



Clinical and genetic diversity in Iranian individuals with *RAPSN*-related congenital myasthenic syndrome

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Abstract

Congenital myasthenic syndromes (CMSs) are genetic disorders affecting motor function with variable symptoms. *RAPSN*-related CMS, caused by mutations in the *RAPSN* gene, leads to muscle weakness. Accurate diagnosis is essential for proper management. This study aims to analyze six Iranian families affected by *RAPSN*-CMS, focusing on clinical manifestations, genetic variants, treatment response, and outcomes. Clinical assessments, genetic analysis, and whole-exome sequencing were performed on the six families to identify *RAPSN* gene mutations. The study examined symptoms, disease severity, age of onset, treatment response, and outcomes. Treatment with pyridostigmine and salbutamol was given to assess its effectiveness. Three homozygous known variants in *RAPSN* gene were identified: c.491G>A in three families, c.264 C>A in two families, and c.-210 A>G in one family. Clinical assessments showed diversity in symptoms and treatment responses. Pyridostigmine and salbutamol treatment improved symptoms and quality of life. This study highlights the significance of molecular diagnosis for *RAPSN*-related congenital myasthenic syndromes (CMS) in Iran, marking the first comprehensive genetic analysis in the region. The identification of specific pathogenic variants underscores the unique genetic landscape of local patients. Furthermore, our long-term follow-up revealed variable treatment responses, emphasizing the need for personalized care strategies. The clinical variability among patients with identical mutations necessitates a multidisciplinary approach for effective management. By enhancing genetic awareness and refining follow-up methods, we aim to improve diagnosis accuracy and interventions, fostering better outcomes for affected families in the Iranian population.

Highlights

- A comprehensive description of clinical and molecular findings in six *RAPSN*-related CMS patients is presented.
- This study represents the first comprehensive examination of *RAPSN*-CMS in Iran.
- The study contributes to the expanding spectrum of clinical and genetic variations in *RAPSN*, identifying previously reported variants, and supports the clinical utility of treatments like pyridostigmine.

Keywords Congenital myasthenic syndrome (CMS) · *RAPSN* gene · Neuromuscular junction (NMJ) · Whole exome sequencing (WES)

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Abbreviations

AchR	Acetylcholine Receptor
ACMG	American College of Medical Genetics
APB	Abductor Polipsis Brevis
BMI	Body Mass Index
CHRNA	Choline receptor nicotinic alpha
COLQ	Collagen Q
CMS	Congenital Myasthenic Syndromes
CNVs	Copy Number Variations
CPK	Creatine Phosphokinase

DTR	Deep Tendon Reflexes
EMG	Electromyography
FDI	First Dorsal Interosseous
MG	Myasthenia Gravis
MRC	Medical Research Council
MuSK	Muscle-Specific Kinase
NCBI	National Center for Biotechnology Information
NCS	Nerve Conduction Studies
NRC	Neuromuscular Research Center
NMJ	Neuromuscular Junction
RAPSN	Receptor-Associated Protein of the Synapse
TPR	Tetratricopeptide Repeat
WES	Whole-Exome Sequencing

Introduction

Congenital myasthenic syndromes (CMSs) are a group of extremely heterogeneous genetic disorders, manifesting variable symptoms, including ptosis, diplopia, and muscular weakness, as well as various types of congenital deformities. Severe forms of CMS can also be associated with respiratory symptoms. It can manifest in the neonatal period, childhood, adolescence, or even adulthood [1, 2]. The prevalence of CMS varies depending on the subtype. Presynaptic forms are the rarest, affecting an estimated 7–8% of patients, while synaptic forms account for approximately 14–15% of patients. The remaining 75–80% of cases are attributable to postsynaptic defects [3, 4]. Variants in more than 35 different genes are responsible for this disorder, some of which are *CHRNE*, *COLQ*, *DOK7*, *CHAT*, and *RAPSN* [5].

The *RAPSN*-related myasthenic syndrome is a form of CMS caused by homozygous or compound heterozygous variants in the *RAPSN* gene [5, 6]. This gene encodes a protein called receptor-associated protein of the synapse (RAPSN) which is a postsynaptic peripheral membrane protein that anchors the nicotinic acetylcholine receptor to the motor endplate [7] and is critical for the formation and maintenance of the neuromuscular junction (NMJ) [8]. *RAPSN* variants can lead to the pathogenesis of CMS by affecting the clustering and maintenance of nicotinic acetylcholine receptors at the NMJ [8].

Proper management of individuals with *RAPSN*-related CMS requires a timely and accurate diagnosis. Diagnosis of these patients can be challenging due to the variability in disease severity and course. This type of CMS can present as an early-onset phenotype with symptoms such as arthrogryposis, muscle hypotonia, apneic crisis, and feeding difficulties, or as a late-onset phenotype presenting with weakness and sometimes wasting of muscles [7, 8]. Treatment options for *RAPSN*-related myasthenic syndrome may

include medications such as pyridostigmine, salbutamol and amifampridine to improve symptoms and quality of life [9].

Until now, a precise genetic examination of this particular disorder in Iran has not been conducted. Consequently, for the first time, we present a comprehensive study of the clinical and paraclinical characteristics, response to treatment, as well as the outcomes of genetic analysis of six Iranian families affected by *RAPSN*-CMS.

Materials and methods

This study was conducted according to the declaration of Helsinki and approved by the ethics committee of the Tehran University of Medical Sciences (Approval ID: IR.TUMS.SHARIATI.REC.1403.001) in Iran. The clinical details were collected from the patient upon obtaining written informed consent. Also, the authors affirm that human research participants provided informed consent for publication of the image in Fig. 2.

Subjects

Six unrelated Iranian CMS families were referred to the Neuromuscular Research Center (NRC), Tehran University of Medical Sciences. The disease seemed to be sporadic, albeit consanguineous marriage was noted in the parents of families 1, 2, 3, 5, and 6 (Fig. 1). Also, the parents of family 4 came from nearby small villages, which suggests a common ancestry (Fig. 1). These families were followed over a mean period of eight years in the neuromuscular clinic in the Shariati hospital, Tehran University of Medical Sciences. We used the Medical Research Council score (MRC) for the motor force examination. Electrodiagnosis was performed for all patients with routine nerve conduction studies and low frequency repetitive nerve stimulation (LFRNS).

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 [10]. Whole-exome sequencing (WES) was performed using SureSelect Human All Exon V6 (Agilent Technologies Inc, Santa Clara, CA, USA) enrichment kit on the DNA of the probands. WES data was analyzed as previously reported workflows [11]. Variant filtering was conducted, focusing on all variants located in exons, exon-splice regions, and splice sites that have a reported minor allele frequency (MAF) of less than 0.01 in public genomic databases. Subsequently, considering the consanguineous marriage observed in family 1, 2, 3, 5 and 6 and possible common ancestor, we first focused on

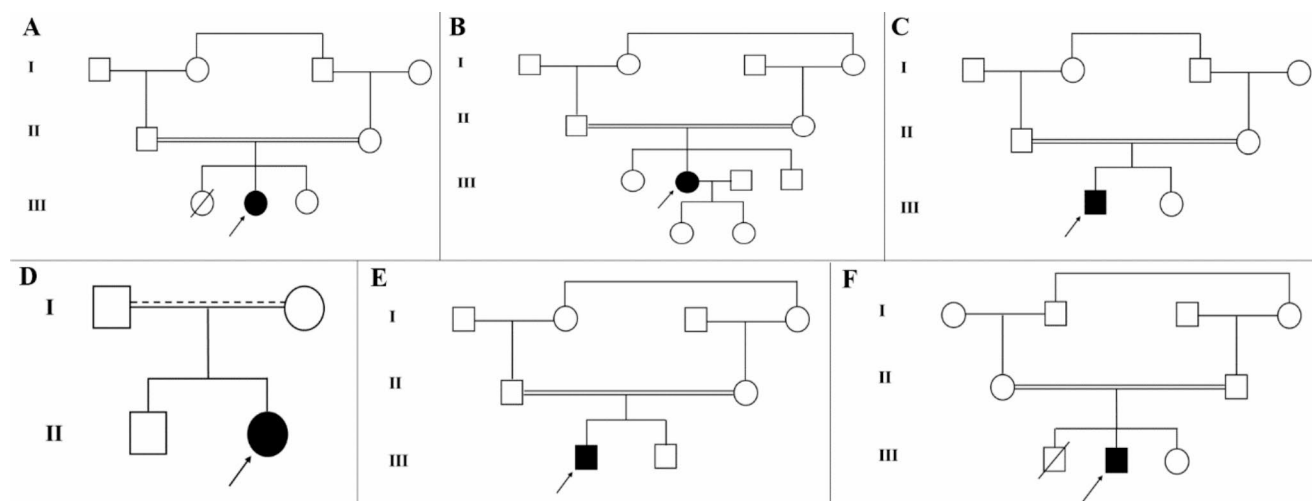


Fig. 1 The Iranian *RAPSN*-related CMS pedigrees: (A) Family 1 (B) Family 2 (C) Family 3 (D) Family 4 (E) Family 5 and (F) Family 6. Arrows show probands. Dashed line shows the parents originated from

nearby small villages, suggesting a common ancestry. Unfilled circles and squares, present normal individuals; black circles and squares indicate CMS patients

the homozygous variants found in the DNA of the probands. However, for all probands, both homozygous and heterozygous variants were evaluated. The remaining variants were analyzed to identify any disease-causing variants linked to known CMS or related neurological disease genes. Finally, several in silico tools were utilized to predict the potential effects of the candidate variants on the encoded proteins, and the variants were classified according to the criteria set by the American College of Medical Genetics (ACMG).

The candidate disease-causing variants in the *RAPSN* gene (NM_005055.5), c.264 C>A:p.(Asn88Lys), c.-210 A>G, and c.491G>A:p.(Arg164His), were confirmed in the probands and evaluated in family members by polymerase chain reaction (PCR) and Sanger sequencing. Sequences were analyzed by comparison with the reference sequence available at NCBI: NC_000011.9, NM_005055.5, and NP_005668.2 for the *RAPSN* gene.

Treatment

All patients were prescribed pyridostigmine (90–240 mg/d) with or without salbutamol (2–12 mg/d). In all cases, pyridostigmine has demonstrated a positive effect, even though some patients did not adhere to salbutamol properly, it has led to satisfactory results when used by them. We have not observed any significant deterioration in any of the patients during the period of follow-up.

Results

Clinical and paraclinical manifestations

Clinical manifestations of all patients are summarized in Table 1. Details of the clinical data of these individuals are meticulously documented herein.

Family 1

The first case was a 29-year-old female (P1) (Fig. 1A) complaining of drooping eyelids, strabismus, and occasional double vision. Her parents were first cousins. Upon birth, she was floppy and had trouble sucking, as well as bilateral drooped eyelids, which gradually improved with age. The development of some motor milestones has been delayed slightly, and she has always been weaker than her peers. She had been hospitalized for dyspnea and weakness followed by respiratory infections and renal disease (renal tubular acidosis) several times in the first decade of her life. When she was nine years of age, her condition was diagnosed as Myasthenia Gravis (MG). She was treated with Pyridostigmine, which resulted in significant improvements in her condition. After the thymectomy at the age of 18, she felt better and stopped her medication, but seven months later, the symptoms worsened and pyridostigmine was restarted. She had three episodes of seizure-like attacks that were controlled with Lamotrigine and Carbamazepine. Similar symptoms do not exist in her family. She had an older sister who died following a seizure two days after birth. The neurological examination revealed Exotropia and ptosis on both sides. Additionally, characteristic facial dysmorphism including hypertelorism and mild micrognathia was

Table 1 Main clinical features of Iranian CMS patients with *R475N* variant

Patients ID	Sex	Age (y)	AAO (y)	AOD (y)	Consanguinity	Delayed milestones	Symptoms at onset	Symptoms at diagnosis	Fatigability	Severity of symptoms	Ocular	Bulbar muscle weakness	Muscular weakness	Deformities		PMH	Treatments received and their response		Course after treatment initiation
														Facial dysmorphism	Other deformities		response to ACEI (pyridostigmine)	Salbutamol	
P1	F	32	at birth	29	1st cousin	yes (motor)	floppy at birth, ptosis, difficulty at sucking, respiratory syndrome	ptosis, yes	yes	mild weakness with severe attacks	Ptosis, strabismus	difficulty sucking at birth	no	hyper-telorism, micrognathia	no	seizure, renal tubular acidosis, thyrotoxicosis	improvement	improvement	improvement
P2	F	63	6	45	1st cousin	no	generalized weakness, speech, dysphagia, jaw weakness	muscle weakness, nasal speech, dyspnea	yes	mild to moderate	Ptosis, diplopia	nasal speech, difficulty swallowing and chewing	facial, neck, limb girdle	NA	high arch palate, jaw deformity, slender tongue	microcytic anemia, eczema, thyrotoxicosis	improvement	improvement	steady with occasional worsening
P3	M	16	at birth	11	1st cousin	no	ptosis, generalized weakness, respiratory symptoms	ptosis, muscle weakness	yes	mild	Ptosis	NR	facial, neck, limb girdle	hyper-telorism, micrognathia	club foot	decreased mental	improvement	NA	improvement
P4	F	36	at birth	29	no#	no	generalized weakness, respiratory symptoms	worsening of weakness, respiratory symptoms	yes	moderate to severe	Ptosis, diplopia	NR	facial, limb girdle	hypertelorism	syndactyly at toes	decreased mental	improvement	NA	steady with treatment
P5	M	36	at birth	27	1st cousin	yes	generalized weakness, ptosis	worsening of weakness, ptosis	yes	mild to moderate	ophthalmoparesis, strabismus, sluggish pupils reflex	NR	facial, neck, limb girdle	micrognathia	DDH, scoliosis	decreased mental	low Apgar at birth	mild improvement	steady with occasional worsening
P6	M	6	at birth	1	1st cousin	yes	floppy at birth, respiratory symptoms, difficulty at sucking	ptosis, difficulty chewing	yes	moderate to severe	Ptosis, mild limitation of ocular movement	respiratory symptoms, difficulty sucking at birth	facial, neck, limb girdle	hyper-telorism, micrognathia	no	decreased mental	improvement	NA	steady with occasional worsening

y: year, M: male, F: female, NR: not reported, NA: not available, Neg: negative, PMH: past medical history, AAO: age at onset, AOD: age of the diagnosis

#: Parents originated from nearby small villages



Fig. 2 Image of the patient (P5). Micrognathia is remarkable

notable. Despite normal muscle force in the face, neck, and all limbs, apparent fatigability was notable. Normal deep tendon reflexes (DTR) were obtained. LFRNS resulted in a significant decremental response (12.3%) in the Anconeus muscle, besides a normal nerve conduction study (NCS) and needle electromyography (EMG). No repetitive Combined Motor Action Potential (CMAP) was found. There were no abnormalities in the serum levels of acetylcholine receptor (ACR) antibody, anti-MuSK antibody, thyroid function test, and creatine phosphokinase (CPK). In addition to pyridostigmine, we added salbutamol to her treatment regimen. She had surgery for exotropia, but after surgery, bilateral esotropia developed. Even though the patient used salbutamol irregularly, her symptoms have significantly improved during the three years that we have been following her.

Family 2

Our next patient was a 45-year-old female (P2) when first seen by us (Fig. 1B) She presented to our emergency department complaining of acute dyspnea. parents are first cousins and are Iranian Jews from the central region of Iran. Since the age of six, she has had mild bilateral drooped eyelids

that worsen in the afternoon. Additionally, she had difficulty chewing, swallowing, and occasionally choking. It was at the age of 12 that she noticed that ascending the stairs was difficult. While she was pregnant, her condition appeared to be deteriorating. She was diagnosed as MG when she was 23 and had significant improvement with pyridostigmine. A thymectomy was performed on her at the age of 30 without any benefit. Over these years, she has received a variety of treatments for MG (plasma exchange, corticosteroid, and azathioprine) without any improvement in her condition. Family history was negative.

Upon examination, in addition to nasal speech, she had mild bilateral ptosis and mild bilateral facial paresis. We found that the tongue was thin and the palate was high-arched. The motor forces were as follows: Neck flexors;4, Neck extensors; 4+, shoulder abduction and elbow flexion;4, elbow extension; 4-, wrist extension;4+, finger extension, first dorsal interosseous (FDI) and abductor pollicis brevis (APB);5, Iliopsoas;4-, Quadriceps;3, foot dorsiflexion; 4+, foot plantarflexion;5. There were no abnormalities in DTRs. Gait was waddling, and Gower's sign was present. Sensory examination was intact.

LFRNS revealed a significant decremental response in the Nasalis muscle (26.3%). There were no repetitive CMAPs and the amplitudes were normal. ACR antibody levels were checked twice and was normal.

We added salbutamol tablet (12 mg daily in divided doses) to her treatment (pyridostigmine) and she has an excellent response to that combination. We have followed her for 18 years and she has generally been in a steady state, but she has experienced some worsening of symptoms after temporarily stopping salbutamol for a few days.

Family 3

The other patient was an 11-year-old male (P3) (Fig. 1C) with drooped eyelids and weakness. Parents are first cousins. His mother reported that he had several episodes of respiratory distress, which resolved after a few days. When the child was 12 months old, the parent noticed bilateral drooping eyelids, which grew worse with activity and fatigue. He was diagnosed with MG at the age of nine and symptoms improved following the administration of pyridostigmine. Additionally, he had a foot deformity that had been corrected by surgery. There are no other people in his family with similar symptoms. On examination, he had hypertelorism and mild micrognathia. Moreover, a mild bilateral facial paresis was observed. Both sides have the following muscle forces: neck flexion and extension;4, Deltoid;4, Biceps and Triceps; 4+, Wrist flexion and extension;5, FDI;4+, finger flexion and extension;5, APB; 5, Iliopsoas;4, Quadriceps;5, Tibialis Anterior;4+ and plantar flexion;5. DTR, sensory examination, and gait were all normal. Some degree of calf and foot atrophy was evident bilaterally.

The results of the laboratory tests did not reveal any noteworthy findings. Serum anti-MuSK and anti-ACR antibodies were negative. An electrodiagnostic examination revealed normal NCS and CMAP amplitudes without evidence of repetitive CMAPs. LFRNS showed a significant decrement (36%) in Anconeus muscle. The patient has remained stable on pyridostigmine during the 5-year follow-up.

Family 4

The fourth patient was a 29-year-old female (P4) (Fig. 1D) with generalized weakness mostly affecting the lower limbs, and occasional double vision and drooping eyelids. She was admitted to the hospital shortly after birth and at the age of 4 months due to pneumonia. Throughout her life, she had been weaker than her peers. The symptoms have progressively worsened over the past five to six years. A diagnosis of MG was made five years ago, and she received treatment with pyridostigmine with partial improvement. The patient suffers from psychological and personality disorders. Two

suicide attempts have been made as well as a history of amphetamine addiction for a few years. Her family history was unremarkable, and her older brother was symptom-free.

Upon physical examination, she had hypertelorism and, her facial muscles were weak. Muscle force was: Deltoid and Triceps; 4+ bilaterally; Biceps; 4 bilaterally; Iliopsoas; 4 bilaterally. A DTR of 3+ was obtained, along with normal sensory tests and gait evaluation. There was syndactyly on the 2nd and 3rd toes.

The results of electrodiagnosis revealed a significant decremental response (30%) in LFRNS in the Anconeus and Trapezius muscles, while the rest of the study had normal results. No repetitive CMAP was detected. There were no abnormal serum levels of CPK, Anti-MuSK antibodies, anti-ACR antibodies, lactate or pyruvate. A combination of Pyridostigmine and Salbutamol was initiated. She was poorly adherent to treatment and discontinued salbutamol due to tremors and was taking pyridostigmine irregularly for four years and had experienced a progression of symptoms until she needed the use of a wheelchair. During the last two years, she had discontinued the amphetamine and consumed pyridostigmine regularly, which resulted in some improvement in her symptoms and the ability to walk on her own.

Family 5

The fifth case was, a 27-year-old male (P5) (Fig. 1E) presented with muscle weakness that had developed since childhood. His parents are first cousins. His Apgar score was low when he was born and he was a floppy baby with shortness of breath in the first few days following birth. Motor milestones were slightly delayed and he had a congenital hip dislocation, which was repaired at ten months of age. He also had scoliosis, which was managed with a brace for four years and was corrected by surgery at the age of 15. His mother states that he has had mildly droopy eyelids since birth and in comparison, to his peers, he was always weaker. His condition deteriorated after he turned seventeen, with worsening with heat and stress and occasional double vision. He was diagnosed with MG at the age of 24, and treatment with pyridostigmine was started which improved and stabilized his condition. Otherwise, his past medical history was negative. He has a sister who is 24 years old and in good health.

Aside from a high-arched palate and marked micrognathia (Fig. 2), remarkable examination findings included bilateral facial weakness, ptosis, sluggish pupil reflex, and slightly limited eye movements. The following muscles were bilaterally weak: Deltoid; 3, Biceps;4, Iliopsoas, Quadriceps, and Tibialis anterior;4-. DTRs were generally brisk, along with intact sensory testing and normal gait patterns.

LFRNS showed significant decrement in the Trapezius (67%) and Anconeus (49%) muscles. Nerve conduction studies were normal and no repetitive CMAP was seen. Electromyography showed short duration, low amplitude motor unit potentials. Serum CK was normal and anti-ACR antibody were negative. He has been under our care for nine years and in serial visits following the initiation of Pyridostigmine and Salbutamol, there has been no significant worsening of the condition.

Family 6

Lastly, we examined the case of a 5-year-old boy (P6) born to a first cousin's parents (Fig. 1F). When he was born, he was floppy and required hospitalization for pneumonia. Additionally, he was fed via a nasogastric tube for the first six months due to difficulty sucking. droopy eyelids were noticed when he was two months old. Motor development has been delayed. After the introduction of pyridostigmine, His symptoms have improved significantly, and he is now able to walk and run on his own. According to the family, some episodes of worsening have occurred following infections. Further, delaying the administration of pyridostigmine led to the return of symptoms.

Mild micrognathia and hypertelorism was noticed. The pupils reacted normally to light. A slight restriction of eye movement as well as ptosis was observed. The neck flexors, bilateral deltoids, biceps, triceps, iliopsoas, hip abductors, and hamstrings all displayed mild weakness ranging from 4/5 to 4+/5. Mild reductions in DTR were observed in the upper limbs. On the right side, there was evidence of scapular winging. The anti-MuSK and anti-ACR antibodies were negative. Both abductor digiti minimi muscles had decremental LFRNS responses. There was no evidence of repetitive CMAP in NCS. The diagnosis of RAPSN-related CMS was confirmed by genetic testing, which detected a homozygous missense mutation in the RAPSN gene. As a result of the treatment with pyridostigmine, the child was able to resume normal motor development and was able to walk and run normally. Except for a few episodes of deterioration during infections or delayed drug consumption, his condition has been generally stable.

Genetic findings

WES results of all probands indicated high-quality sequencing. Filtering of WES data resulted in the detection of the disease-causing variants in all six probands; All of them carried the variants in the *RAPSN* gene. The proband of families 1 and 6 carried a known homozygous variant; *RAPSN*(NM_005055.5):c.264 C>A;p.(Asn88Lys), the proband of family 2 carried another known homozygous regulatory variant; *RAPSN*(ENST00000298854.2):c.210 A>G, and the probands of families 3, 4, and 5 shared the same known homozygous variant; *RAPSN*(NM_005055.5):c.491G>A;p.(Arg164His) (Table 2). MAF of c.264 C>A variant in gnomAD was 0.00156 and on the basis of ACMG (American College of Medical Genetics) criteria, this variant was categorized as Pathogenic by rules PM3, PP1, PS3, PM2, and PM1. Next variant, c.-210 A>G, was a regulatory variant that not detected in gnomAD and predicted as Likely Pathogenic by ACMG (rules PM3, PP1, PM2 and BP7). The third variant; c.491G>A had a frequency of 0.00000808 in gnomAD and was classified as Likely Pathogenic by rules PM2, PM5, and PM1. These three variants were not detected in exomes data of 1000 unrelated healthy Iranian individuals (<http://iranome.com/>). By using GermlineCNVcaller for CNVs (copy number variations) analysis, no pathogenic CNV was detected as a disease-causing variant in our probands.

The identified variants were co-segregated with the disease status in available members of families; affected individuals of each family carried the variants in the homozygous state, while unaffected individuals harbored at least one normal allele.

Discussion

RAPSN variants were initially described in recessive forms of CMS by Ohno et al. in.

2002 [12]. Rapsyn is a protein found in the postsynaptic region, has a molecular weight of 43 kilodaltons, and is necessary for AChRs clustering in the postsynaptic membrane [13].

This protein consists of various distinct regions that serve different functions; a myristoylated N-terminal which

Table 2 Molecular findings of *RAPSN*-related CMS patients (NM_005055.5)

Patients ID	Variant						
	cDNA level	Protein change	Exon	dbSNP	Zygosity	ACMG classification (Franklin)	Variant type
P1	c.264 C>A	p.Asn88Lys	2	rs104894299	Hom	pathogenic	missense/ known
P2	c.-210 A>G	-	5'UTR	rs786200905	Hom	likely pathogenic	regulatory/ known
P3	c.491G>A	p.Arg164His	2	rs374588028	Hom	likely pathogenic	missense/ known
P4	c.491G>A	p.Arg164His	2	rs374588028	Hom	likely pathogenic	missense/ known
P5	c.491G>A	p.Arg164His	2	rs374588028	Hom	likely pathogenic	missense/ known
P6	c.264 C>A	p.Asn88Lys	2	rs104894299	Hom	pathogenic	missense/ known

is essential for the interaction with the membrane, seven tetratricopeptide repeats that facilitate Rapsyn self-aggregation and its ability to bind to the cytoplasmic portion of the muscle-specific kinase (MuSK). Additionally, the coiled-coil domain interacts with the cytoplasmic loops of AChR subunits. Lastly, the C-terminal domain binds to the cytoskeletal protein beta-dystroglycan, which in turn links the Rapsyn-AChR complex to the cytoskeleton [14] (Fig. 3).

In this study, three different *RAPSN* variants were found. Variants c.264C>A and c.491G>A, both are located in exon 2 of the *RAPSN* gene and the TPR3 and TPR5 repeat of Rapsyn protein, respectively (Fig. 3). The variant p.Asn88Lys is a frequent cause of *RAPSN*-related CMSs in European patients [15] and is not frequent in non-European countries [16], but it is remarkable that we found this variant in two out of six patients (~33%). Patient 2 displayed another known variant, c.-210A>G, located in the promoter region of the *RAPSN* gene, which is 38 bp before the transcription start site. Therefore, -38A>G is the old annotation for c.210A>G. As it is reported, this variant can cause impaired transcriptional activities of the promoter region. The -38A>G promoter variant in the *RAPSN* gene has significant consequences for the expression and function of the RAPSIN protein, which is crucial in the clustering of acetylcholine receptors at the neuromuscular junction. This specific mutation alters the middle E-box sequence within the promoter, changing it from CA4CTG to CAGCTG. As a result, the core dinucleotide of the middle E-box becomes identical to that of the adjacent 3' E-box, potentially disrupting the binding affinity for transcription factors that regulate *RAPSN* expression. This may lead to impaired expression of *RAPSN*, resulting in insufficient clustering of acetylcholine receptors, which can contribute to the pathological mechanisms underlying congenital myasthenic syndrome [17]. Interestingly, the -38 A-G mutation is assumed to be a founder mutation in affected individuals of Persian and Iraqi Jewish origin [18].

It is essential to recognize that the p.Asn88Lys variant may not exclusively be attributable to a European founder

effect, as our findings indicate that this variant might also be present in Iranian populations, although potentially at lower frequencies. Additionally, both p.Arg164Cys and p.Asn88Lys variants, which were more prevalent in this study, are located in the early region of TPR (Tetratricopeptide repeat) domain. This suggests that this region may be a hotspot within the domain.

This realization paves the way for further exploration of the genetic landscape of *RAPSN*-CMS in Iran. To substantiate this hypothesis, we propose undertaking comprehensive genetic screening within Iranian CMS families, which could confirm or refute the prevalence of these variants in our population. Establishing a clearer genetic framework for *RAPSN*-CMS within this demographic is crucial, and we recommend implementing targeted screening for these three key variants in clinical settings. This approach could greatly contribute to the timely identification of *RAPSN*-CMS patients within our population, ultimately leading to more personalized and effective strategies for diagnosis and treatment. By focusing on the unique genetic characteristics of the Iranian population, we can enhance patient care, ensuring that it is informed by a deeper understanding of the underlying genetic factors associated with this condition.

CMS is frequently misdiagnosed or not diagnosed at all. This disorder closely mimics other neuromuscular diseases, and physicians may be less knowledgeable about them compared to other conditions. Additionally, some patients show few classic clinical signs that could lead to a CMS diagnosis, and not all medical facilities have the advanced EMG capabilities needed for accurate diagnosis [19]. The utilization of next generation sequencing (NGS) especially WES in diagnosing CMS highlights the technology's significance, same we used WES as a diagnostic tool during our cohort [20, 21]. Identifying the specific variant is critical, as treatment effectiveness varies based on the type of CMS; in fact, using acetylcholine esterase inhibitors can be harmful for certain forms of CMS. The clinical improvement of our patients demonstrates that WES was instrumental in both diagnosis and treatment. The expense of this technology is justified

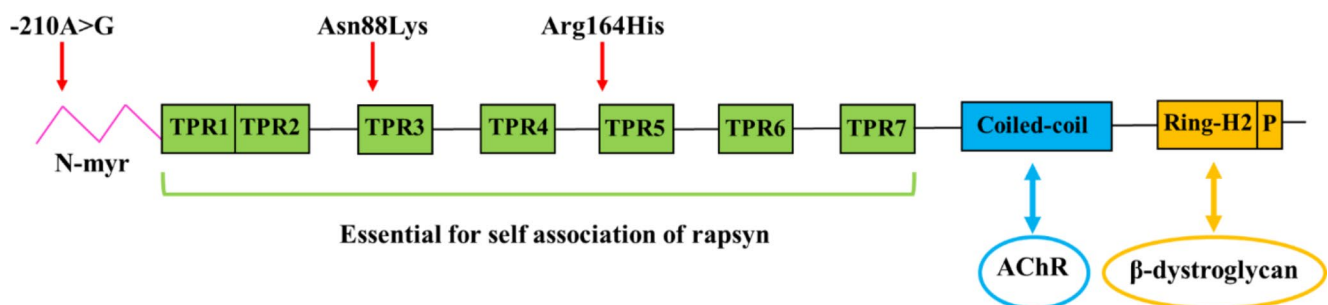


Fig. 3 This figure represents structure of the domains of Rapsyn with identified variants in this study. As it is shown, Rapsyn carries a myristoylation signal at the N-terminus which is required for membrane association and has seven tetratricopeptide repeats (TPRs) that sub-

serve self-association, a coiled-coil domain, responsible for interacting with AChR, a RING-H2 domain that binds to the cytoplasmic domains of β-dystroglycan and mediates the MuSK-induced phosphorylation of AChR, and a serine phosphorylation site

when considering the potential savings from reduced future diagnostic testing and avoided hospitalizations, making it a valuable option for patients with undiagnosed conditions suspected of having a genetic basis [19].

Clinical assessment

There is currently no genotype-phenotype correlation with *RAPSN* variants, and there is a significant heterogeneity in the severity of clinical manifestations even among patients harboring identical mutations [13]. In the present case report, we describe the clinical and molecular characterization of six cases of genetically confirmed CMS in Iranians caused by *RAPSN* variants. We have followed them for several years and reviewed the course of their disease as well as their response to treatment.

Clinical manifestations of this disease are most commonly present at birth or in infancy but may present as late as the fifth decade [22, 23]. Similarly, the symptoms in five of our patients appeared at birth, whereas one particular case experienced the onset of symptoms during her childhood (Table 1). Unlike some other studies like the report published in 2003 [23], we did not observe any cases of late-onset disease after the first decade.

Earlier studies have shown that the *RAPSN*-CMS case may present with a different severity [14, 22], as we also observed in our cases. A case (P1) was found to have only ocular symptoms. The severity of symptoms was mild to moderate in four of the patients (P1,2,3 and 5), while two of the cases experienced severe symptoms (P4, P6). One of the patients (P4), after discontinuation of treatment, became wheelchair-bound for a while. The course of our patients was steady with occasional worsening, which is consistent with cohorts from 2009 to 2016 [14, 22].

All patients of this study presented ocular symptoms with ptosis in all, along with diplopia in four (P1,2,4 and 5) (Table 1). The high prevalence of ocular symptoms is in accordance with the findings of Milone et al. [22].

Bulbar muscle weakness was reported in three of the patients (P1, P2, P6). Although all of the reported cases by Natera-de Benito D, et al. [14], had muscle hypotonia and sucking difficulties, only two of our cases showed that (P1,P6). Bulbar weakness leading to difficulty chewing was seen in two cases (P2, P6) which were mild to moderate similar to some of the previous studies [14, 22] and unlike severe dysphagia reported in the study by Ioos et al. 2004 [24].

As in the cohort of *RAPSN*-CMS in 2009, all patients (except P1 who had only ocular symptoms) had proximal weakness as severe as or more severe than distal weakness [22]. As described previously, the presence of out-of-proportion foot dorsiflexion weakness is characteristic of

the late-onset phenotype [23]; however, only in one of our cases, a mild atrophic Tibialis anterior was present with mild proximal weakness as well. There was a marked increase in muscular weakness during fever and infection in our cases, as reported in previous studies [24, 25].

CMS patients are known to exhibit phenotypic heterogeneity [14], as was seen in three of our cases (P3, P4, P5), and two others with a similar mutation (P1, P6). They presented with different symptoms, severity levels, and clinical course (Table 1). One of them had delayed motor milestone development (P5). We have discovered several new findings through this study. In addition to the pupil's slow reaction to light in P5, thin tongue in P2 has never been reported before. Despite experiencing episodes of difficulty breathing in all of our cases, they did not report episodes of apnea [22, 26, 27]. Contrary to this heterogeneity, there may be an overlap in signs and symptoms between the subtypes of CMS, since sluggish pupils and thin tongue that were seen in our patients, are more frequent in the *COLQ* and *DOK7* subtypes of CMS, respectively [20, 28].

Paraclinical assessment and response to treatment

The electrodiagnosis and laboratory workup were performed on all patients. For CMS diagnosis, a slow RNS with a decremental response of more than 10% or an abnormal single-fiber electromyography is required [9]. To exclude autoimmune myasthenia, anti-AChR and anti-MuSK antibodies should be tested. The *RAPSN*-CMS patients are seronegative [22, 23, 26, 29], as were our cases. In four cases, slow RNS showed a clear decremental response whereas in one case (P1), a mild decline was apparent, which is in agreement with the existing research (Table 1) [14, 22, 26].

The early detection of both early-onset and late-onset forms of *RAPSN*-CMS is crucial. Early-onset types respond significantly to treatment, whereas late-onset types may be misdiagnosed as MG or myopathy, resulting in unnecessary investigations or treatment with medications that may have adverse effects, such as corticosteroids or other immunosuppressive drugs [9, 22] as P2 and P4 had consumed corticosteroids without experiencing positive outcomes. The misdiagnosis of MG even resulted in two cases undergoing thymectomy.

AChE inhibitors, beta agonists, and 3,4-diaminopyridine were found to be effective in treating the patients [22, 24, 26]. Our patients responded favorably to the treatment with pyridostigmine and salbutamol. The symptoms of some patients deteriorated following the discontinuation or reduction of these drugs. While fluoxetine has been reported to worsen the *RAPSN*-CMS [30], in our experience, Escitalopram and Sertraline did not cause any deterioration. In agreement with reported data [14, 22, 26], our patients

on treatment remained stable, with some worsening due to precipitating factors. In one of the patients (P2), who had previously been stable, symptoms progressively worsened during her pregnancy. In light of this finding, it is even more important to closely monitor RAPSN-CMS patients during pregnancy. Further data is required to better understand the effect of pregnancy on RAPSN-CMS patients.

Conclusion

Our analysis of six Iranian families reveals diverse clinical manifestations of RAPSN-related CMS, contributing to the understanding of its genetic variations and supporting treatments like pyridostigmine. It underscores the need for comprehensive genetic testing for accurate diagnosis and management of CMS. This research advances knowledge and treatment of CMS in Iran and aids global efforts against this rare disorder.

Limitations

There were some limitations to this study. Since most of the patients were followed as MG by other general neurologists, we have not observed any of them since the early stages of their illness. We do not have any information about fetal problems in our patients. 3,4-Diaminopyridine is not easily available in our country and we have not prescribed it to patients and have no information about its effects. There were problems with follow-up since the patients came from far away provinces and regular follow-up was impossible. Some patients had poor adherence to salbutamol and we did not assess its effects properly.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval 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.

Competing interests The authors declare no competing interests.

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