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Congenital myasthenic syndromes in the Thai population: Clinical findings and novel mutations

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Abstract

Congenital myasthenic syndromes (CMS) comprise a heterogeneous group of genetic disorders of the neuromuscular junction. Next generation sequencing has been increasingly used for molecular diagnosis in CMS patients. This study aimed to identify the disease-causing variants in Thai patients. We recruited patients with a diagnosis of CMS based on clinical and electrophysiologic findings, and whole exome sequencing was performed. Thirteen patients aged from 2 to 54 years (median: 8 years) from 12 families were enrolled. Variants were identified in 9 of 13 patients (69%). Five novel variants and two previously reported variant were found in the *COLQ*, *RAPSN* and *CHRND* gene. The previously reported c.393+1G>*A* splice site variant in the *COLQ* gene was found in a majority of patients. Five patients harbor the homozygous splice site c.393+1G>*A* variant, and two patients carry compound heterozygous c.393+1G>*A*, c.718–1G>*T*, and c.393+1G>*A*, c.865G>*T* (p.Gly289Ter) variants. The novel variants were also found in *RAPSN* (p.Cys251del, p.Arg282Cys) and *CHRND* (p.Met481del). Molecular diagnosis in CMS patients can guide treatment decisions and may be life changing, especially in patients with *COLQ* mutations.

Keywords: Neuromuscular junction; Myopathy; Novel variants; Whole exome sequencing; *COLQ*.

1. Introduction

Congenital myasthenic syndromes (CMS) are a heterogeneous group of genetic disorders caused by mutations in genes encoding proteins at the neuromuscular junction (NMJ) that lead to impaired neuromuscular transmission and fatigable muscle weakness. The prevalence is approximately 0.1–1/100,000 population, with variations according to ethnicity [1–6]. To date, slow channel syndrome in a large Thai family is the only published CMS case series in Thailand [7]. CMS has a wide range of clinical manifestations. The most severe form presents as early as the prenatal period, whereas the milder form can present during adolescence or in adulthood. Symptoms include fatigable muscle weakness, ptosis, ophthalmoplegia, swallowing dysfunction, respiratory dysfunction, and scoliosis. CMS was initially classified as presynaptic, synaptic, or postsynaptic syndrome based on clinical, electrophysiological and endplate electromicroscopic findings [8–10]. However, these findings may not always be able to clearly delineate each subtype. The advent of next generation sequencing (NGS) fascilitates the genetic diagnosis of CMS and led to the discovery of novel causative genes. Therapies for CMS include acetylcholinesterase inhibitors (pyridostigmine), potassium channel blockers

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(3,4-diaminopyridine (3,4-DAP)), acetylcholine receptor (AChR) open channel blockers (fluoxetine, quinidine), and β2-adrenergic receptor agonists (ephedrine, salbutamol). Responses to treatment depend on the CMS subtype, and therapy that is effective for one type of CMS may aggravate another type. Obtaining molecular diagnosis is, therefore, crucial for therapeutic decisions [11–13]. NGS is increasingly used for molecular diagnosis in CMS patients. The responsible genes frequently described in CMS include *CHAT, COLQ, CHRNA1, CHRNB1, CHRND, CHRNE, RAPSN, MUSK, DOK7*, and *SCN4A*. To date, there are at least 32 genes known to be associated with CMS [1],[14],[15]. The frequency of pathogenic variants in each gene varies by ethnicity [5],[15–17]. Here, we report clinical and genetic findings in thirteen Thai CMS patients.

2. Materials and methods

2.1. Study design and patient population

A descriptive, cross-sectional study was performed in patients with CMS at three tertiary care centers (Siriraj Hospital, Ramathibodi Hospital, and King Chulalongkorn Memorial Hospital) from August 2017 to January 2019. The Institutional Review Board approved this study. The diagnosis of CMS was based on clinical symptoms and a decremental electromyographic response on repetitive nerve stimulation (RNS). Clinical features included childhood-onset or fatigable weakness of extra-ocular, facial, bulbar, axial, limb or respiratory muscles. The decremental electromyographic response on RNS was defined as a greater than 10% decrease in the amplitude or area of the fourth compared to the first compound muscle action potential (CMAP) on slow (2–3 Hz) RNS [10]. RNS findings were categorized into three patterns as follows: Pattern 1, if there is a decremental response of CMAPs at 2–3 Hz stimulation only; Pattern 2, if there is a decremental response of CMAPs at 2–3 Hz stimulation with repetitive CMAPs; Pattern 3, if there is a decremental response of CMAPs at 2–3 Hz stimulation only after 10 Hz stimulation for 5 min was applied or the presence of facilitation after a ten-second exercise. Informed assent and/or consent was/were obtained from each patient and/or parent. Detailed clinical data including the age of onset, presenting symptoms, motor milestones, patterns and degree of weakness, electrophysiological data, laboratory investigations (serum creatine kinase (CK), muscle biopsies, AChR antibodies), treatment responses, and disease course were obtained by reviewing the medical record. Responses to treatment were described according to motor power grading or respiratory function or developmental outcome. The outcome was evaluated after months or years of observation and was assessed as improvement, stable, worsening, or exacerbation of weakness. Improvement and worsening were defined when there were significant improvement and worsening of clinical parameters mentioned previously. Stable was defined when there was only subjective or mild objective clinical improvement. Exacerbation of weakness was defined when there was a history of acute motor deterioration aggravated by external causes, such as illness or medication.

2.2. DNA extraction, whole exome sequencing, and bioinformatic analysis

After informed consent was obtained, three milliliters of peripheral blood was taken from patients and their available parents. Genomic DNA was extracted from peripheral blood leukocytes using a Puregene Blood Kit (Qiagen, Hilden, Germany). DNA samples were sent to Macrogen Inc., Seoul, South Korea for whole exome sequencing (WES). The sequencing libraries were enriched by SureSelect Human All Exon V5 Kits (Agilent Technologies, Santa Clara, CA). The captured libraries were sequenced using Illumina HiSeq 2000 Sequencer (Illumina, Inc., San Diego, CA). Sequence reads in FASTQ sequencing files were aligned with the Human Reference Genome hg19 from UCSC using Burrows-Wheeler Alignment (BWA) software (http://bio-bwa.source forge.net/). Single nucleotide polymorphisms (SNPs) and INDELS were detected by SAMTOOLS (http://samtools.source forge.net/), and annotated by dbSNP&1000G. Trio-WES analysis was performed, and all SNVs and INDELS were filtered to include splicing and exonic variants. Variants were subsequently filtered out if they were present in our in-house database of 1864 unrelated Thai exomes.

The coding missense, nonsense, frameshift, and splice-site variants in the 32 genes associated with CMS according to the gene table of neuromuscular disorders (http://www.musclegenetable.fr/4DACTION/Blob_groupe2) were first analyzed. The candidate pathogenic variants were selected according to the following criteria: (1) population frequency $<1\%$ in gnomAD (https://gnomad.broadinstitute.org/) or Thai Exome database; (2) variants predicted to have a functional impact on coding regions (predicted missense, nonsense, consensus donor/acceptor splice site mutations, and insertions/deletions; and/or, (3) variants determined to be damaging, probably damaging, or disease causing by one or more of the following *in silico* predictive mutation impact software programs: SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and/or Mutation Taster (http://www.mutationtaster.org/). If no candidate variants in the 32 genes were identified, variants of other relevant genes were filtered using the aforementioned criteria. All candidate variants were evaluated by clinical geneticists and neurologists, and they were classified according to American College of Medical Genetics and Genomics (ACMG) interpretation guidelines [18].

3. Results

We identified 13 CMS patients from 12 families. The age of patients ranged from 2 to 54 years, with the median age of 8 years. All patients were ambulatory.

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Table 1
Molecular genetic findings in 10 patients with congenital myasthenic syndromes.

Patient ID	Mutation analysis	Gene	Nucleotide change	Zygosity	Parental mutation status	Allele frequency (gnomAD/dbSNP)	Pathogenicity prediction (SIFT/Polyphen-2/MT@)	Classification[b]
1	WES singleton	*COLQ*	c.393+1G>*A*	Homozygous	Not performed	0.00000398/rs1085307792	N/A	Pathogenic
2	WES singleton	*COLQ*	c.393+1G>*A*	Homozygous	Not performed	„	N/A	Pathogenic
3	WES trio	*COLQ*	c.393+1G>*A*	Homozygous	Parents are heterozygous	„	N/A	Pathogenic
4	Sanger sequencing	*COLQ*	c.393+1G>*A*	Homozygous	Not performed	„	N/A	Pathogenic
5	WES trio	*COLQ*	c.393+1G>*A*	Homozygous	Parents are heterozygous	„	N/A	Pathogenic
6	WES singleton	*COLQ*	c.393+1 *G>A* **c.718–1G>*T***	Compound heterozygous	Not performed	0.00000398/rs1085307792 None/none	N/A	Pathogenic Pathogenic
7	Sanger sequencing	*COLQ*	c.393+1G>*A* **c.865G>*T* (p.Gly289Ter)**	Compound heterozygous	Paternal allele Maternal allele	0.00000398/rs1085307792 None/none	N/A	Pathogenic
8	WES trio	*RAPSN*	**c.844C>*T* (p.Arg282Cys) c.752_754delGCT (p.Cys251del)**	Compound heterozygous	Paternal allele Maternal allele	0.00000406/rs75184547 None/none	D./P.D./D.C. N/A	VUS VUS
9	WES trio	*CHRND*	**c.1441_1443delATG (p.Met481del)** c.932+5G>*A*	Compound heterozygous	Paternal allele Maternal allele	None/none 0.00000398/rs797045474	D.C.[a] N/A	VUS Likely pathogenic
10	WES singleton	*DNM2*	c.496C>*T* (p.Arg166Trp)	Heterozygous	Not performed	0.00000398/rs1366377441	D/P.D./D.C.	VUS

D: damaging; D.C.: disease causing; P.D.: probably damaging; N/A: not applicable; VUS: variant of uncertain significance; WES: whole exome sequencing. SIFT: sorting intolerant from tolerant (http://sift.jcvi.org/); Polyphen-2: prediction of functional effects of human SNPs (http://genetics.bwh.harvard.edu/pph2/);. *MT@*: Mutation Taster (http://www.mutationtaster.org/); dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/); gnomAD: Genome aggregation database (https://gnomad.broadinstitute.org/).
Novel variants are demonstrated in **bold.**
[a] PROVEAN (Protein Variation Effect Analyzer; http://provean.jcvi.org/index.php) was used to predict the effect of an in-frame deletion.
[b] According to the American College of Medical Genetics and Genomics interpretation guidelines [18].

3.1. Molecular genetic findings

Molecular diagnosis could be made in 9 of 13 patients (69%). The mutations were mostly found in the *COLQ* gene (7/13), followed by *RAPSN* (1/13) and *CHRND* (1/13). The genetic findings are summarized in Table 1.

3.2. Clinical findings and treatment responses

Ten of thirteen CMS patients presented with congenital hypotonia and various degrees of respiratory insufficiency during infancy. Most of them had ptosis, ophthalmoplegia, and delayed motor milestones. All patients had normal or low CK value. Ten patients had been tested for AchR antibodies (patients 1–5, 7- 9, and 11–12), and the results were negative except in patient 7 who had low titers (0.50 nmol/L) of AchR antibodies (normal $<$ 0.45 nmol/L). Muscle histopathology available in seven patients (patients 1–3, 6, 10, 12, and 13) revealed nonspecific myopathic change such as fiber size variation and atrophic fibers.

Before genetic testing was available, 12 patients (except patient 8) initially went through trials of pyridostigmine. Of those, two patients (one family) improved, two patients experienced exacerbation (more frequent respiratory failure episodes or more extremity weakness), and eight patients reported stabilization of symptoms. Patient 8 was the only one who received therapy after the identification of causative mutations in the *RAPSN* gene. The second-line drugs included ephedrine, salbutamol, or fluoxetine. Seven patients who received ephedrine showed improvement in overall strength. Salbutamol was prescribed in three patients. One of those had significant improvement in respiratory function, one had improvement in overall strength, and the other had no significant improvement. Clinical characteristics, responses to medications, and the progression of disease are summarized in Table 2.

3.2.1. COLQ-CMS

Seven patients (54%) harbor *COLQ* mutations. All of them had symptom onset within the neonatal period. The presenting symptoms were congenital hypotonia, generalized weakness, and recurrent pneumonia. Two to twenty-four episodes of recurrent pneumonia (most of which required mechanical ventilation) occurred throughout their lives. Ptosis, ophthalmoplegia, facial weakness, bulbar weakness, and neck muscle weakness were present in all patients with *COLQ*

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Table 2
Clinical characteristics in 13 patients with congenital myasthenic syndromes (The full table is available online).

Patient ID	Family ID	Sex	Current age (years)	Consanguinity	Age of onset	Proximal weakness/ Distal weakness	Ptosis/ Ophthalmoplegia	Facial weakness/ Bulbar weakness	Neck weakness/ Scoliosis or hyperlordosis	Respiratory involvement	Delayed motor milestones	RNS pattern[#]	Previous drug response				Disease course
													Pyridostigmine	Ephedrine	Salbutamol	Fluoxetine	
1	1	M	5	Y	Birth	+/+	++/+++	+/+	++/+	+++	Y	1	No IM	IM	N/A	N/A	IM
2	2	M	5	N*	Birth	+/+	++/+++	+/+	++/+	+++	Y	1	No IM	IM	No IM	N/A	IM
3	3	F	6	N*	2 weeks	+/+	+++/++	+/+	+/-	+++	Y	1	EX	N/A	IM	N/A	IM
4	4	M	13	N	Birth	+++/+	++/+++	++/+	++/++	+++	Y	2	No IM	IM	N/A	N/A	IM
5	5	M	3	N	10 days	++/-	++/+++	+/+	++/-	+	N	1	EX	IM	N/A	N/A	IM
6	6	F	14	N	Birth	+/+	+++/++	+/++	++/+++	+++	Y	1	No IM	IM	N/A	N/A	IM
7	7	F	3	N	Birth	++/+	++/+++	+/+	+/-	+++	Y	2	No IM	IM	N/A	N/A	IM
8	8	M	2	N	Birth	+/-	+/+	+/+	+/-	+	Y	1	N/A	N/A	N/A	N/A	EX
9	9	M	8	N	1 month	+/-	++/+++	+/+[$]	+/-	-	N	1	No IM	IM	N/A	No IM	IM
10	10	M	18	Y	2 months	+/+	+/+	+/-	+/++	+	Y	2	No IM or EX	N/A	IM	N/A	IM
11	11	F	34	N	5 years	++/-	+/-	++/+	+/-	-	N	1	Some IM	N/A	N/A	Some IM	WO
12	11	M	33	N	19 years	+/+	+/-	++/-	+/-	-	N	1	IM	N/A	N/A	N/A	WO/ST
13	12	F	54	N	30 years	+/-	+/-	-/-	+/-	-	N	1	No IM	N/A	N/A	N/A	WO

[#] RNS findings had 2 patterns as follows: 1) Pattern 1, there is a decremental response of CMAPs at 2-3 Hz stimulation only; 2) Pattern 2, there is a decremental response of CMAPs at 2-3 Hz stimulation with repetitive CMAPs
* Parent's hometown distance is within 2 kilometers.
[$] History of severe oropharyngeal incoordination needed gastrostomy feeding that resolved within years
-: no weakness; +: mild weakness; ++: moderate weakness; +++: severe weakness
CMAPs: compound muscle action potentials; EX: exacerbations of weakness; F: female; IM: improvement; M: male; N: no; N/A: not applicable; RNS: Repetitive nerve stimulation test; ST: stable, WO: worsening; Y; yes

mutations. Four patients had spinal scoliosis or hyperlordosis. None of the patients in our cohort showed a slow pupillary response.

One of the two patients (patients 3 and 6) who underwent a neostigmine test showed a positive response (patient 3). All patients had electrodecremental responses of the RNS test. Repetitive CMAPs could be elicited in two patients. Pyridostigmine was initiated before the genetic diagnosis was made. Two patients (patients 3 and 5) experienced exacerbations of respiratory insufficiency, and the rest of the patients showed no improvement. Ephedrine or salbutamol was prescribed as second-line medication in six patients, and

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all showed significant improvement in respiratory strength and overall strength. Especially patient 3 who experienced ten respiratory failures, after pyridostigmine was replaced by salbutamol she had never suffered from respiratory failure again.

3.2.2. RAPSN-CMS

Patient 8 harbored compound heterozygous mutations in *RAPSN*. He presented with congenital hypotonia with mild respiratory distress at birth. He developed facial weakness, ptosis, and ophthalmoplegia during infancy. Gross motor delay, waddling gait, and Gowers' sign were also observed. He had episodes of worsening weakness related to acute illnesses and two episodes of pneumonia without respiratory failure. Chromosome analysis and lysosomal enzyme acid alpha-1,4-glucosidase activity were normal. The test for AchR antibodies was negative. After the molecular diagnosis was made, pyridostigmine was prescribed when exacerbation of weakness occurred.

3.2.3. CHRND-CMS

Patient 9 carried compound heterozygous mutations in the *CHRND* gene. He presented with severe gastroesophageal reflux disease (GERD) and pharyngeal incoordination. Gastrostomy tube has been in place since one month of age. Ptosis, ophthalmoplegia, mild facial weakness, and neck and proximal muscle weakness were apparent by one year of age. Pyridostigmine did not result in clinical improvement. Ephedrine was added and the patient showed improvement in bulbar symptoms. He is currently 8 years old and is able to remove his gastrostomy tube and take solid food orally. Ptosis and ophthalmoplegia are still present.

3.2.4. Genetically uncharacterized-CMS

Patient 10 is an 18-year-old male presented with fluctuating ptosis and subtle ophthalmoplegia since 2 months of age. He suffered from recurrent pneumonia and respiratory insufficiency during his first 2 years of life. Proximal muscle weakness was evident by age 12 years. He underwent spinal fixation for lumbar scoliosis. His parents are fourth-degree cousins. There are no other similarly affected family members. Physical examination revealed hyposthenic built, short stature, ptosis, ophthalmoplegia, and mild proximal muscle weakness. Mitochondrial DNA sequencing was negative for pathogenic mutations. The electrophysiologic study revealed electrodecremental response with repetitive CMAPs on RNS and evidence of chronic myopathy. Muscle biopsy revealed a nonspecific myopathic change (mild to moderate fiber size variation and no fibers with internal nuclei). Pyridostigmine was prescribed and later discontinued due to complaints of dysarthria. Therapy with salbutamol resulted in subjective improvement in muscle strength.

Three patients (patients 11–13) had the clinical pattern of chronic (10–30 years) progressive myopathy in common.

Patients 11–12 are siblings. Patient 12 is the only one who has had a muscle biopsy and the result was a nonspecific myopathy. Due to their facial features and chronicity of disease, the diagnosis has long pointed to hereditary myopathies until RNS was performed and both revealed decremental responses.

Patient 13 is a 54-year-old woman who had slow progressive proximal muscle weakness within 20 years. She reported a history of ptosis and went through upper blepharoplasty due to cosmetic concerns since she was 40 years old. The CK level and thyroid function test were normal. Autoantibodies results were negative. The muscle pathology of the deltoid had a few small and large group of type 1 fibers among randomly distributed fiber types, which were inconclusive. CMS was later impressed mainly by the decremental response on RNS and the chronicity of disease without clinical fluctuation and typical relapsing-remitting clinical course of autoimmune myasthenia gravis. However, a possibility of seronegative myasthenia gravis could not be excluded in this patient.

4. Discussion

We report on clinical and genetic findings of thirteen Thai patients with CMS. The disease-causing variants could be identified in 9 of 13 patients (69%). The patients with identified mutations had symptom onset during infancy. Five novel variants and two previously reported variant were found in the *COLQ*, *RAPSN* and *CHRND* genes. *COLQ* mutations are the most common, accounting for 54% of all cases. The c.393+1G>*A* variant in the *COLQ* gene is the most frequent in our cohort. Seven unrelated patients carry the c.393+1G>*A* variant. Five patients harbor the homozygous splice-site c.393+1G>*A* variant, and two patients carry compound heterozygous c.393+1G>*A*, c.718–1G>*T* and c.393+1G>*A*, c.865G>*T* (p.Gly289Ter) variants. The c.393+1G>*A* variant has been previously reported in 2 Chinese siblings with compound heterozygous c.367–3T>*G*, c.393+1G>*A* variants in the *COLQ* gene [19]. Although a significant proportion of Thais are of Chinese descent, based on history taking (three-generation pedigree, birth places) all of our patients and their available parents were Thai in origin. The c.393+1G>*A* variant (rs1085307792) is found in one of 251,346 alleles in the gnomAD (anonymous Asian male) but absent in 2152 individuals (4304 alleles) in Thai exome database from which 5 patients and 4 of their parents were excluded. The variant has not been previously reported in larger cohorts from other ethnic groups. This suggests the c.393+1G>*A* variant might be a founder mutation in the Thai population. A more extensive population study with haplotype analysis is needed to confirm this hypothesis.

The *COLQ* gene mutation is associated with motor endplate acetylcholinesterase deficiency. The patients had an onset of respiratory insufficiency within the neonatal period. Ophthalmoplegia, ptosis, neck weakness, and scoliosis were also present. Another hallmark of *COLQ* mutations is a slow pupillary response that can be found in 25% of patients [10],[20–22]. This finding was not observed in our cohort.

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Repetitive CMAPs is also a clue for diagnosing *COLQ*-CMS but may not be elicited until adolescence [19]. Worsening of symptoms after pyridostigmine is observed in cases with *COLQ* mutations. Patients 3 and 5 experienced exacerbation of weakness, and the rest of our study patients showed no improvement from pyridostigmine. Ephedrine and salbutamol are effective in most patients [3],[10],[23–25]. All *COLQ*-CMS patients in our cohort reported improvement after treatment with ephedrine or salbutamol. Our findings suggest that ephedrine or salbutamol should be considered as the first drug of choice in Thai patients. Pyridostigmine should also be avoided in Thai CMS patients without molecular diagnosis or with features of *COLQ, DOK-7* and slow channel syndrome [7],[26]. The clinical phenotypes of our CMS patients with *COLQ* mutations compared with previous reports are summarized in Table 3 (Supplementary material).

CMS patients with *RAPSN* mutations have been reported to have two main phenotypes: late-onset with fatigable limb weakness or early-onset characterized by arthrogryposis, high-arched palate, and facial, cervical, and bulbar weakness [27–30]. Patient 8 had an early onset of symptoms without arthrogryposis. Facial weakness, ptosis, ophthalmoplegia, bulbar weakness, and limb weakness were mild, and he was able to catch up with his milestones. Episodic apnea aggravated by infections is a distinctive feature that has been reported in *CHAT*-, *SLC5A7*- and *RAPSN*–CMS patients [13]. Patient 8 experienced two episodes of exacerbation of weakness without apnea. These findings expand the phenotype of *RAPSN*–CMS.

The *CHRND* gene encodes for delta subunit nicotinic AChR. Mutations in *CHRND* are known to cause CMS with variable severity. Dominant gain of function mutations in the ligand-binding or pore domain can result in prolonged synaptic currents or slow channel syndrome, clinically resembling acetylcholinesterase deficiency. Patients usually present in the first decade of life. There are involvement of the cervical, scapular, and dorsal forearm muscles. The ocular muscles are usually spared. Some patients could have mild, and asymmetric ptosis. Patients with monoallelic or bi-allelic loss of function mutations are usually severely affected and have high mortality in infancy or in early childhood. Individuals harboring null mutations in both alleles probably die in utero [10]. Patient 9 harbors one previously reported splice-site c.932+5G>*A* variant and one novel in-frame c.1441_1443delATG (p.Met481del) mutation [31]. This novel in-frame deletion involves amino acid methionine located at the transmembrane domain of delta subunits of the acetylcholine receptor. The methionine residue is highly conserved across different species. The PROVEAN (Protein Variation Effect Analyzer; http://provean.jcvi.org/index.php) predicts the variant to be disease-causing. The effect of the variant to the acetylcholine receptor function remains to be explored. Clinically, patient 9 had severe feeding difficulty during the infancy period which subsequently resolved at the age of one year. However, his ptosis and ophthalmoplegia were still present. Pyridostigmine did not improve or aggravate the disease. He showed some responses to ephedrine and is currently ambulatory at the age of 8 years.

Heterozygous *DNM2* mutations are known to be associated with centronuclear myopathy and Charcot-Marie-Tooth disease [32,33]. Although *DNM2* mutations are not widely known to cause NMJ defects, there is increasing evidence of the expanding spectrum of myopathy-myasthenic overlap [34–38]. Gibbs et al. demonstrates that deficits in neuromuscular transmission are a significant component of *DNM2*-myopathy pathology and that therapeutics targeting the neuromuscular junction may provide effective treatment [34]. Patient 10 retrospectively had the clinical sign and symptoms of CMS since infantile period, but they were unrecognized until muscle weakness and scoliosis was evident during adolescence. His muscle pathology is not typical of centronuclear myopathy. Repetitive CMAPs on RNS in patient 10 was a clue for *COLQ*-CMS or slow channel syndrome but have not been reported in *DNM2* patients [8]. However, singleton whole exome sequencing revealed a missense variant (c.496C>*T*; p.Arg166Trp) in the *DNM2*. No other variants in genes associated with fatigable weakness (HP:0003473), EMG: impaired neuromuscular transmission (HP:0100285) and myopathy (HP:0003198) was found in this patient, which includes *COLQ* and slow channel syndrome genes. The variant is rare and present in one of 250,946 alleles in gnomAD (https://gnomad.broadinstitute.org/). The sequence change replaces the arginine residue with tryptophan at the codon 166 of the DNM2 protein. Prediction programs predict it to be disease-causing. The missense c.496C>*T* (p.Arg166Trp) variant identified in the *DNM2* gene in patient 10 should be taken into consideration. However, the evidence is inadequate to conclude its causative role for CMS. The variant's pathogenicity and its etiologic role in patient 10 need further investigations.

5. Conclusions

We report the use of WES in the diagnosis of CMS in the Thai population. Molecular diagnosis is crucial for therapeutic decision making. Mutations in the *COLQ* gene were identified in the majority of our patients. Our findings also suggest that ephedrine or salbutamol should be considered as the first drug of choice and pyridostigmine should be avoided in Thai CMS patients.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2020.08.362.

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