NA
EMG/NCV	MP	MP	NA	NA	NA	MP	MP	NA	MP	MP	MP
Muscle pathology	NS	CFTD	NA	MD	NA	NS	NS	NS	NS	MD	MD
Treatment response											
Pyridostigmine	NE		NE	E	E	E	NE	E	E	E	E
Salbutamol	E	E	E	E		E	E	E	E		E
3,4 Diaminopyridine	E		E	E		NE	E				E

Case 3-1 and 3-2 are siblings.
F, female; M, male; Def, deficiency; FIS, floppy infant syndrome; DD, developmental delay; CMP, congenital myopathy; MG, myasthenia gravis; CMS, congenital myasthenic syndrome; LGMD, limb girdle muscular dystrophy; CMD, congenital muscular dystrophy; MP, myopathy; MD, muscular dystrophy; CFTD, congenital fiber type disproportion; CK, creatine-kinase; EMG, electromyography; NCV, nerve conduction velocity; –, negative; +, mild; ++, moderate; +++, severe; NA, not available; NS, nonspecific; E, effective; NE, not effective.

maximal motor performance achieved in others (case 2-4) was rolling over at diagnosis. These three patients (case 2-4) underwent therapeutic tracheostomy for the management of severe respiratory distress. Four patients had moderate to severe scoliosis with a Cobb angle of 30 degree or more and one of them (case 1) underwent surgical correction at her age of 13 years. Pyridostigmine was not effective or somewhat worsen in two patients (case 1 and case 3-1) but partially effective in case 3-2 (6 mg/kg/day). Salbutamol therapy was effective in all patients with *DOK7* and *AGRN* mutations. Three patients were under the combination therapy of salbutamol (1-2 mg/kg/day) and 3, 4 diaminopyridine (0.5-1 mg/kg/day), showing variable degrees of improvement. At age 15 years, case 1 was able to climbing stairs and independent in her daily activities. Case 2 started to stand with assist at his age of 5 years. Two brothers with *AGRN* mutations could walk independently (case 3-1) or with assist (case 3-2) since early childhood. Case 4 with *RAPSN* mutations presented with ptosis at birth. He developed facial weakness and mild motor developmental delay during infancy and was diagnosed as myasthenia gravis with the positive response to neostigmine test at his age of 2 years. The test for acetylcholine receptor (AchR) antibodies was negative but ptosis had been well controlled with oral pyridostigmine (6 mg/kg/day). The molecular diagnosis was made at his age of 11 years.

2. Postsynaptic acetylcholine receptor deficiency (*CHRNE*)

Case 5 harbored a heterozygous c.850A>C mutation of *CHRNE*. He presented with generalized hypotonia with transient respiratory distress at birth and developed gross motor delay, facial weakness, and ptosis during infancy and childhood. At age 13 years, he reported mild fluctuations of weakness, usually related to exertion, and the electrophysiologic study revealed abnormal electrodecremental response to repetitive nerve stimulation (Jolly test positive). The molecular diagnosis was made at his age of 15 years. Drug treatment with pyridostigmine (4 mg/kg/day) and salbutamol (0.5 mg/kg/day) resulted in improvement in muscle strength, but 3.4 diaminopyridine was not effective.

3. Endplate acetylcholinesterase deficiency (*COLQ*)

Case 6 carried compound heterozygous c.107-2A>G and c.1354C>T mutations of *COLQ*. He presented with gross motor delay during early infancy and developed proximal dominant generalized weakness in childhood. There was a diurnal variation, with worsening of weakness in the afternoon. Jolly test was positive but pyridostigmine did not improve muscle weakness. After the molecular diagnosis was confirmed at his age of 16 years, he showed significant improvement with the combination therapy of salbutamol (0.2 mg/kg/day) and 3.4 diaminopyridine (0.5 mg/kg/day).

4. High-affinity presynaptic choline transporter (*SLC5A7*)

The homozygous c.886G>A mutations of SLC5A7 were identified from an Emirati boy (case 7). He had a history of respiratory distress during neonatal period and showed persistent ptosis since birth. He was clinically diagnosed with CMS but previous sequencing result of *DOK 7, CHRNE, RAPSN*, and *CHAT* analysis in UK was negative. The NGS based targeted panel analysis revealed *SLC5A7* mutations at his age of 5 years. He showed significant improvement with the combination of pyridostigmine (6 mg/kg/day) and salbutamol (0.2 mg/kg/day) therapy.

5. Protein glycosylation defects (*GFPT1*)

Three different compound heterozygous variants of *GFPT1* genes were identified in case 8-10 (c.520G>A and c.766G>C, c.706A>T and c.1549A>C, and c.128A>T and c.706A>T). All patients presented with a limb girdle muscular dystrophy phenotype having proximal limb but no facial muscle weakness. Case 8 and case 9 began having difficulty walking and climbing stairs in the childhood. Case 10 presented with gross motor delay during infancy and developed proximal limb weakness and severe scoliosis during childhood. The electrophysiologic study revealed chronic myopathic changes in all cases. Serum creatine kinase was mildly elevated and muscle biopsy revealed chronic degenerative process with a pathologic diagnosis of muscular dystrophy in two patients. After molecular diagnosis of CMS, all patients benefited from pyridostigmine therapy and two patients showed additional improvement from salbutamol.

Discussion

We report detailed clinical and genetic features of eleven cases with CMS. All patients shared the clinical features of muscle weakness, but the age of onset, distribution and severity of weakness, and response to treatment were very diverse according to the genes harboring the mutations. Typical clinical features were generalized muscle weakness presenting in the neonatal period or early infancy. Diurnal variation of weakness was not so much distinct during infantile and early childhood period and often appeared after childhood as an important clinical clue. Presence of diurnal fluctuations should raise clinical suspicion of

CMS even in patients diagnosed with myopathy previously [8]. Ptosis was recognized in five patients but often combined with facial weakness, leading to difficulty in differentiating it from other hereditary myopathy faces. Seven patients had respiratory distress at birth and three patients underwent tracheostomy for managing the persistent respiratory insufficiency. Eight patients had scoliosis and three of them were treated surgically. Paying attention to the posture of patients and continued assessment of spine throughout lifespan is essential.

Laboratory findings were not very helpful in the differential diagnosis of CMS in our cohort. Two cases showed Jolly test negative and case 6 showed once negative and once positive result on repeated tests. All seven patients who underwent electrodiagnostic tests showed myopathic findings with short-duration and low-amplitude motor unit potentials. This is thought to result from functional loss of myofibers caused by chronic neuromuscular transmission failure [9]. Muscle biopsy was performed in nine patients and showed nonspecific myopathic findings with myofiber size variations and minimal degenerating changes in five cases. However, one patient (case 2) was pathologically diagnosed as a congenital myopathy with type 1 fiber predominance and three patients (case 3-2, case 9, and case 10) were diagnosed as muscular dystrophies before molecular diagnosis of CMS. Even serum creatine kinase were mildly elevated in two cases with *GFPT1* mutations.

The response to treatment depends on the subtype of CMS [2,3,10,15,18]. Traditionally, pyridostigmine, a competitive acetylcholinesterase inhibitor, was the mainstay of treatment for the neuromuscular junction disorders. Oral pyridostigmine can be given at an initial dose of 0.5 to 1 mg/kg every 4 to 6 hours and the maximum recommended total daily dose is 7 mg/kg. Consistent with previous reports, our patients with defects of acetylcholine receptor, presynaptic choline transporter, and protein glycosylation had much benefited from pyridostigmine. But some subtypes such as endplate acetylcholinesterase deficiency due to *COLQ* mutations and *DOK7*-related CMS are known to refractory or deteriorate with pyridostigmine. Patients with *DOK7* and *COLQ* mutations in our cohort derived no benefit or worsened with pyridostigmine. Whereas salbutamol were very effective in patients with *DOK7* and *COLQ* deficiency and partially beneficial in other subtypes of CMS. Oral salbutamol can be given at an initial dose of 4 mg/day and titrated up to 12 mg/day. A 3,4-Diaminopyridine (amifampridine; Ruzurgi, available at Korean Orphan & Essential Drug Center) is a potassium channel blocker working on the presynaptic nerve terminal [15]. The pediatric starting dose is 0.25-0.5 mg/kg/day and titrated up to 1 mg/kg/day total dose if necessary. It was a useful add-on therapy for the patients with mutations of *DOK7, AGRN, COLQ*, and *GFPT1* in our experience.

Our study suggested the importance of molecular diagnosis of CMS to warrant appropriate treatment. Due to the wide heterogeneity of clinical and genetic features, the diagnosis of CMS remains challenging even for experienced clinicians. The number of causative genes continues to grow and the clinical differentiation of subtypes is usually very difficult. The advent of NGS technologies opened a new era of molecular genetic diagnosis in various neuromuscular disorders. Since molecular diagnosis is crucial for the therapeutic decision making in CMS, it is important for clinicians to recognize clinical clues and confirm the genetic analysis earlier.

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