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CASE REPORT

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Core myopathy in two siblings with a biallelic variant in the CACNA1S gene—A case series study

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Key Clinical Message

Homozygous variants of Calcium Voltage-Gated Channel Subunit Alpha1 S (CACNA1S) gene mutation were previously identified as causes of periodic paralysis and congenital early-onset myopathy, while it could be manifested as a late-onset congenital core myopathy.

Abstract

Calcium Voltage-Gated Channel Subunit Alpha1 S (CACNA1S) gene mutation has been linked to various neuromuscular conditions in recent years. Congenital myopathy with core-like features is one of the cardinal associations reported previously, causing severe respiratory insufficiency and death in neonates. Informed consent was received from the patients. Subsequently, peripheral blood leukocytes were utilized to extract genomic DNA. Moreover, exome enrichment was implemented through the Twist Human Core Exome Kit (Twist Bioscience) and exome sequenced using Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Sanger sequencing using BIG Dye Terminators confirmed the presence of the final variant. Finally, the candidate variants were classified based on the American College of Medical Genetics and Genomics (ACMG) guidelines. In this report, we describe two siblings, who presented with childhood and late-onset progressive muscle weakness, and had a homozygous variant in exon 2 of the CACNA1S gene defined as c.188C>A (p.Ala63Asp) (NM_000069.3). The SIFT, Polyphen2, CADD PHRED, and Mutation Taster analysis tools classified the variant as pathogenic/damaging. The muscle biopsy of the younger brother revealed intermyofibrillar network pattern disruption as cytoplasmic core-like lesions. The muscle magnetic resonance imaging (MRI) reported grade IIa and IIb fatty changes. Finally, the electromyography (EMG) findings suggested a myopathic change pattern. This report illustrates the clinical variability in CACNA1S-related myopathy by reviewing prior reports and adding newly found aspects, additionally expanding the gene defects associated with core myopathy.

KEYWORDS
CACNA1S, calcium voltage-Gated Channel subunit Alpha1 S, congenital neuromuscular disorders, core myopathy

1 | INTRODUCTION

Congenital myopathies are a large group of genetic disorders characterized by slowly progressive muscle weakness, which could be detected immediately after birth to late adulthood with enormous disabilities. The congenital myopathies have been classified based on the histopathological features into five main groups: Nemaline myopathy, Core myopathies, Centronuclear myopathies, Myosin storage myopathy, and Congenital fiber-type disproportion. Each of these categories shared clinical features. Although many genes can cause the same clinical features, a single gene may also demonstrate heterogeneous phenotypes.[1] Rapid advances in genetic screening for congenital neuromuscular disorders signified new therapeutic options.

The CACNA1S (Cav1.1) gene encodes the α1s subunit of the dihydropyridine receptor (DHPR), a voltage-gated calcium channel at the T-tube with a critical role in the excitation–contractions coupling (ECC). ECC occurs at the triad, formed by the interaction between the T-tubule and the sarcoplasmic reticulum (SR). The DHPR in the T-tubule and the ryanodine receptor (RYR) in the SR interfaced lead to calcium release which participates in a variety of cellular processes, especially, muscle contractions.[2,3] CACNA1S mutations were linked to malignant hyperthermia susceptibility 5 [MIM 601887], Hypokalemic Periodic Paralysis type 1 [MIM 170400], hyperCKemia, exercise-induced rhabdomyolysis, thyrotoxic periodic paralysis susceptibility 1(TPP) [MIM188580], and statin-associated myopathy. Recently a new phenotype has been associated with mono or biallelic variants in the CACNA1S gene. It was demonstrated by congenital hypotonia, progressive muscle weakness, respiratory insufficiency, ophthalmoplegia, and cognitive delay. In addition, there were symptoms of mild facial muscle weakness and oropharyngeal involvement. Magnetic resonance imaging (MRI) could help in the differential diagnosis.[4] Besides, muscle biopsy was highlighted with an alveolar aspect of the intermyofibrillar network on NADH-TR staining, centralized nuclei, fiber size variability, core-like features, uniformity of type 1, and a dystrophic process.

Here, two siblings from an Iranian family with a biallelic variant in CACNA1S were reported. One of them presented with late-onset progressive muscle weakness. To the best of the author's knowledge, few reports have been found so far for *CACNA*-related core myopathy and far too little attention has been paid to it. The clinical features of our cases were milder than most of the previously reported cases. Both cases had no intellectual disability and mild or no facial weakness. One of our cases had milder respiratory involvement, no ophthalmoplegia, or oropharyngeal muscle weakness. Therefore, the current report attempts to expand the clinical variability in CACNA1S variants associated with core myopathy.

2 | CASE

Proband IV-3 (Figure 1A) is a 29-year-old female with proximal muscle weakness for 6 years. She had difficulty in rising from a seated position and climbing stairs. However, there was normal development during infancy, childhood, and early adolescence. Symptoms related to bulbar weakness or exercise intolerance were not observed. Neurological examination revealed normal visual acuity and fundi. Besides normal sensory examination, hip flexors, knee extensor, and shoulder abductor demonstrated mild muscle weakness (Medical Research Council Scale for Muscle Strength [MRCMS] score:4/5), whereas the bulbar and other limb muscles were at normal strength. Upper and lower extremities' deep tendon reflexes were defined as 1 plus. The plantar response was flexor. There was no appreciable fatigue from repeated efforts. All the laboratory examinations including CK were in the normal range, in addition to the normal nerve conduction study (NCS) (Table 1). In electromyography (EMG) findings, myopathic motor unit action potential (MUAPs) without any spontaneous activity were observed suggesting a myopathic change pattern (Table 2). The T1-weighted images were used to grade a 6-point Mercuri scale representing muscle fatty changes.[5] The muscle MRI taken from proximal muscles of the upper extremities demonstrated grade IIa fatty changes (early confluence) in the biceps, triceps, brachialis, subscapularis, and deltoid muscles. In the distal muscles of the upper extremities grade IIb (fatty infiltration 30%–60%) was noted. Lower extremities proximal and distal muscles MRI fatty changes were similar to upper extremities. In addition, semimembranosus and biceps femoris were hypertrophied (Figure 2).

Proband IV-1 is a 15-year-old brother of Proband IV-3 (Figure 1A). He had a history of chronic and slowly progressive muscle weakness with onset in his childhood, therefore he was unable to participate in physical

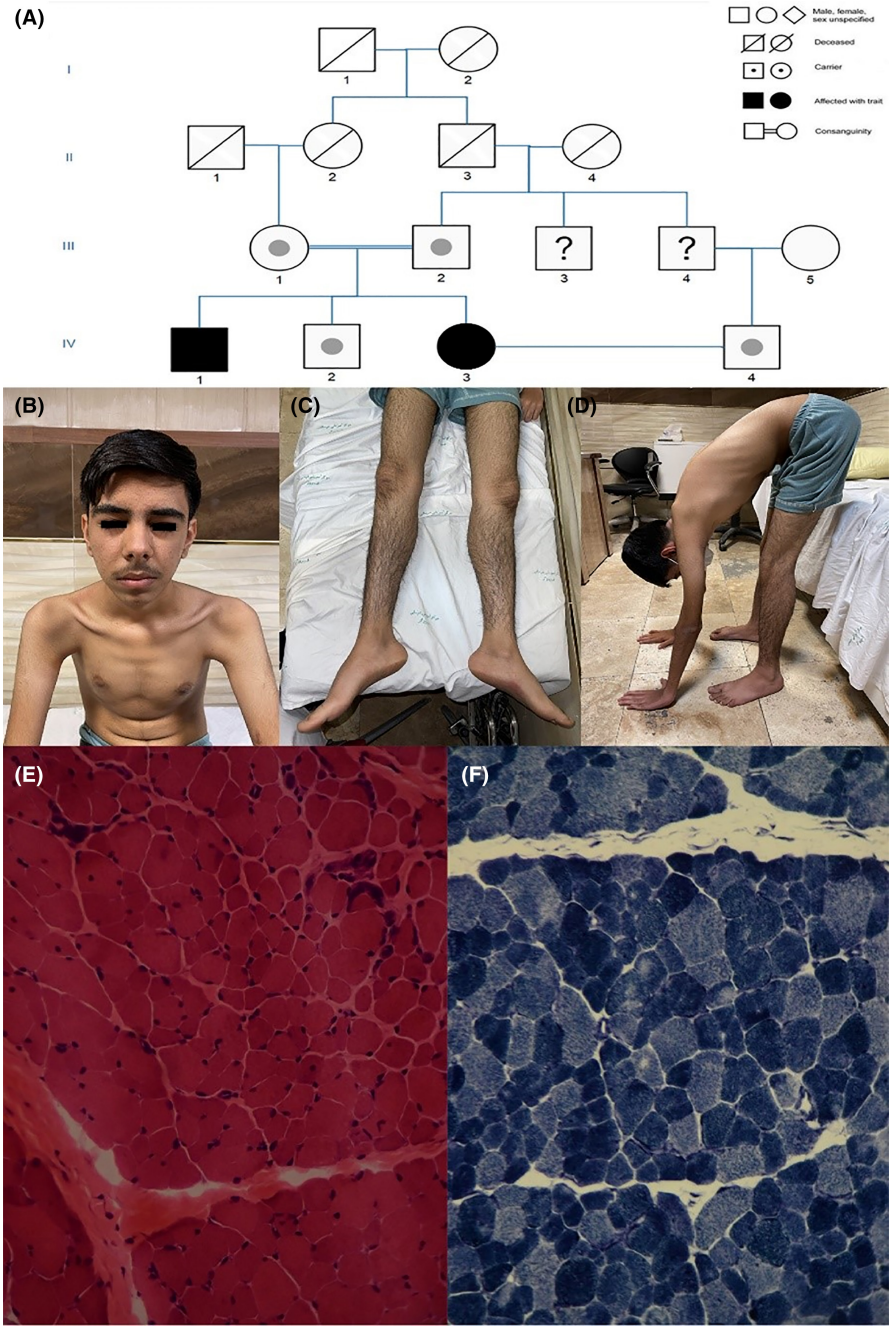

FIGURE 1 (A) Proband IV-1 pedigree (B) mild facial weakness (C) muscle wasting (D) spine hyperlaxity are the main phenotypic findings. Structural abnormalities are slight myopathic atrophy with fiber size variation and round atrophic fibers and a few centralized nuclei muscle fibers (less than 3 per 100 muscle fiber) (E) (H&E ×400), Good differentiation of muscle fibers with intermyofibrillar network disruption as the presence of core lesions in some dark type 1 fibers (F; NADH-TR ×400).

activities like other same-aged children. He walked independently at 2 years old. Aside from mild episodic swallowing problems and dyspnea, his mental and fine motor skills developed normally. On physical examination, his body mass index (BMI) was 17, and miniature muscles, high arch palate, bilateral ptosis, and facial weakness were detected (Figure 1B,C). There was notable weakness in neck flexor, pelvic, and shoulder girdles muscles with marked difficulties in rising from a chair. MRCMS-scores were: shoulder abduction: 3/5, forearm flexion: 4/5, forearm extension: 3/5, wrist flexion: 4/5, wrist extension: 3/5, fingers flexion: 4/5, fingers abduction: 4/5, fingers extension: 3/5, forearms pronation: 3/5, thigh flexion: 3/5, thigh extension: 3/5, thigh abduction: 3/5, leg flexion: 4/5, leg extension:5/5, foot flexion: 4/5, and foot extension: 3/5, all symmetrically. There was evidence of ligamentous laxity in his spine, which was depicted by an increased range of motion known as hypermobility without complaints of back pain, tingling, or numbness (Figure 1D). His laboratory tests and EMG-NCS were similar to his sister's (Proband IV-3).

An open muscle biopsy was undertaken from the left biceps of Proband IV-1 and showed type 1 fiber predominance and atrophy associated with intermyofibrillar network pattern disruption as cytoplasmic core-like lesions. Slight endomysial fibrosis was seen around some round

TABLE 1 Nerve conduction study of the proband IV-3.

Sensory					
Nerve	**Onset latency (ms)**	**Peak latency (ms)**	**Negative peak amplitude (μV)**	**Peak to peak amplitude (μV)**	**Velocity (m/s)**
Left median	2.60	3.23	31.5	60.4	54
Right Ulnar	1.93	2.50	57.4	37.8	57
Left sural	2.60	3.28	26.2	24.5	54
Right superficial peroneal	1.61	2.40	26.3	35.1	67
Motor					
Nerve	Latency (ms)	Amplitude (mV)	Duration (ms)	Latency difference (ms)	Velocity (m/s)
Left median (Wrist)	2.92	6.2	7.81	-	-
Left median (Elbow)	6.15	6.2	8.07	3.23	61
Right median (Wrist)	2.81	5.0	7.34	-	60
Right ulnar (Wrist)	2.34	7.2	5.73	-	-
Left peroneal (Ankle)	3.07	1.3	6.67	-	-
Left peroneal (Fib head)	9.32	1.1	6.67	6.25	50
Left tibial (Ankle)	4.90	6.3	5.05	-	-
Left tibial (Pop fossa)	11.88	5.3	4.74	6.98	48
Right tibial (Ankle)	4.90	8.8	5.52	-	-

TABLE 2 Electromyography of proband IV-3.

	Spontaneous					**MUAP**			**Recruitment**
	IA	**Fib**	**PSW**	**Fasc**	**H.F.**	**Amp**	**Dur**	**PPP**	**Pattern**
Right Deltoid	Normal	None	None	None	None	Normal	1-	2+	1+
Right Biceps brachii	Normal	None	None	None	None	Normal	1-	1+	Reduced
Right Extensor digitrum communis	Normal	None	None	None	None	Normal	1-	2+	Reduced
Right first dorsal interosseous	Normal	None	None	None	None	Normal	1-	2+	Reduced
Left Gluteus maximus	Normal	None	None	None	None	Normal	1-	2+	1+
Left Quadriceps	Normal	None	None	None	None	Normal	1-	2+	Reduced
Left Tibialis anterior	Normal	None	None	None	None	Normal	1-	2+	Reduced
Left Gastrocnemius (Medial head)	Normal	None	None	None	None	Normal	1-	2+	Reduced

Abbreviations: Amp, amplitude; Dur, duration; Fasc, fasciculation; Fib, fibrillation; H.F, harmonic focus; IA, insertion activity; MUAP, motor unit action potential; PPP, polyphasic potential; PSW, positive sharp wave.

atrophic fibers (Figure 1E,F). The above histochemical pathologic findings were compatible with congenital myopathy with cores.

3 | MATERIALS AND METHODS

After receiving written, informed consent, genomic DNA was extracted from peripheral blood leukocytes using the salting out method.[6] Twist Human Core Exome Kit (Twist Bioscience) was used for exome enrichment. The captured libraries were sequenced on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Following the FastQC quality check, the reads were aligned to the human genome assembly GRCh37 (hg19) using the Burrows-Wheeler Aligner (v0.7.17).[7] After trimming the SAM files with Picard tools (v2.21.8), the BAM files were aligned with the GATK (v4.14.1).[8] The identified variants were then annotated using the ANNOVAR tool.[9]

The tertiary analysis focused on the 626 genes associated with neuromuscular disorders. Our in-house-developed bioinformatics pipeline was used for variant analysis. Filtering out frequent variants (based on a minor

allele frequency [MAF] higher than 0.01) using public variant frequency databases such as TopMed, NHLBI GO Exome Sequencing Project, the database of single nucleotide polymorphisms (dbSNP), GnomAD browser, and the ethnicity-specific database, Iranome (http://www.iranome.ir), was part of the analysis pipeline. Variants were prioritized based on gene ontology, clinical significance, and bioinformatics prediction scores. The mean depth of coverage for the exons of the human genome based on CCDS Release 23 was 177.75× with 97.7% and 97.3% coverage at 10× and 20×, respectively. The candidate variants were then classified based on the American College of Medical Genetics and Genomics (ACMG) guidelines.[10] Sanger sequencing using BIG Dye Terminators confirmed the presence of the final variant.

4 | RESULTS

Whole exome sequencing and variant data analysis revealed a homozygous variant in exon 2 of the CACNA1S gene defined as c.188C > A (p.Ala63Asp) (NM_000069.3). Sanger sequencing confirmed the presence of the above-mentioned homozygous variant in two probands of the family and the heterozygous state for the parents (Table 3). Neurological examinations didn't demonstrate any abnormality in heterozygote family members. This variant has not been observed in the population databases (NHLBI Exome Variant Server, gnomAD, TOPMed) and in-house population database (Iranome), indicating it is not a common benign variant in these populations. There is no report for this variant in Clinvar.

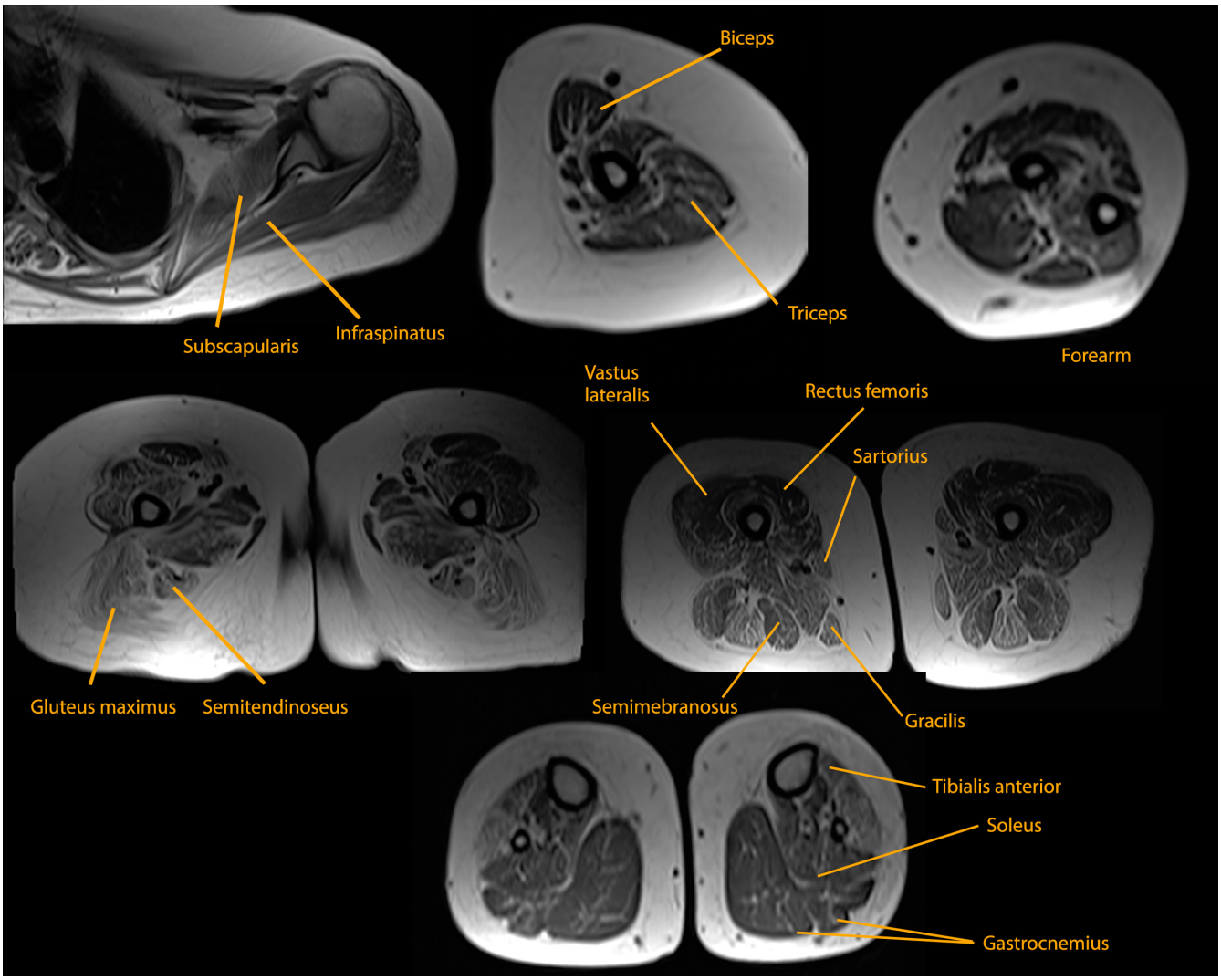

FIGURE 2 T1-weighted Muscle MRI of the Proband IV-3 in the axial view.

TABLE 3 Characteristics of the identified variant in this study.

Gene	HGNC symbol: CACNA1S	
	Ensembl version: ENSG00000081248.10	
	Genomic location: chr1 (hg38): 201039512 – 201112566 [negative strand]	
	Cytogenetic location: 1q32.1	
Transcript coordinates	Ensembl transcript ID: ENST00000362061.4	
	MANE: NM_000069.3	
	Transcript Support Level (TSL): 1	
	APPRIS: P2	
UniProt peptide	Q13698	
Identified Variant	Type: Single nucleotide variant, Zygosity: Homozygous	
	Variant length: 1 bp	
	Consequence: missense variant	
	RefSeq: NM_000069.3(CACNA1S):c.188C>A:p.Ala63Asp	
Alteration position	Chromosome: chr1:201110234G>T	
	gDNA: g.2333C>A	
	cDNA: cDNA.415C>A	
	CDS: c.188C>A	
	Exon: 2/44	
	AA: Ala63Asp	
Sequence snippet	Original gDNA	CATCTTGCTCACCATCTTTG[C]CAATTGTGTGGCCCTGGCCG
	Altered gDNA	CATCTTGCTCACCATCTTTG[A]CAATTGTGTGGCCCTGGCCG
	Original cDNA	CATCTTGCTCACCATCTTTG[C]CAATTGTGTGGCCCTGGCCG
	Altered cDNA	CATCTTGCTCACCATCTTTG[A]CAATTGTGTGGCCCTGGCCG
	wt-AA sequence	KPFETIILLT IF[A]NCVALAV
	mu-AA sequence	KPFETIILLT IF[D]NCVALAV

Abbreviations: AA, Amino Acid; APPRIS, Annotating principal splice isoforms; CDS, Coding sequence; Cys, Cysteine; Gly, Glycine; HGNC, HUGO Gene Nomenclature Committee; MANE, The Matched Annotation from the NCBI and EMBL-EBI; mu, mutated; wt, wildtype.

The pathogenicity of the mutation was suggested as a deleterious mutation with 0.001, 0.943, 26.6, and 0.99 scores for SIFT, Polyphen2, CADD PHRED, and Mutation Taster. Moreover, the PhyloP and PhastCons were specified 188C as a highly conserved nucleotide in the sequence of this gene (Table 3). Eventually, the proteomics analysis for evaluating the protein structure changes after mutation reported less stability and high changes in the alpha fold of the protein structure with −0.64 Kcal/mol calculated for ΔΔG and 126 for amino acid change score, which suggested that this mutation could destabilize the protein structure. These results were confirmed by pLDDT analysis, which suggested Ala63 as a crucial amino acid in the CACNA1S structure (Figure 3).

CACNA1S gene pathogenic and likely pathogenic variants have been linked to Hypokalemic periodic paralysis type 1 [MIM#170400], Malignant hyperthermia susceptibility 5 [MIM#601887], and TPP susceptibility 1 [MIM#188580]. In addition, heterozygous, compound heterozygous, and homozygous variants of the CACNA1S gene were identified as causes of congenital early-onset myopathy[11–13] (Table 4).

5 | DISCUSSION

In recent years, with easier access to genetic testing, many causative genes for congenital myopathy have been detected. Recently, there have been a few articles that identified mutations in the CACNA1S genes and reported 15 congenital myopathy patients from nine families.[11–13] Mutations are segregated through recessive or dominant modes of inheritance. Four patients from three families showed autosomal dominant inheritance

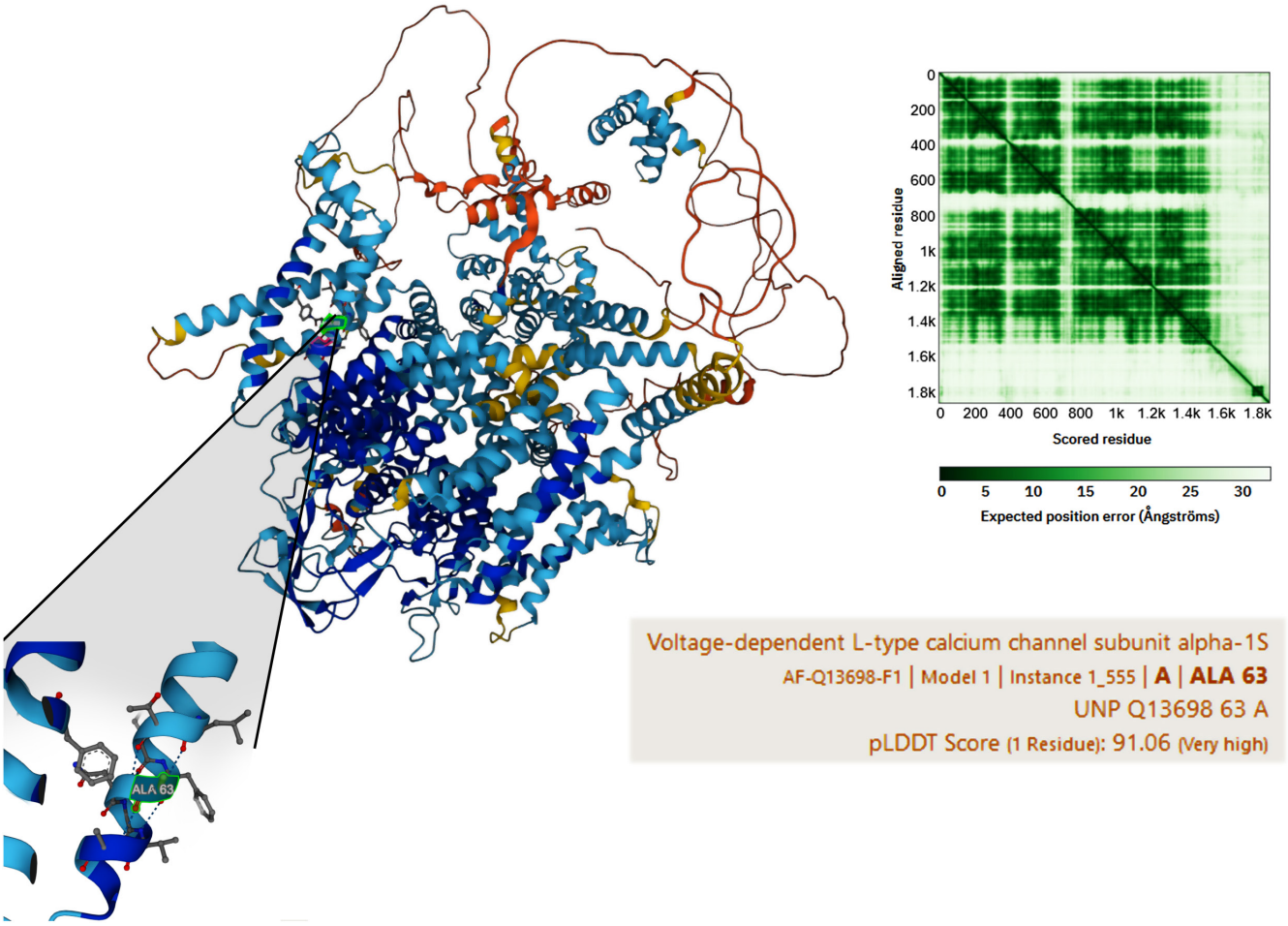

FIGURE 3 pLDDT analysis of the CACNA1S protein. The predicted aligned error is depicted in the green chart.

and the remaining 11 patients from six families showed autosomal recessive inheritance. There was not any significant difference in clinical phenotype with recessive or dominant inheritance. Onset was before 1 year of age in 12 out of 15 patients and only three of the previously reported patients had onset of symptoms during childhood. Cases of this report have had an onset of disease at 23 years for proband IV-3, and during childhood for proband IV-1. Perhaps most importantly, CACNA1S-associated core myopathy has not been reported in adults. Severe respiratory insufficiency is a major finding in previously reported patients causing early death in two patients. To manage the respiratory complications mechanical ventilator was used, nevertheless, the respiratory insufficiency was so severe in some cases resulting in being taken to the neonatal intensive care unit. However respiratory complications in our cases are considerably milder, detectable only by extensive pulmonary tests with moderate involvement in FVC, FEV1, and abnormal apnea tests. Besides, in the Yis et al study, pes equinus deformity was reported in all three cases, while it was not presented in our cases. Mild facial weakness and oropharyngeal involvement were consistent features of previous patients, both were present in the IV-1 subject, manifested by intermittent difficulty in swallowing. In the previous cases mentioned by Yis et al, the swallowing problems were managed using percutaneous gastric feeding tube and tracheostomy. A high-arched palate was seen in almost all previously reported cases (13/15) and was present in patient IV-1. Ptosis was seen in one other patient besides the proband IV-1. One striking finding was hyperlaxity in the spine which was not reported in the previous cases. Scoliosis and ophthalmoplegia were seen in several patients previously and were absent in all examined members of this family. In all the cases there was no cardiac involvement and only two patients had elevated serum creatine kinase values (Table 5). Moreover, among clinical presentations of previous core myopathy due to CACNA1S mutation absent suck, deep tendon reflexes, and Moro reflex were noted.

In the prior reported case series, histochemical pathologic analyses of muscle biopsies showed various morphopathological features mainly classified as a form

TABLE 4 Results of in silico analysis and pathogenic prediction tools.

Analysis	Tools and in silico parameters	Score (criteria)	Result/classification
Pathogenicity	MetaRNN	0.872	Between 0.841 and 0.939 (moderate pathogenic)
	SIFT	0.001	Deleterious/ low confidence
	Mutation Taster	0.99	Disease-causing
	Polyphen2	0.943	Probably Damaging/ pph2_prob
	ACMG rules	PVS1, PM2, PP3, PP4	Pathogenic/Class I
	CADD PHRED	26.6	Pathogenic
Conservation	PhastCons	1.00	Highly conserved/buried residue
	PhyloP	6.464	
	PhyloP100way_vertebrate	6.464	
	PhyloP17way	0.675	
	SiPhy_29way_logOdds	11.297	
Protein stability	I-Mutant 2.0	ΔΔG: −0.64Kcal/mol	ΔΔG < 0 /destabilizing mutation
	Amino acid change score	126	Ranging from 0 to 215

Abbreviations: ACMG/AMP, American College of Medical Genetics and Genomics/Association for Molecular Pathology; D, Deleterious; PMut, Pathogenic Mutation prediction.

of congenital myopathy or mild dystrophic-like process (Table 6). Different forms of congenital myopathies, that have been reported before by mutation in this gene, are composed of nemaline myopathy, core myopathies, centronuclear myopathy, and congenital fibers type disproportion. The alveolar aspect of the intermyofibrillar network pattern on NADH-TR staining and uniformity of type 1 fibers, endomysial fibrosis, as well as grouping of large and small size fibers, suggestive of denervation with reinnervation, are also reported before.[12,13] In our case, the muscle biopsy of Proband IV-1 showed histopathologic findings compatible with congenital myopathy with cores.

Other congenital myopathies that could mimic the clinical presentation of the core myopathy are congenital fiber-type disproportionate, nemaline, myotubular, and mitochondrial myopathies. Congenital fiber-type disproportionate and myotubular myopathies are characterized by joint deformities, weakness, and respiratory problems. Nemaline myopathies are presented by respiratory problems, weakness, and hypotonia. Mitochondrial myopathies often present by weakness, ptosis, and development delays. While congenital myopathies share similar symptoms, diagnosis mainly relies on muscle biopsy, as the gold standard, and genetic tests.[14]

Although the phenotype is more severe in the younger brother, both patients had a biallelic variant in the CACNA1S gene. In this study, the p.Ala63Asp variant in the CACNA1S gene was found as a homozygous state in two probands of a consanguineous Iranian family. At the same position, the other mutation (p.Ala63Ser) has been reported as a variant of uncertain significance in ClinVar (RCV000397333). Based on PhyloP100way and GERP prediction tools Ala63 is a highly conserved residue (PhyloP100way = 6.279, GERP = 4.86). The majority of recessive inheritance previously reported cases were the result of compound heterozygote variants, which include frameshift mutations located at various sites of the protein and missenses in the pore-forming or voltage-sensing domains. All these patients had substantial axial involvement, early-onset hypotonia, and increasing muscular weakening.[12] The only previously reported homozygous missense mutation in myopathy patients was (p.Arg789His). This family's probands had early onset severe myopathy, and only one proband is still living. Two of the probands died of respiratory insufficiency in their third month of life. The patient who survived also showed scoliosis, congenital pes equinus deformity, and mild cognitive impairment.[13] The dihydropyridine receptor's α1s subunit (an 1873 amino-acid protein) is encoded by the CACNA1S gene. This subunit

TABLE 5 Clinical features of reported patients with CACNA1S-related myopathy.

Study	Hunter et al., 2016	Schartner et al., 2016	Yis et al., 2019	Present cases	
Case	**Case 1**	**Case 2–12**	**Case 13–15**	**Case 16 (Proband IV-3)**	**Case 17 (Proband IV-1)**
Sex	M	7 M, 4 F	1 M, 2 F	F	M
Age at examination	9 months	5–60 years	3 months-5 years	29 years	15 years
Demise	–	–	2 × 3 months 1 × alive at 5 years	–	–
Perinatal complications	In vitro fertilization conception	3 x Reduced fetal movement	1 x Reduced fetal movement 2 x respiratory failure at birth	–	–
Age of onset	Neonatal	3 x antenatal 4 x neonatal 1 infancy 3 x childhood	3 x Antennal/ neonatal	23 years	Childhood
Dysmorphism	Dolichocephaly, high arched palate, micrognathia	11/11 High-arched palate 1/11 ptosis	1 x high-arched palate	–	High arched palate ptosis
Hypotonia	+	11/11	3/3	–	NA
Developmental delay	+	9/11	3/3	–	+
Contracture	Flexion In Lower Limbs	NA	3 x Pes equinus	–	–
Muscle wasting	–	7/11	NA	–	–
Facial weakness	+	11/11 mild	+	–	+
Oropharyngeal weakness	+	8/11 swallowing difficulties	3 x swallowing difficulties	–	swallowing difficulties
Neck weakness	+	NA	NA	–	+
Limb weakness	+	11/11	+	+	+
Scoliosis	–	6/11	1 x scoliosis	–	–
Gait	NA	NA	NA	Normal	Normal
Ophthalmoplegia	+	4/11	3/3	–	–
DTR	NA	NA	1 x absent, 2 x NA	+1	+1

(Continues)

TABLE 5 (Continued)

Study	Hunter et al., 2016	Schartner et al., 2016	Yis et al., 2019	Present cases	
Case	**Case 1**	**Case 2–12**	**Case 13–15**	**Case 16 (Proband IV-3)**	**Case 17 (Proband IV-1)**
Respiratory complications	labored and paradoxical breathing	6/11	3/3	abnormal PFT	Intermittent dyspnea+ abnormal PFT
CPK	<400	Elevated in 1/11	Normal	Normal	Normal
EMG	NA	NA	Myopathic changes	Myopathic changes	Myopathic changes
Muscle MRI	NA	4 x muscle atrophy 2 x fatty replacement 6 x anterior involvement	NA	Grade IIa fatty changes	NA
Cognition	Normal		1 x Cognitive delay, 2 x NA	Normal	Normal
Other			Brain MRI: mild ventricular enlargement, thin corpus callosum.		Hyperlaxity in spine

Abbreviations: F, female; M, male; NA, not available; PFT, pulmonary function test.

TABLE 6 Histological findings and genetic variants of patients with variants in CACNA1S with core myopathy.

Study	Hunter et al., 2015	Schartner et al., 2016							Yis et al., 2019	Present case
Case	1	2	3	4,5	6	7–9	10	11–12	13–15	16 (Proband IV-1)
Muscle Biopsy	Considerable myofiber size variation of both type I and type II myofibers, polygonal small and large fibers, and occasional internal nuclei. COX enzymatic activity staining demonstrated moderate architectural alterations in the form of coarse whorled fibers. EM revealed normal appearance, abundance, and distribution of mitochondria; no rods or cores.	Centralized nuclei; focal disorganization; fiber size variability; alveolar aspect of the intermyofibrillar network Uniformity of type I fibers	Centralized nuclei; focal disorganization; fiber size variability; alveolar aspect of the intermyofibrillar network Uniformity of type I	NA*	Fiber size variability endomysial connective tissue around most fibers. The predominance of type I fiber	Fiber size variability; alveolar aspect of the intermyofibrillar network Predominance of type I fiber	Rare internalized nuclei. Fiber size variability; alveolar aspect of the intermyofibrillar network Uniformity of type 1 fibers	Internalized nuclei; core-like structures; fiber size variability; endomysial fibrosis	Marked variation in fiber size and shape; increased nuclear internalization. grouping fascicules of large and small myofibers	Type 1 fibers predominance and atrophy with intermyofibrillar network pattern disruption as cytoplasmic core-like lesions Slight endomysial fibrosis round atrophic fibers
Variants	c.4947delA/ c.3795G > T	c.1189_1190del/ c.4967del	c.4453C > T/ c.4967del	c.825C > A/ c.2371delC	c.298G > T/ c.3795G > T	c.2225C > A	c.2224C > T	c.4099C > G	c.2366G > A	c.188C > A
mutation	Compound heterozygote	Compound heterozygote	Compound heterozygote	Compound heterozygote	Compound heterozygote	Dominant de novo	Dominant de novo	Dominant de novo	homozygote	homozygote

*Not available.

is composed of six transmembrane segments in each of its four homologous domains (I–IV).[15] The p.Ala63Asp is located in the first ion transport domain (50–345) of the CACNA1S protein whereas the location of (p.Arg789His) is cytoplasmic loop II-III of Cav1.1, which is essential for conveying the excitation-contraction coupling to Ca^{2+} release by the gating of RYR1. Comparing the location of these two variants can confirm the difference in phenotypic severity between our probands and p.Arg789His.

Currently, there are no possible treatments for core myopathy. One of the suggested treatments is targeting ECC as the theoretical pathophysiology of core myopathies. Accordingly modulating calcium channels to prevent muscle damage by reducing excess calcium influx. In addition, decreasing depletion of the SR is another possible treatment strategy. Eventually, since there is a significant decrease in Ca^{2+} release induced by KCl depolarization, increasing the amount of Ca^{2+} release either by receptor agonists or protein engineering is another possible treatment strategy for managing central core myopathy.[12] Other novel techniques mentioned correction of transcription from missense mutation techniques including missense-correcting tRNAs, transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeat (CRISPR)–Cas-associated nucleases.[16,17]

6 | CONCLUSION

In conclusion, we have characterized the late-onset homozygote CACNA1S congenital myopathy, with distinct clinical features. One of our patients presented with a lesser degree of muscle weakness, oropharyngeal involvement, and respiratory symptoms than previously reported cases. The identification of the CACNA1S mutation allows for improved molecular diagnosis, genetic counseling, and prognosis not only for early-onset myopathy but in adults with Limb-girdle type of weakness or core myopathy. It also points to CACNA1S and ECC as therapeutic targets for developing treatments which could be facilitated by the already extensive knowledge accumulated on DHPR.

AUTHOR CONTRIBUTIONS

Tara Khoeini: Resources; writing – original draft; writing – review and editing. **Ariana Kariminejad:** Formal analysis. **Yalda Nilipour:** Investigation. **Armin Ariaei:** Formal analysis; writing – review and editing. **Hossein Najmabadi:** Formal analysis. **Mojtaba Arabshahi:** Investigation. **Mehrshid Faraji Zonooz:** Formal analysis. **Bahram Haghi Ashtiani:** Project administration; resources; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest concerning this research and.

DATA AVAILABILITY STATEMENT

The data were available upon reasonable request from the corresponding author.

CONSENT

The written informed consents were obtained from the patients in advance of writing the manuscript.

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