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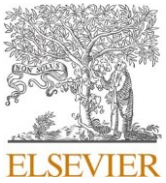

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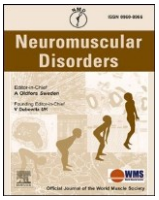

Research paper

CHRNE-related congenital myasthenic syndrome in Iran: Clinical and molecular insights

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A R T I C L E I N F O

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A B S T R A C T

Variants in the *CHRNE* gene can lead to a condition called congenital myasthenic syndrome (CMS), which affects the neuromuscular junction (NMJ). *CHRNE* mutations are the most common cause of CMS. Seventy-seven patients with a possible diagnosis of CMS were referred to the neuromuscular clinic of Shariati Hospital affiliated with the Tehran University of Medical Sciences. We performed whole-exome sequencing (WES) to determine the underlying defect in a group of individuals with a possible diagnosis of CMS. Clinical features and morphological and molecular data on 33 patients with mutations in *CHRNE* were described. Age of onset, age at diagnosis, consanguinity, family history, motor milestone delay, ophthalmoparesis, generalized fatigue, dysphagia, neurophysiologic findings, and response to treatment of the patients were assessed. Nineteen *CHRNE* variants including 10 novel ones were identified. The most common mutations were c.1327del; (p.Glu443LysfsTer64) in four different families and c.1252–1267dup; (p.Cys423SerfsTer38) in three families. Clinical onset was mostly at birth or under one year with bilateral fatigable ptosis, ophthalmoplegia, bulbar weakness, and proximal muscle weakness. All patients were treated with pyridostigmine ± salbutamol, which resulted in improvement of motor function, dysphagia, and breathing.

1. Introduction

Congenital myasthenic syndromes (CMS) are a group of heterogeneous inherited disorders, caused by 35 different genes encoding various neuromuscular junction (NMJ) proteins [1–4]. CMS are classified into presynaptic, synaptic space, and post-synaptic syndromes based on the location of the encoded protein defect at the NMJ. Post-synaptic CMS defects include primary acetylcholine receptor (AChR) deficiency, AChR kinetic defects (slow and fast-channel), endplate development and maintenance defects, and congenital glycosylation defects [5]. The AChR is composed of five subunits; two α, one ß, one δ, and one γ present before the 33rd week of gestational age in humans, and in adult-type AChR γ is replaced with ε subunit [6]. The *CHRNE* gene (OMIM #100,725) codes for this ε (epsilon) subunit of the AChR and is located on chromosome 17p13.2 [7]. *CHRNE* variants are the most common cause of CMS, which account for 30 %–50 % of all CMS and 75 %–80 % of all AChR deficiency-related CMS cases [8–11]. Mutations of *CHRNE* fall into two main groups: kinetic mutations with or without minor AChR deficiency and low-expressor mutations with or without minor kinetic effects, which is termed primary AChR deficiency [12].

The disease associated with *CHRNE* variants can vary in terms of its clinical characteristics and severity among different families. Some of the patients may only experience ptosis, while other affected individuals may show more severe clinical manifestations of generalized myasthenia [7].

CMS should be considered in the differential diagnosis of patients with early-onset fatigable muscle weakness, with a positive family history, negative Myasthenia Gravis antibody testing, and repetitive nerve stimulation (RNS) study showing decremental responses of 10 % or more in the amplitude of compound muscle action potential (CMAP) or single-fiber EMG studies compatible with an NMJ dysfunction [5,9]. CMS can be effectively treated, and response to treatment can vary depending on the underlying specific variants. Pharmacological treatment with acetylcholinesterase inhibitors and β2-adrenergic receptor agonists has shown favorable results in patients with *CHRNE* variant causing AChR deficiency [13].

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In the current study, we describe the clinical, neurophysiological, and molecular features as well as the response to treatment of a series of Iranian *CHRNE*-related CMS cases.

2. Materials and methods

2.1. Subjects

Seventy-seven patients with a possible diagnosis of CMS were referred to the neuromuscular clinic of Shariati Hospital affiliated with the Tehran University of Medical Sciences. Their clinical features, electrodiagnostic studies, and negative anti-AChR and anti-MuSK antibodies were evaluated between 2005 and 2023. Whole-exome sequencing (WES) was performed for each proband and 33 CMS patients from 27 different families with *CHRNE* variant were included in the study. Current age, age of onset, age at diagnosis, consanguinity, family history, motor milestone delay, ophthalmoparesis, generalized fatigue, dysphagia, neurophysiologic findings, and response to treatment of the patients were assessed (Tables 1 and 2). Response to treatment was evaluated in follow-up clinic visits using myasthenia gravis activities of daily living (MG-ADL) scale at baseline and after 3 months (Table 3).

MG-ADL is an outcome measurement tool that evaluates the functional performance of daily activities of patients with MG. It consists of eight items that two items assess ocular, three bulbar, one respiratory, and two limb-related impairments. Each item is given a score between 0 and 3; thus, the total score range is from 0 to 24 points. A higher score demonstrates the patient's poor condition [14].

2.2. Genetic analysis

To identify the underlying genetic defect, DNA was isolated from the peripheral blood of probands and their family members by the standard salting-out protocol. Whole-exome sequencing (WES) was performed on the DNA of the probands. WES data was analyzed as previously reported workflows [15]. The candidate disease-causing variants in the *CHRNE*

Table 1
Clinical features of Iranian patients with *CHRNE* variant.

Patient ID	Sex	Consanguinity /Family history	AAO (m)	Current age (y)	Age at diagnosis (y)	Motor / Milestone delay	Ophthalmoparesis / Ptosis	Proximal/ Muscle weakness	Neurophysiologic findings
P1.1	M	Y/Y	24	25	13	N	N/Y	Y	P
P1.2	M		at birth	23	11	N	Y/Y	Y	P
P2.1	F	Y/Y	12	15	3	N	Y/Y	Y	P
P2.2	M		9	22	10	N	Y/Y	Y	P
P3	M	Y/Y	at birth	27	23	N	Y/Y	Y	P
P4	M	Y/N	2	13	11	Y	Y/Y	Y	P
P5	M	Y/N	24	10	6	N	Y/Y	Y	P
P6	M	Y/Y	at birth	27	23	N	N/Y	N	Ne
P7	F	N/N	at birth	31	25	N	Y/Y	Y	P
P8.1	F	Y/Y	at birth	26	25	N	Y/Y	Y	P
P8.2	F		at birth	34	32	N	Y/Y	Y	P
P9.1	M	Y/Y	at birth	18	17	N	Y/Y	Y	P
P9.2	M		at birth	13	12	N	Y/Y	Y	P
P1.1	M	Y/Y	at birth	5	2	N	Y/Y	Y	Ne
P1.2	F		at birth	13	10	N	Y/Y	Y	P
P11	F	Y/N	48	26	17	N	Y/Y	Y	P
P12	M	Y/N	at birth	17	9	Y	Y/Y	Y	Ne
P13	F	Y/N	4	13	8	N	Y/Y	Y	P
P14	F	N/N	12	25	23	N	Y/Y	Y	P
P15.1	F	Y/Y	at birth	33	20	N	Y/Y	Y	P
P15.2	M		at birth	40	22	N	Y/Y	Y	P
P16	F	Y/N	72	35	28	N	Y/Y	N	P
P17	M	N/N	6	39	38	N	N/Y	Y	P
P18	M	N/N	72	19	19	N	Y/Y	Y	P
P19	M	N/N	16	7	6	N	Y/Y	Y	P
P20	F	Y/N	at birth	11	11	Y	Y/Y	Y	P
P21	F	Y/N	at birth	13	5	N	Y/Y	Y	P
P22	F	N/Y	at birth	8	7	N	N/Y	Y	P
P23	F	Y/N	48	15	4	N	Y/Y	Y	P
P24	M	Y/N	at birth	4	4	N	Y/Y	Y	P
P25	M	Y/N	12	13	13	Y	Y/Y	Y	P
P26	M	Y/Y	12	36	26	N	Y/Y	Y	P
P27	F	Y/N	12	22	20	N	Y/Y	Y	P

M: male, F: female, y: years, m: month, AAO: age at onset, Y: yes, N: no .

Table 2
Molecular findings of Iranian patients with *CHRNE* (NM_000080.4) variants.

Patient ID	Variant cDNA level	Variant Protein level	Exon No.	dbSNP	Zygosity	ACMG	Variant type	
P1.1	c.601G->*A*	p.Glu201Lys	6	–	Hom	VUS (PM2, PP3)	Missense	Novel
P1.2								
P2.1	c.1441C->T	p.Arg481Ter	12	rs775550642	Hom	Likely pathogenic (PVS1, PM2)	Nonsense	Novel
P2.2								
P3	c.130dup	p.Glu44GlyfsTer3	2	rs762368691	Hom	Pathogenic (PM3, PP1, PVS1, PM2)	Frameshift	Known
P4	c.164T->*A*	p.Val55Asp	2	–	Hom	Likely pathogenic (PS4, PM2, PP3)	Missense	Novel
P5	c.442T->*A*	p.Cys148Ser	5	rs1597621396	Hom	Pathogenic (PM3, PP1, PS3, PP3, PM2)	Missense	Known
P6	c.991C->G	p.Arg331Gly	9	–	Hom	VUS (PM2, PM5, PP3)	Missense	Novel
P7	c.1252_1267dup	p.Cys423SerfsTer38	11	rs1597613479	Hom	Pathogenic (PS4, PVS1, PM2)	Frameshift	Known
P8.1	c.183_187dup	p.Leu63ProfsTer3	2	rs776927709	Hom	Pathogenic (PM3, PS3, PVS1, PM2)	Frameshift	Known
P8.2								
P9.1	c.1327del	p.Glu443LysfsTer64	12	rs763258280	Hom	Pathogenic (PM3, PS3, PVS1, PM2)	Frameshift	Known
P9.2								
P1.1	c.1090dup	p.Arg364ProfsTer33	10	rs1156634884	Hom	Pathogenic (PS4, PVS1, PM2)	Frameshift	Known
P1.2								
P11	c.1364A->*T*	p.Asp455Val	12	–	Hom	Likely pathogenic (PP3, PM2)	Missense	Novel
P12	c.916A->*G*	p.Arg306Gly	8	–	Hom	Pathogenic (PS4, PM2, PM5, PM1, PP3)	Missense	Novel
P13	c.1252_1267dup	p.Cys423SerfsTer38	11	rs1597613479	Hom	Pathogenic (PS4, PVS1, PM2)	Frameshift	Known
P14	c.992G->*A*	p.Arg331Gln	9	rs760022829	Hom	Pathogenic (PM3, PP1, PS3, PM2, PM5, PP3)	Missense	Known
P15.1	c.571A->*T*	p.Lys191Ter	6	–	Hom	Likely pathogenic (PVS1, PM2)	Nonsense	Known
P15.2								
P16	c.1319_1326+15del	–	11	rs1208462125	Hom	Pathogenic (PM3, PVS1, PM2)	Splice	Known
P17	c.710G->C	p.Arg237Pro	7	–	Hom	VUS (PM2, PP3, PM1)	Missense	Novel
P18	c.1326G->*A*	p.Glu442=	11	–	Hom	VUS (PM2, PP3)	Splice	Novel
P19	c.1327del	p.Glu443LysfsTer64	12	rs763258280	Hom	Pathogenic (PM3, PS3, PVS1, PM2)	Frameshift	Known
P20	c.164T->*A*	p.Val55Asp	2	–	Hom	Likely pathogenic (PS4, PM2, PP3)	Missense	Novel
P21	c.601G->*A*	p.Glu201Lys	6	–	Hom	Likely pathogenic (PVS1, PM2)	Missense	Novel
P22	c.1327del	p.Glu443LysfsTer64	12	rs763258280	Hom	Pathogenic (PM3, PS3, PVS1, PM2)	Frameshift	Known
P23	c.502_503delinsAA	p.Ser168Asn	6	–	Hom	VUS (PM2)	Missense	Novel
P24	c.46+1G->*A*	–	Int 1	rs746199600	Hom	Likely pathogenic (PVS1, PM2)	Splice	Novel
P25	c.1327del	p.Glu443LysfsTer64	12	rs763258281	Hom	Pathogenic (PM3, PS3, PVS1, PM2)	Frameshift	Known
P26	c.502_503delinsAA	p.Ser168Asn	6	–	Hom	VUS (PM2)	Missense	Novel
P27	c.1252–1267dup	p.Cys423SerfsTer38	11	rs1597613479	Hom	Pathogenic (PS4, PVS1, PM2)	Frameshift	Known

Hom: homozygous, VUS: variants of uncertain significance, Int: intron, ACMG: American college of medical genetics.

Table 3
Response to treatment in congenital myasthenic syndrome with CHRNE mutation.

Variable	Pyridostigmine alone [14]	Pyridostigmine plus salbutamol [17]	*p-value
Gender; M/F	10/4	6/11	0.55
Age, year	18.5 ± 11.08	21.7 ± 9.7	0.39
Age assessed, year	13.6 ± 10.5	19.3 ± 9.6	0.12
Age of onset, Month	14.2 ± 17.9	11.4 ± 21.9	0.69
MG-ADL pretreatment	7.0 ± 2.7	9.5 ± 3.1	0.02
Mg-ADL after treatment	4.4 ± 2.2	5.8 ± 2.6	0.15

M/F: Male/ Female; MG-ADL: Myasthenia Gravis Activities of Daily Living.
* p-value <0.05 is significant.

gene were confirmed in the probands and evaluated in available family members by polymerase chain reaction (PCR) and Sanger sequencing. Sequences were analyzed by comparison with the reference sequence at NCBI: NC_000017.10, NM_000080.4, and NP_000071.1 for the *CHRNE* gene.

2.3. Statistical analysis

Statistical analysis was performed with SPSS statistical software (version 24.0; SPSS, Inc., Chicago, IL, USA). Quantitative data were stated as mean ± SD and qualitative data were summarized as frequency and percentage. An independent sample *t-test* was used to evaluate continuous data, and a chi-squared test was applied to relate categorical variables and qualitative data. A pair *t-test* was performed to evaluate before and after treatment. P values <0.05 were considered statistically significant.

3. Results

A total of 33 patients (18 males and 15 females) with a mean age of 20.6 ± 10.1 years (range of 4 to 40 years) from 27 different unrelated Iranian families were evaluated. The mean age of disease diagnosis was 16.8 ± 10.06 years. Consanguinity was reported in 21 families and 10 families had more than one affected family member. Table 1 shows the clinical data of the patients.

3.1. Molecular findings

In total 19 *CHRNE* variants including 10 novel variants were identified (Table 2 and Fig. 1). Among them, there were nine missense, five frameshift, two nonsense, and three variants which could potentially influence splicing. Based on the ACMG (American College of Medical Genetics) criteria, nine variants were categorized as pathogenic, five as likely pathogenic, and five as variance of uncertain significance (VUS) (Table 2). Variant c.1327del; (p.Glu443LysfsTer64) was found in four different families (P9, 19, 22 and 25). Variant c.1252–1267dup; (p. Cys423SerfsTer38) was seen in three families (P7, 13 and 27) and variants c.502–503delinsAA; (p.Ser168Asn) and c.164T->*A*; (p.Val55Asp) each were detected in two different families (Table 2).

3.2. Clinical findings

3.2.1. Clinical presentation

The onset of clinical symptoms was at birth or under one year of age in 25 (75.8 %) patients and over one year in eight patients (24.2 %). The mean age of disease onset was 15.6 ± 27.02 months (range 0–120 months). All patients had normal height and weight with intact cognitive function. Bilateral ptosis was seen in all patients (100 %). Other symptoms included ophthalmoparesis 29 (87.9 %); bilateral facial

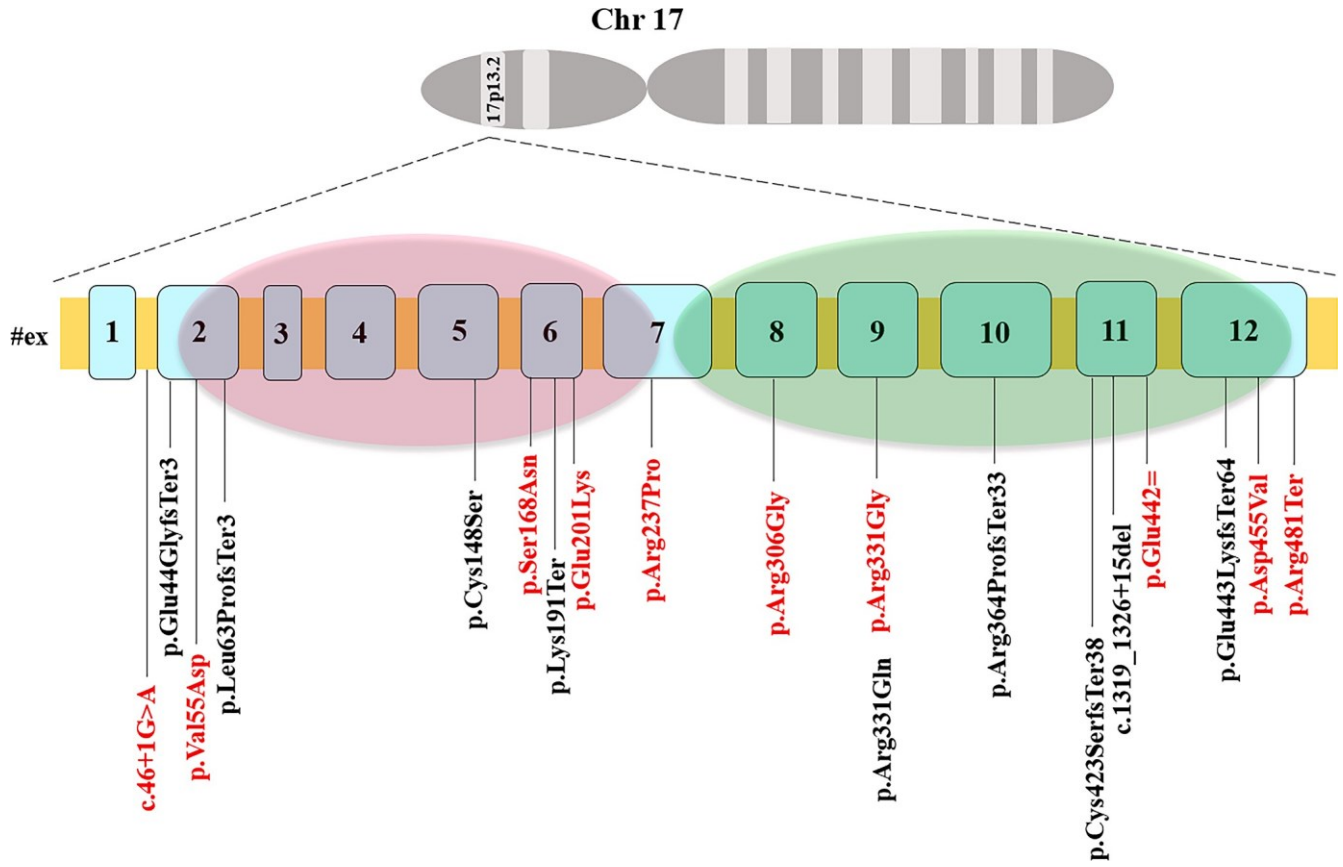

Fig. 1. Schematic diagram of *CHRNE* gene showing the location of different variants identified within the cohort of patients. The blue boxes represent the exons and the red and green shadows show LGIC_ECD_nAChR_E and Neur_chan_memb domains, respectively. The novel variants are marked in red.

weakness 24 (72.7 %); axial weakness 17 (56.6 %); bulbar dysfunction 17 (51.5 %); and diplopia 17 (51.5 %). Ptosis was fluctuating and fatigable on examination. Diplopia was intermittent and brief and was reported by patients when asked on the MG-ADL questionnaire.

None of the patients showed significant swallowing or breathing problems requiring ventilation or tracheostomy. None of the patients had skeletal deformity or scoliosis. Three patients had joint contractures (P15.1, 15.2, 21). Most of the patients had mild to moderate weakness in the proximal muscles of the upper and lower limbs (93.9 %). Two patients had no proximal muscles weakness (P6 and P16). Only one patient became wheelchair-bound at the age of ten years (P21), and the rest retained independent ambulation.

P4 and P20 who carry c.164T>*A*; p.Val55Asp variant, and P12 with c.916A>*G*; p.Arg306Gly variant, reported delay in motor milestones. Moreover, P16 and P18 with variants of c.1319_1326+15del and c.1326G>*A*; p.Glu442=, respectively, had facial deformity with an elongated face and high-arched palate.

3.3. Neurophysiology

Three Hz RNS was performed in all CMS patients and 30 (90.9 %) of patients showed the presence of significant decrement (10 % or more in the amplitude of CMAP). Patients 6, 10, and 12 had normal RNS and demonstrated increased jitter in single-fiber EMG. No incremental response was detected. Nerve conduction studies (NCSs) and electromyography (EMG) were normal.

3.4. Response to treatment

All patients received cholinesterase inhibitors (pyridostigmine) after diagnosis of CMS. In this study, 14 patients (42.4 %) were treated only with pyridostigmine, 17 patients (51.5 %) were on dual therapy with concomitant use of salbutamol and pyridostigmine and two patients (6.1 %) received 3, 4-Diaminopyridine (DAP) and pyridostigmine. All patients treated with only pyridostigmine (30–180 mg daily, orally) had some improvement. When the symptoms of the patients were suboptimal with pyridostigmine treatment, salbutamol (0.1 mg kg^{-1}) was added to the treatment strategy. Fifteen of 17 patients improved further on dual therapy with pyridostigmine and salbutamol. Two patients did not attend for follow-up. There was no significant association between age of onset, age of assessment, and sex with type of treatment (Table 3). The mean MG-ADL of all patients was 8 ± 3.1 before treatment and 5 ± 2.6 after treatment ($p<.0001$). On the other hand, the mean score of MG-ADL pre-treatment with pyridostigmine alone and pyridostigmine plus salbutamol was different (7. 0.0 ± 2.7 and 9.5 ± 3.1, $p=.02$, respectively). The mean MG-ADL after treatment decreased in patients receiving pyridostigmine alone or pyridostigmine plus salbutamol (4.4 ± 2.2 and 5.8 ± 2.6, $p=.15$, respectively). There was no significant association between the mean score of MG-ADL and the type of treatment regimens (Table 3).

4. Discussion

In this study, we describe the clinical features, molecular findings, neurophysiology, and response to treatment of *CHRNE*-CMS patients. To our knowledge, this is the first time such a large number of *CHRNE*- CMS patients have been reported from Iran. The frequency of *CHRNE* mutation in our study was 38.9 %. In a study in Israel, the frequency of *CHRNE* was reported as 20 %, which was lower than *RAPSN* and *COLQ* mutations [16]. Estephan et al. reported the frequency of CHRNE mutation 38.1 % in CMS Brazilian patients [17]. However, in several other studies including our series (unpublished data); the most common gene

involved in CMS has been reported to be *CHRNE* [18–21]. After 2011, whole-exome sequencing (WES) has effectively enabled the discovery of causative genes linked to CMS [22]. Therefore, over 169 variants responsible for CMS have been reported (HGMD professional 2023.4).

We described 19 different *CHRNE* variants in 33 subjects of which nine variants (P.3, 4, 5, 7, 8, 9, 10, 12, and 14) have been reported in clinvar database (https://www.ncbi.nlm.nih.gov/clinvar/?gr=0). Ten variants in families 1, 2, 4, 6, 11, 12, 17, 18, 23, and 24, are novel and have not been reported before (Table 2 and Fig. 1). All of the variants were inherited in an autosomal recessive manner. The *CHRNE* homozygous nonsense mutation c.571A>*T*; (p.Lys191Ter) has been reported previously in a family in Iran [23]. The most frequent mutation in the present study was c.1327del mutation (four families 9, 19, 22, and 25). In a study in Turkey, the most common *CHRNE* mutation was 1219 + 2T>*G* (23.5 %) and c.1327del (11.8 %) [24].In the southern region of Brazil, a study revealed c.130dupG (p.Glu44Glyfs*3) *CHRNE* mutation in up to 33 % of families with CMS [17]. In a study in North Africa, CHRNE 1293insG mutation was found in 14 families (about 60 %) of patients [25]. Our study demonstrated that the missense variants are more prevalent (~47 %). Moreover, the frequency of frameshift, splice, and nonsense variants are about 36 %, 15 %, and 10 %, respectively. Patient 18 showed a novel synonymous variant, c.1326G>*A*, p. Glu442=, which is predicted to lead to an abnormal splicing by SpliceAI and Pangolin scores (https://spliceailookup.broadinstitute.org/), as Acceptor Loss: Δ score = 0.58, Donor Loss: Δ score = 0.68 and Splice Loss: Δ score = 0.84. Also, P24 carried a novel splice variant, c.46+1G>*A* in intron 1, with scores: Donor Loss: Δ score = 0.67 and Splice Loss: Δ score = 0.44 (Table 2) [26].

The clinical and demographic characteristics of CMS, such as the age of onset, neurological symptoms, severity, and response to treatments, occasionally exhibit variation, depending on the specific molecules and functions that are affected [27]. The age at onset of the patients in this study varies from birth to 6 years old. Sixteen patients (48.5 %) had clinical symptoms at birth. Richard et al. reported the onset of clinical features in 10 of 14 patients was in neonatal period [25]. The early symptoms of *CHRNE* patients were ptosis, ophthalmoparesis, and mild to moderate proximal weakness. Very few numbers of patients had severe muscle weakness that required a wheelchair or were dependent on a bed. Respiratory involvement and severe swallowing disorder were not prominent symptoms of the disease. Like most CMS patients, fatigability and fluctuation were the obvious symptoms of the disease. Patients usually improved on treatment with pyridostigmine, salbutamol, and 3, 4-DAP concerning muscle weakness and bulbar dysfunction. Estephan et al. reported clinical manifestations of *CHRNE* mutation in Brazilian patients [17]. The onset of symptoms was before 2 years of age. The most common clinical characteristics were ocular muscle impairment, ptosis, limb weakness, facial involvement, bulbar symptoms, and the patient's response to pyridostigmine [17]. Kastreva et al. reported 91 Bulgarian Roma CMS patients associated with c.1327delG mutation in the CHRNE gene with variability in the severity of the clinical manifestations [28]. Similar to our study, in the Spanish CMS cohort in 2017, c.1327delG was the most common variant reported [29]. A study in North Africa on fifty-one patients with mutation in the CHRNE gene recognized mild to moderate weakness of oculobulbar and limbs, with a benign disease course and good response to cholinesterase inhibitors [25] Two *CHRNE*-CMS families have been previously reported from Iran, a homozygous missense mutation of c.973G>*T* and a homozygous nonsense mutation c.571A>*T* [23,30]. The clinical symptoms of the former were hypotonia and bilateral ptosis without involvement of mental, facial, and bulbar muscles [30]. Our patients have no joint hyperlaxity or contractures except patient 21 and patients 15.1 and 15.2, which were previously reported by Soltanzadeh et al. [23]. Salih et al. reported clinical characteristics of three siblings from one family with homozygous duplication mutations c.123_127dup in exon 2 of the *CHRNE* gene, with ptosis, restricted ocular motility, mild proximal weakness, and difficulty swallowing [15]. Ardissone et al. reported four Italian *CHRNE* patients with mild to severe phenotypic features. One family had p.Thr159Pro mutation in the *CHRNE* gene and the second presented p.Ser235Leu mutation along with p.Thr159Pro mutation. None of the patients had respiratory weakness. One patient responded to salbutamol significantly [31]. Gülen Gül Mert et al. reported the obvious symptoms of *CHRNE* as bilateral ptosis and ophthalmoparesis without severe respiratory failure, and all patients responded to pyridostigmine and salbutamol significantly [32].

More severe symptoms including significant disability and skeletal and chest deformity moreover, life-threatening respiratory problems have not been reported in other studies. Patients with *CHRNE* mutations who have milder symptoms usually have an optimal response to treatment with pyridostigmine, but salbutamol seems to be a useful adjuvant in *CHRNE*-CMS patients with more profound motor weakness and swallowing disorder.

In conclusion, knowing this rare disorder, as well as clinical and genetic findings within a particular geographical area should make physicians aware for accurate diagnosis and treatment.

Ethics approval and consent to participate

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Consent for publication

Informed consent was obtained from all participants.

CRediT authorship contribution statement

Narges Karimi: Writing – review & editing, Writing – original draft, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Aida Ghasemi:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation. **Akram Panahi:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Bentolhoda Ziaadini:** Writing – review & editing, Visualization, Project administration, Data curation, Conceptualization. **Shahriar Nafissi:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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