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CACNA1S-associated triadopathy presenting with myalgia, muscle weakness, and asymptomatic hyperCKemia

Si Luo, Min Zhu, Dandan Tan, Yusen Qiu, Meihong Zhou and Daojun Hong

***Abstract*:** *CACNA1S* variants can alter the structure and function of the calcium channel, resulting in abnormal calcium influx and homeostasis. It is well established that pathogenic variants in *CACNA1S* can lead to hypokalemic periodic paralysis, malignant hyperthermia, and congenital myopathy. Nevertheless, the clinical presentations and disease progression of exertional myalgia and weakness associated with *CACNA1S* variants remain elusive. In this study, four affected individuals from an autosomal-dominant family were described, exhibiting symptoms of severe exertional myalgia, followed by flaccid weakness or rhabdomyolysis, along with asymptomatic hyperCKemia during the interictal period. Long exercise test showed a late decrease in compound muscle action potential amplitude. Muscle MRI revealed edema-like changes in the early stage, and fatty degeneration and substitution in prolonged disease courses, while closely aligned with the features of chronic myopathy. Ultrastructural examination revealed dilation of the sarcoplasmic reticulum and myofibrillar structural disarrangement. Genetic screening identified a c.3724A>G (p.Arg1242Gly) mutation in the *CACNA1S* gene. A literature review revealed that 15 patients exhibited the exertional myalgia and weakness phenotype associated with CACNA1S mutations, presenting similar clinical, electrophysiological, radiological, and pathological features. As the disease progressed, these patients developed severe muscle weakness, ultimately leading to wheelchair dependency. This exertional myalgia-weakness phenotype represented a unique *CACNA1S*-related phenotype that broadened the spectrum of *CACNA1S*-associated myopathy, bridging between periodic paralysis and congenital myopathies. The similarities between *CACNA1S*-associated myalgia-weakness and RyR1-associated myalgia-weakness underscored a shared pathogenesis of excitatory-contractile coupling at the triad of skeletal muscle.

Plain language summary

CACNA1S-linked muscle disorder with pain, weakness, and asymptomatic high creatine kinase levels

The *CACNA1S* gene is linked to several muscle conditions, including hypokalemic periodic paralysis (HypoPP), malignant hyperthermia, and congenital myopathy. In this study, four people from a family with an inherited pattern of the disease were examined. They experienced severe muscle pain after exercise, followed by muscle weakness or muscle breakdown (rhabdomyolysis), and had elevated levels of a protein called CK in their blood when they were not having symptoms. Genetic testing found a mutation (change) in the *CACNA1S* gene: c.3724A>G (p.Arg1242Gly). A review of other cases showed that 15 people with similar symptoms—exercise-related muscle pain and weakness—also had mutations in the *CACNA1S* gene. These patients showed similar signs in tests of their muscle function, imaging scans, and tissue samples. This pattern of muscle pain and weakness is a new type of *CACNA1S*-related muscle disease, expanding our understanding of how mutations in this gene can cause different kinds of muscle problems. It helps link conditions like periodic paralysis and congenital myopathies.

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Introduction

Triadopathy is a group of muscle disorders that affect the structure and function of the triad, which consists of the transverse tubule (T tubule) membrane and the two flanking terminal cisternae of the sarcoplasmic reticulum (SR). This essential substructure of skeletal muscle is responsible for regulating excitation–contraction coupling.[1] At the triad, electrical impulses travel down in the membrane and activate the dihydropyridine receptor (DHPR) in the T-tubule, resulting in the opening of the skeletal muscle ryanodine receptor (RyR1) calcium release channels in the terminal SR.[2]

Malignant hyperthermia, central core myopathies, centronuclear myopathies, and tubular aggregate myopathy are potential phenotypes of triadopathies, depending on the different pathogenic genetic origins.[3] Among the genetic forms, *CACNA1S* encodes for the skeletal muscle type of Cav1.1 protein which is the pore-forming subunit of the DHPR. *CACNA1S* variants can alter the structure and function of the DHPR channels, resulting in abnormal calcium influx and homeostasis.[4,5] Pathogenic variants of *CACNA1S* are mostly known to cause hypokalemic periodic paralysis (HypoPP). However, some pathogenic *CACNA1S* variants have been associated with congenital myopathy with hypotonia, delayed motor development, and progressive muscle weakness.[6] More recently, *CACNA1S* variants have been reported to cause an excitation–contraction myopathy characterized by exertional myalgia stiffness and cramp, muscle weakness, and rhabdomyolysis.[7] However, the case number of exertional myopathy associated with *CACNA1S* is very limited, and some similar patients have been overlooked in the previous literature.[8] Therefore, further research will facilitate the clarification of the evolution process and phenotype spectrum of the disease, thereby enhancing the diagnosis of *CACNA1S*-related myalgia.

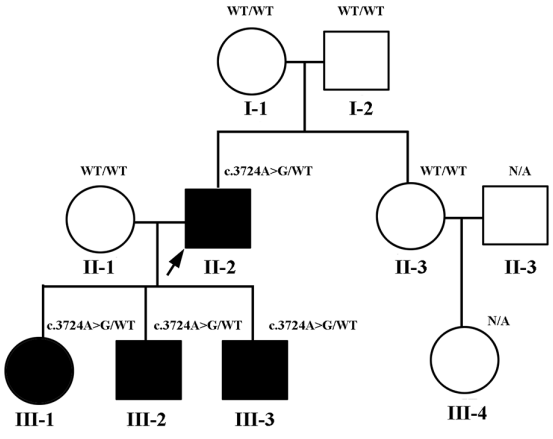

Figure 1. Pedigree of the family. The square represents male and the circle represents female. The filled symbol indicates affected. The arrow indicates the proband.

In this study, we described the clinical characteristics, electrophysiological changes, MRI features, and histopathological changes in a family with an autosomal dominant inherited pattern, in which four patients presented with clinical triad signs of exercise-induced myalgia, muscle weakness, and asymptomatic hyperCKemia. In addition, we summarized all patients with *CACNA1S*-related myalgia in the literature. This study would broaden the clinical spectrum of *CACNA1S*-related phenotypes, aiming to gain an understanding of the pathogenesis behind triadopathy associated with calcium channels.

Materials and methods

Subjects

In an autosomal-dominant inherited family (Figure 1), a total of four affected individuals (II2, III1, III2, and III3) and four unaffected individuals (I1, I2, II1, and II3) were recorded or interviewed. A detailed medical history was obtained from the subjects and their relatives. Information regarding age of onset, progression of disease, and other clinical manifestations was collected.

The clinical data and tissue samples of all patients were acquired after written consent signed by each individual in compliance with the bioethics laws of China as well as the Declaration of Helsinki. The research was approved by the ethics committee of the first affiliated hospital of Nanchang University (IITS2024796).

Muscle MRI

Axial planes of the thigh and leg muscles were imaged in the index patient using 3.0-T MR scanners. Conventional T1 weighted image (T1WI) sequences were obtained to observe fatty infiltration with the following parameters: repetition time (TR) = 500 ms, echo time (TE) = 8 ms, matrix 512 × 512. The short-time inversion recovery sequences were obtained to evaluate muscle edema with the following parameters: TR = 6100 ms, TE = 70 ms, inversion time = 180 ms, matrix 512 × 512. The slice thickness was 5 mm with a slice gap of 1 mm and the field of view was 36 × 48 cm.

Muscle biopsy

The index patient underwent two muscle biopsies at 24 (right bicep) and 38 (left tibialis) years of age, respectively. The tissue was frozen and then cut into 8 µm sections. These sections were stained according to standard histological and enzyme histochemical procedures with hematoxylin-eosin (H&E), modified Gomori trichrome, periodic acidic Schiff, oil red O, adenosine triphosphatase (ATPase), nicotinamide adenine dinucleotide tetrazolium reductase (NADH), succinate dehydrogenase, cytochrome c oxidase, and nonspecific esterase. For immunohistochemistrical staining, the primary antibodies included CD3, CD4, CD8, CD20, CD68 (all from ZSGB-Bio, 1:1), MHC-I (Dako, R7000), and MAC (Dako, M0777). For electron microscopy, the muscle specimen was fixed in 2.5% glutaraldehyde in phosphate buffer and postfixed in 1% osmium tetroxide in the same buffer. The specimen was then dehydrated and embedded in Epon 812 resin. The ultrathin sections of muscle tissue were double-stained with uranyl acetate and lead citrate and then observed with an electron microscope (JEM-1230 JEOL Inc., Tokyo, Japan).

Genetic screening

Genomic DNA was extracted from peripheral blood samples. The whole-exome sequencing (WES) was commercially supported by GrandOmics Lab (Beijing, China). In brief, targeted exon enrichment was performed using SureSelect Human All Exon V5 (Agilent Technologies). The exon-enriched DNA libraries were subjected to paired-end sequencing with the Hiseq2000 platform (Illumina, Inc.). Sequence data were mapped with BWA and SAMTOOLS onto the hg19 human genome as a reference. Calls with variant quality less than 20 were filtered out, and 95% of the targeted bases were covered sufficiently to pass our thresholds for calling single nucleotide polymorphisms (SNP), nonsynonymous/splice acceptor and donor site, insertions or deletions (NS/SS/InDel) variants in the dbSNP v137, ESP6500, and 1000 Genome were removed. Synonymous changes were filtered using SIFT software (http://sift.jcvi.org). Variant pathology was classified according to American College Medical Genetics and genomics criteria (ACMG) guidelines. Sanger sequencing with specific primers was conducted to confirm the *CACNA1S* mutation in the patients and their available family members.

Literature review

We searched the literature in multiple databases including PubMed, EMBASE, Scopus, Web of Science, EBSCO, and Google Scholar database using the keywords "*CACNA1S* gene," "myalgia," "muscle pain," "muscle stiffness," "muscle cramp," "hyperCKemia," "malignant hyperthermia," and "rhabdomyolysis." All included cases had definite *CACNA1S* mutations, and subsequently, the clinical characteristics and laboratory results of all patients were summarized.

Results

Clinical features

The index patient (Figure 1) was a 38-year-old man who experienced episodic exertional myalgia and weakness, fluctuating hyperCKemia, and recurrent rhabdomyolysis since adolescence. He achieved a milestone in motor development, while his performance in physical education during elementary school was inferior to that of his peers. At the age of 13 years, he experienced severe myalgia and muscle stiffness, predominantly affecting his legs, after engaging in prolonged running. This myalgia subsequently resulted in his inability to walk, which gradually improved over approximately 1 week of rest. At the age of 14 years, he had a similar episode after lifting heavy objects. His serum creatine kinase (CK) rose to over 10,000 IU/L (normal range: 25–175 IU/L), and his blood potassium level was normal during the attack. Within a week, the myalgia and weakness completely resolved, but the CK level remained at approximately 3000 IU/L. At the age of 15 years,

he endured severe myalgia, muscle stiffness, and muscle weakness after a strenuous exercise. Rhabdomyolysis was subsequently diagnosed based on a high CK level of 37,940 IU/L, along with positive myoglobin and tea urine. Since the age of 20 years, the patient has experienced two to three episodes of myalgia every year; each episode was associated with exertional or febrile activity. During each acute episode, the patient also exhibited muscle weakness, accompanied by a significant increase in CK levels. After recovery, muscle weakness generally improved, yet the patient persistently exhibited an asymptomatic hyperCKemia of 2000–3000 IU/L. Besides episodic exertional myalgia, the duration of muscle weakness gradually extended. In severe conditions, it can lead to difficulty in climbing stairs at the age of 30 years and standing after squatting at the age of 33 years. Upon admission at the age of 38 years, he still complained of severe myalgia that occurred periodically after exercises. The patient had difficulty climbing stairs, getting up from chairs, walking on uneven floors, and washing his hair. The neurological examination on admission revealed the function of the extraocular, facial, and bulbar muscles was normal. Additionally, the limb muscles had a normal size and shape, with no signs of atrophy or hypertrophy. Muscle strength according to the MRC (Medical Research Council) scale rated as forearm flexion 4/4 (right/ left), wrist extension 5/5, finger flexion 5−/5−, hip flexion 3+/3+, knee extension 4/4, knee flexion 4/4, foot dorsiflexion 5−/5−, and foot plantar flexion 5/5. Tendon reflexes were reduced. The proband underwent a general anesthesia procedure at the age of 35 without experiencing any symptoms related to malignant hyperthermia, such as skeletal muscle rigidity or elevated body temperature.

Laboratory studies showed that the level of serum CK was 10,153 U/l, and the serum potassium level was normal. The results for thyroid function, HIV, plasma acylcarnitines, lactate, serum carnitines, and urine organic acids were within the normal range or negative. Motor and sensory nerve conduction studies were normal. Needle electromyography (EMG) of the right deltoid and quadriceps muscles showed a small amount of spontaneous fibrillation potentials. Motor unit potentials were characterized by short duration and low amplitude with early recruitment. The long exercise test revealed that the compound muscle action potential (CMAP) decreased by 17% at 5 min postexercise, progressively decreased over time, and ultimately reached a decrease of approximately 50% from its peak at 105 min postexercise (Figure 2).

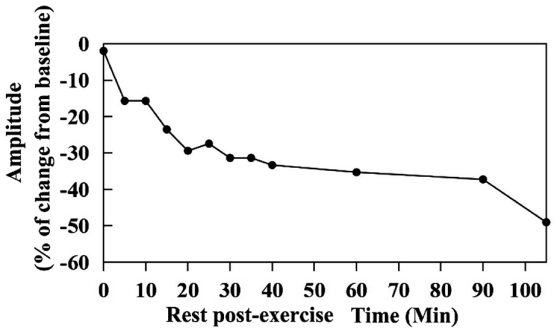

Figure 2. Long exercise test in the index patient showed that the CMAP amplitude decreased by 17% at 5 min postexercise, and ultimately reached a decrease of 50% from its peak at 105 min.
CMAP, compound muscle action potential.

The family over two generations consisted of four affected members (Figure 1). All patients experienced exertional myalgia, followed by flaccid weakness or rhabdomyolysis, with asymptomatic hyperCKemia in the interictal period. The elder sister (III-1), a 16-year-old girl, typically exhibited all the characteristics of her father. At the age of 3 years, she first experienced lower-limb muscle pain after exercise, resulting in her inability to walk. She recovered in about a week, with the serum CK level rising to over 30,996 IU/L. Since then, she had several similar episodes of symptoms following vigorous physical activities, occasionally accompanied by muscle stiffness. Additionally, during the interictal periods, the patient exhibited no clinical symptoms, yet her serum CK levels remained between 2000 and 3000 IU/L. The elder son (III-2), 12 years old, presented with similar clinical features as his sister. At the age of 10 years, he first experienced severe muscle pain after playing a basketball match, which was followed by muscle weakness lasting up to several days, accompanied by a serum CK level exceeding 16,490 IU/L. Afterward, the patient had recurrent episodes of exertional myalgia, muscle weakness, and occasionally muscle soreness and stiffness. In the interictal periods, he had no clinical symptoms, while the serum CK levels ranged between 2000 and 3000 IU/L. The young son (III-3), a 9-year-old boy, complained of episodic myalgia and muscle fatigue in his lower legs after exercise. At the age of 2 years, he first suffered from muscle pain after

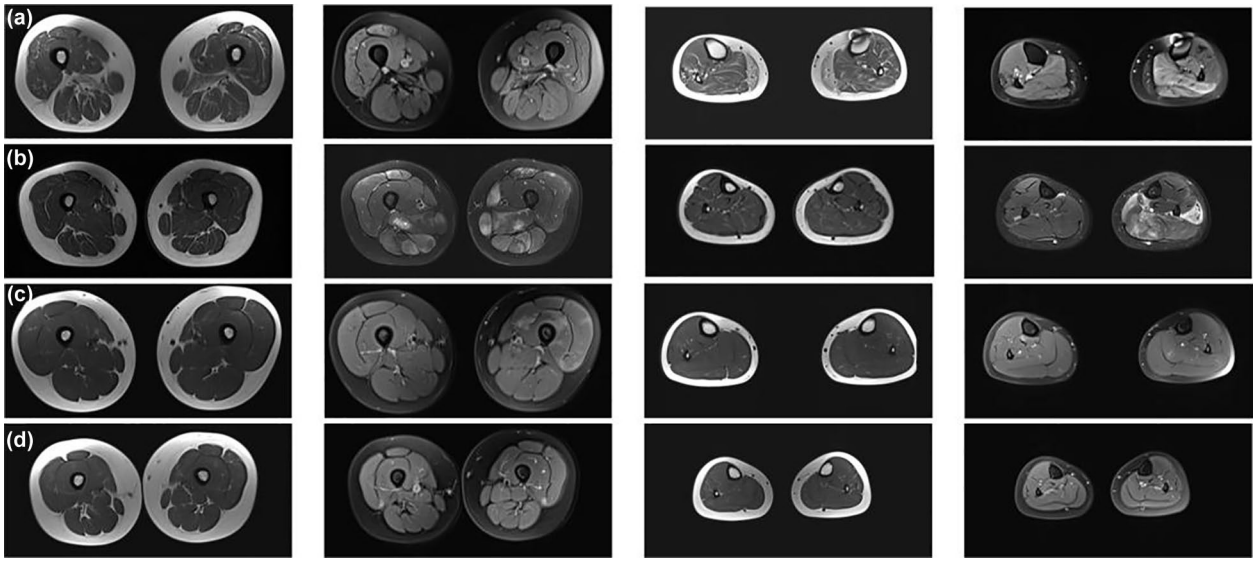

Figure 3. Muscle MRI changes in the four affected patients. Muscle MRI of the proband revealed fatty degeneration and edema (a). Muscle MRI of patient III-1 showed no evidence of fatty infiltration in the lower limbs, whereas significant muscle edema was observed in muscles (b). Muscle MRI of patient III-2 revealed focal edema in the left quadriceps femoris muscle, while the leg muscles showed no abnormalities (c). Muscle MRI of patient III-3's lower limb showed no significant abnormalities (d).

exercise, and the serum CK level was over 11,682 IU/L. When he was asymptomatic, the serum CK level remained at approximately 2000 IU/L.

Muscle MRI changes

The muscle MRI of the proband revealed severe fatty infiltration in the adductor magnus and medial head of gastrocnemius muscles, with slight fatty infiltration in the quadriceps femoris, hamstring, and soleus muscles. However, moderate muscle edema was only observed in the right rectus femoris and the left soleus muscle (Figure 3(a)). The muscle MRI of patient III-1 showed no evidence of fatty infiltration in the lower limbs, whereas significant muscle edema was observed in the thigh and leg muscles (Figure 3(b)). The thigh muscle MRI of patient III-2 revealed focal edema in the left quadriceps femoris muscle, while the leg muscles showed no abnormalities (Figure 3(c)). The MRI of the patient III-3's lower limb showed no significant abnormalities (Figure 3(d)).

Myopathological findings

After experiencing rhabdomyolysis at the age of 24 years, the proband underwent a biopsy of his biceps muscle. The muscle biopsy, as revealed by H&E staining, demonstrated numerous atrophic fibers, increased diameter variation of the muscle fibers (Figure 4(a)), and degenerative necrosis of the fibers, occasionally accompanied by infiltration of phagocytic cells (Figure 4(b)). NADH staining showed that some muscle fibers were darkly stained, accompanied by abnormalities in the structure of the SR (Figure 4(c)). No pathological vacuoles and tubular aggregates were observed in the fibers. Upon this admission, the patient underwent another muscle biopsy of the left anterior tibial muscle. The muscle section revealed myofiber atrophy and hypertrophy, accompanied by connective tissue proliferation, many fresh necrotic myofibers (Figure 4(d)), and infiltration of massive inflammatory cells in the necrotic myofibers and perimysium (Figure 4(e)). Many muscle fibers exhibited a homogeneous distribution of small vacuoles (Figure 4(f)), devoid of abnormal aggregation of lipid droplets, glycogen (Figure 4(g)), mitochondria (Figure 4(h)), or lysosome (Figure 4(i)). NADH staining showed atypical tubular aggregation in some muscle fibers (Figure 4(j) and (k)). A small number of myofibers exhibited a slight positive immunostaining for MHC-I (Figure 4(l)), indicating that the immune response was involved in the pathological processes of muscle fibers.

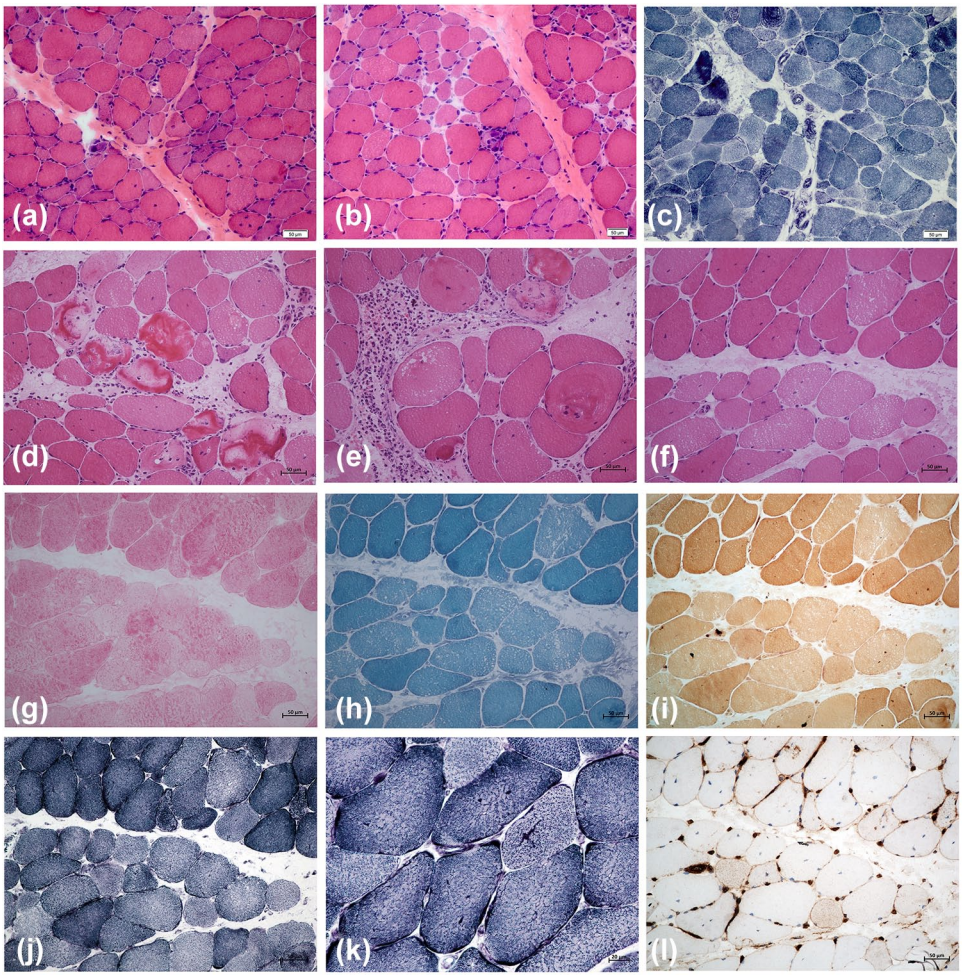

Figure 4. Myopathological features of two biopsies of the index patient. The first biopsy at age 24 revealed regenerative and degenerative changes of muscle fibers (a), occasionally accompanied by infiltration of phagocytic cells (b); and abnormalities in the structure of the sarcoplasmic reticulum on NADH stain (c). The second biopsy at age 38 showed chronic myopathy changes with many fresh necrotic myofibers (d); inflammatory cells infiltration in myofibers and perimysium (e); and numerous small vacuoles in myofibers (f), devoid of glycogen (g), mitochondria (h), and lysosome (i). NADH stain showed atypical tubular aggregation in some muscle fibers (j) and abnormalities of the sarcoplasmic reticulum (k). MHC-I was positive in some myofibers (l).
NADH, nicotinamide adenine dinucleotide tetrazolium reductase.

Ultrastructural examination revealed that numerous dilated tubular SR of various sizes were observed among myofibrils and in subsarcolemmal regions (Figure 5(a)). Many fused bubbly SR, which was considered subsequently to be an enlarged vacuole, and large vacuoles were observed in transverse sections (Figure 5(b)). The border zone between a vacuole and a muscle fiber contained various dense materials including glycogen granules, vesicles, calcium, and related proteins (Figure 5(c)). Additionally, an increased number of mitochondria with normal shapes were observed in the subsarcolemmal spaces and among myofibrils. Furthermore, Z-line disorganization and streaming among some myofibrils were observed (Figure 5(d)).

Genetic analysis

WES revealed a heterozygous variant c.3724A>G (p.Arg1242Gly) in exon 30 of the *CACNA1S* gene (NM_000069.3) in the index patient (Figure 6(a)). The variant was not found in the parent (Figure 6(b) and (c)). The heterozygous variant was also identified in the three affected offspring (Figure 6(d)–(f)), while it was negative in the healthy family members II-1 and II-3, indicating a family co-segregation. The variant had

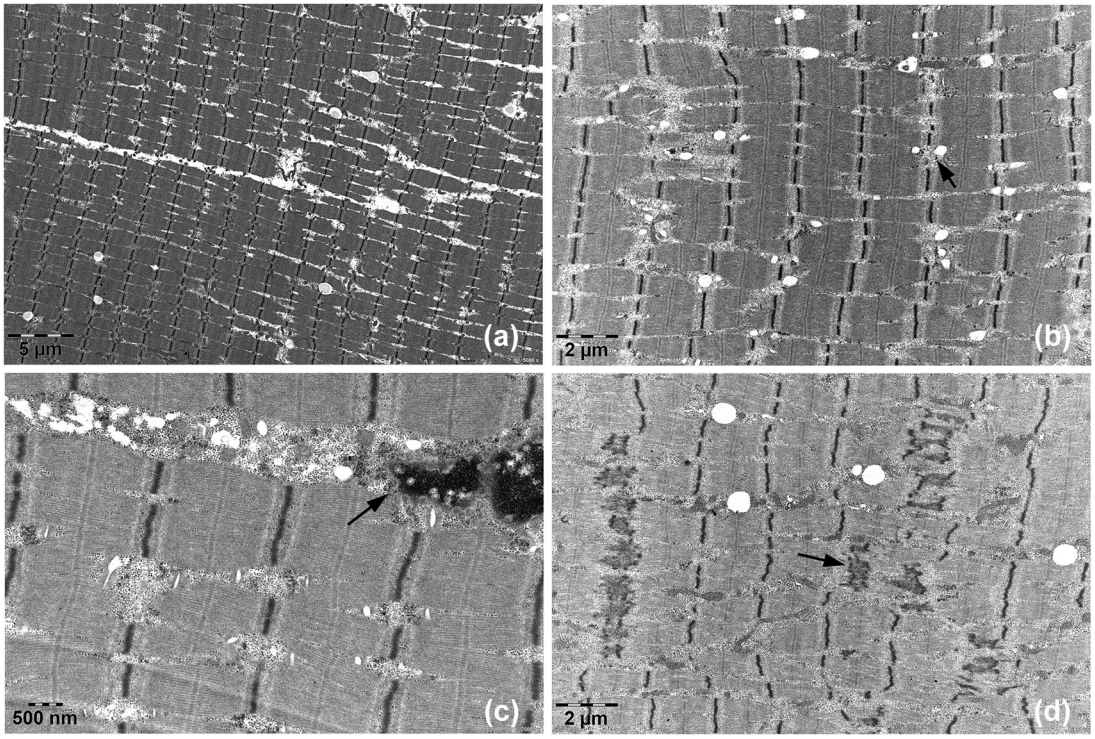

Figure 5. Ultrastructural examination revealed numerous dilated tubular SR of various sizes in myofibrils (a). Many fused bubbly SR with large vacuoles in transverse sections (b). The border zone between a vacuole and a muscle fiber contained various dense materials (c). Z-line disorganization and streaming in some myofibrils (d). SR, sarcoplasmic reticulum.

not been previously found in the gnomAD database (http://gnomad.broadinstitute.org), whereas it had been reported in two unrelated families. A homology search in different species demonstrated that the amino acid at residue Arg1242 is evolutionally highly conserved (Figure 6(g)). The variant was predicted to be damaging by several in silico tools. Of note, functional studies revealed that in cell lines expressing c.3724A>G, the inward current through the central α pore significantly decreases, together with the presence of outward omega currents. Collectively, the significance of the variant was evaluated as pathogenic according to the *ACMG* criteria (PS1 + PS2 + PS3 + PP1). No other variants associated with channelopathies were found in the genetic screening.

Literature review

We conducted a retrospective analysis of all reported cases of exercise-induced muscle pain and weakness linked to *CACNA1S* mutations, encompassing a total of 15 patients,[7–11] including those from this study (Table 1). These patients comprised 12 males and 3 females, with a median age of onset being 10 (2, 20) years old. All patients initially exhibited symptoms of muscle pain and weakness induced by exercise, labor activity, fever, or cold. Specifically, 14 patients (14/15, 93.3%) had muscle weakness or fatigue, 13 (13/15, 86.7%) complained of muscle pain and/or cramp, and 5 (5/15. 33.3%) became dependent on wheelchairs or walking aids in an advanced stage of the disease. Additionally, some patients developed symptoms such as ptosis, facial muscle weakness, dysphagia, and abdominal pain. Of the 12 patients who underwent CK testing, 8 had significantly elevated CK levels exceeding 10,000, manifesting as malignant hyperthermia, rhabdomyolysis, or asymptomatic hyperCKemia. EMG was conducted on seven patients, revealing a late decrease in CMAP in long exercise tests in five of them. Ten patients underwent muscle MRI, with four patients showing normal results, four exhibiting varying degrees of selective muscle degeneration and atrophy, and five displaying muscle edema. Nine patients underwent muscle biopsies,

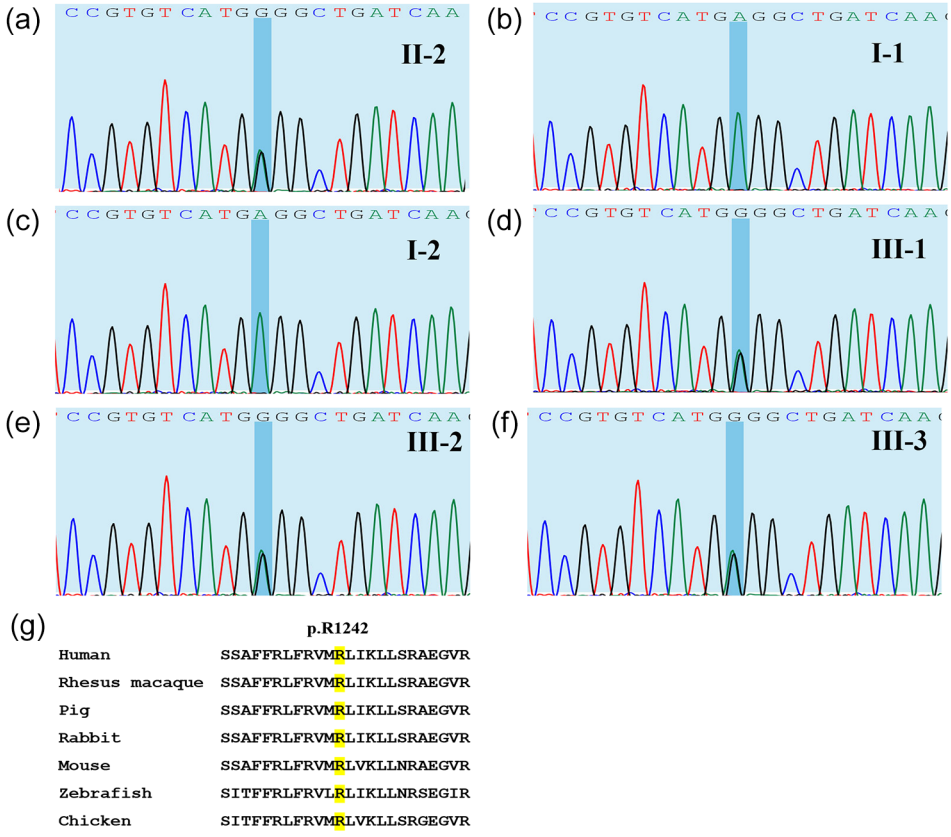

Figure 6. Genetic analysis in the family. (a) The proband had a heterozygous c.3724A>G variant in the *CACNA1S* gene. The mother (I-1, b) and the father (I-2, c) of the proband were wild genotype. The daughter (III-1, d), the son (III-2, e), and the little son (III-3, f) all carried a heterozygousc.3724A>G variant. Residue arginine 1242 had high evolutionary conservation (g).

all revealing nonspecific myopathic changes characterized by an increase in internalized nuclei, variations in muscle fiber diameter, atrophic fibers, and NADH staining indicating varying degrees of irregularity in oxidative enzyme activity, primarily affecting type II muscle fibers. Genetic testing revealed heterozygous missense mutations in the *CACNA1S* gene in all patients. Specifically, nine patients carried the mutation c.3724A>G (p.Arg1242Gly), four patients carried the mutation c.2893G>C (p.E965Q), one patient possessed the mutation c.1583G>T (p.Arg528Leu), and one patient harbored the mutation c.1678G>T (p.Ala560Ser).

Discussion

CACNA1S variants emerged as the principal genetic determinant of HypoPP, encompassing up to 60% of HypoPP patients. Typically, these individuals experienced recurrent episodes of limb weakness during their adolescence, with only a minority progressively developing permanent muscle weakness following recurrent attacks.[6] However, the phenotypes linked to *CACNA1S* variants also exhibited significant heterogeneities, indicating a complicated relationship between genetic variations and phenotypic manifestations.[7,9] In this study, we summarized a group of patients presenting with severe exertional myalgia, frequently accompanied by symptoms such as muscle stiffness, cramp, asymptomatic hyperCK-emia, recurrent rhabdomyolysis, and eventually muscle weakness. Notably, the proband underwent general anesthesia surgery but did not exhibit any symptoms associated with malignant hyperthermia, including muscle stiffness or elevated body temperature. This observation suggested that this myopathy syndrome was distinctly different from malignant hyperthermia and

Table 1. The summarizations of patients with exercise-induced muscle pain and weakness associated with *CACNA1S* mutations.

Case	Sex/AAO (years)	Presenting symptoms	Main symptoms	Highest CK (IU/L)	EMG	Muscle MRI	Muscle pathology	Mutations	References
1	M/14	Muscle fatigue and aching	Exercise-induced myalgia, cramp and stiffness, muscle weakness, ptosis, facial weakness, dysphagia	Normal	Immediate increase and late decrease in CMAP[a]	Normal	NA	c.2893G>C (p.E965Q)	Periviita et al.[9]
2	M/30	Muscle fatigue and aching	Exercise-induced myalgia, cramp and stiffness, muscle weakness, ptosis, dysphagia	Normal	Late decrease in CMAP[a]	Fatty degenerative changes	Myopathic findings	c.2893G>C (p.E965Q)	Periviita et al.[9]
3	M/51	Muscle fatigue and aching	Exercise-induced myalgia, cramp and stiffness, muscle weakness, ptosis	Slight elevation	Immediate increase and late decrease in CMAP[a]	Normal	Fiber grouping, increased nuclei	c.2893G>C (p.E965Q)	Periviita et al.[9]
4	M/27	Muscle fatigue and aching	Exercise-induced myalgia, cramp and stiffness, muscle weakness, ptosis	Normal	Late decrease in CMAP[a]	Normal	Myopathic findings	c.2893G>C (p.E965Q)	Periviita et al.[9]
5	M/36	Exercise-induced rhabdomyolysis	Rhabdomyolysis, hyperCKemia	17,658	NA	NA	Core-like structures	c.1583G>T (p.Arg528Leu)	Anandan et al.[7]
6	M/20	Exercise-induced muscle pain	Myalgia, abdominal pain, hyperCKemia	17,974	NA	NA	NA	c.1678G>T (p.Ala560Ser)	Vivante et al.[10]
7	M/2	Episodic muscle weakness	Exercise or febrile-induced muscle pain, cramp, and weakness	33,000	NA	NA	Nonspecific myopathic changes	c.3724A>G (p.Arg1242Gly)	Edizadeh et al.[8]
8	M/infancy	Delayed motor development	Exercise or cold-induced muscle pain, cramp, and weakness	NA	NA	NA	Atypical muscle dystrophy	c.3724A>G (p.Arg1242Gly)	Fan et al.[11]
9	M/at birth	Paralysis	Respiratory failure	NA	NA	NA	NA	c.3724A>G (p.Arg1242Gly)	Fan et al.[11]
10	F/10's	Episodic muscle cramp	Exercise or febrile-induced muscle pain, cramp, and weakness	NA	Myopathic changes	Fatty degeneration, muscle atrophy, and edema	Chronic myopathic changes	c.3724A>G (p.Arg1242Gly)	Fan et al.[11]
11	F/at birth	Floppy and delayed motor development	Exercise or cold-induced muscle pain, cramp, and weakness	15,500	Myopathic changes	Fatty degeneration, muscle atrophy, and edema	Nonspecific myopathic changes	c.3724A>G (p.Arg1242Gly)	Fan et al.[11]

(Continued)

Table 1. (Continued)

Case	Sex/AAO (years)	Presenting symptoms	Main symptoms	Highest CK (IU/L)	EMG	Muscle MRI	Muscle pathology	Mutations	References
12	M/13	Exercise-induced muscle pain and cramping	Exercise-induced muscle pain, cramp, and weakness, hyperCKemia	37,940	Myopathic changes, late decrease in CMAP[a]	Fatty degeneration and edema	Myopathic findings and vacuoles	c.3724A>G (p.Arg1242Gly)	This study
13	F/3	Exercise-induced myalgia, hyperCKemia	Exercise-induced muscle pain and weakness, hyperCKemia	30,996	NA	Muscle edema	NA	c.3724A>G (p.Arg1242Gly)	This study
14	M/10	Exercise-induced myalgia, hyperCKemia	Exercise-induced muscle pain and weakness, hyperCKemia	16,490	NA	Muscle edema	NA	c.3724A>G (p.Arg1242Gly)	This study
15	M/2	Exercise-induced myalgia, hyperCKemia	Exercise-induced muscle pain and fatigue, hyperCKemia	11,682	NA	Normal	NA	c.3724A>G (p.Arg1242Gly)	This study

[a]Long exercise test.
AAO, age at onset; CAMP, compound muscle action potential; CK, creatine kinase; EMG, electromyography; NA, not available.

instead pointed to a possible novel phenotype of myalgia-weakness linked to dysfunction in muscle excitation–contraction coupling.

The average age of onset for patients with this phenotype is 14.53 ± 14.92 years; however, there is considerable individual variation, varying from as early as 0 years to as late as 51 years. The condition was more prevalent among males, with a lower proportion of female patients. Myalgia served as the most distinguishing feature of this clinical phenotype, with severe cases potentially resulting in disability. Exercise was the primary trigger for most cases of myalgia, while it can also be induced by febrile or cold factors.[7] Concurrently, myalgia was frequently accompanied by muscle stiffness or cramp, as well as a notable elevation in CK level. In the early stage of the disease, most patients typically experience isolated myalgia. As the disease progresses slowly, the duration of myalgia might become prolonged or permanent, often accompanied by symptoms of muscle fatigue or weakness. Some patients might develop permanent muscle weakness affecting the proximal limb muscles. Aged patients with advanced disease might eventually require auxiliary devices for walking or become dependent on wheelchairs. During the myalgia and weakness, most patients exhibited significant elevations in CK level, with a few individuals progressing to rhabdomyolysis or malignant hyperthermia. Additionally, some patients maintained asymptomatic hyperCKemia with moderately elevated CK levels during the interictal period. Furthermore, a minority of patients might exhibit a combined phenotype of periodic paralysis or atypical congenital myopathy, manifesting as ptosis, facial muscle weakness, dysphagia, upper-limb hypertrophy, floppy, and delayed motor development. Therefore, this exertional myalgia-weakness phenotype represented a unique *CACNA1S*-related phenotype that lay somewhere between periodic paralysis and congenital myopathy. Given the severity of its long-term prognosis, early recognition of this phenotype held significant implications for the long-term management of patients.

The findings of traditional neurophysiological studies in patients with myalgia-weakness phenotype were inconsistent. Some patients showed a myogenic pattern, and some patients were not significant, while no myotonic discharges had been recorded in these cases. Among patients

who underwent long exercise tests, some exhibited an immediate increase followed by a late decrease in CMAP amplitude, resembling hyperkalemic periodic paralysis associated with sodium channel mutations, and the others only demonstrated a late decrease in CMAP amplitude, akin to the classical HypoPP phenotype resulting from *CACNA1S* mutations.[9,11] Therefore, long exercise test screening for patients with early-onset myalgia can be instrumental in identifying potential diagnoses of skeletal muscle channelopathy.

Muscle MRI could show fatty degeneration and substitution in the leg muscles of patients with prolonged disease courses, while only edema-like changes could be observed in the muscles of patients at the early stage.[9,11] Given that the *CACNA1S*-related myalgia-weakness phenotype clinically resembled a myopathy, the features of muscle MRI changes aligned more closely with those associated with chronic myopathy. The characteristics of muscle MRI corresponded to the features of muscle pathological alterations. The majority of patients exhibited nonspecific chronic myopathy-like pathological changes, characterized as great variations in fiber diameter, increased internal nuclei, and disarranged SR on NADH staining. Additionally, a few patients might show tube aggregating and core-like changes.[7,9,11] Our research revealed dynamic pathological alterations in the patient's muscles, initially manifesting as atrophy, degeneration, and necrosis of individual muscle fibers. However, as the patient's muscle weakness progressed, myofibrillar structural disarrangement, dilation of the SR, as well as degeneration and inflammatory responses were observed. These pathological changes in the muscles explained the clinical phenotypic process associated with the myalgia-weakness phenotype.

The c.3724A>G (p.Arg1242Gly) mutation identified in this study is situated in the exon 30 of the *CACNA1S* gene. This mutation has been reported in two families, including an instance of de novo mutation, indicating that it serves as a hotspot mutation in the *CACNA1S* gene.[11] The *CACNA1S* gene encodes the α1 subunit of the L-type voltage-gated calcium channel in skeletal muscle, crucial for muscle excitatory contraction.[12,13] Within the voltage-sensitive S4 segment of each transmembrane domain, four positively charged arginine residues serve as voltage sensors, mediating conformational changes in the channel structure during membrane depolarization.[14–16] Mutation of arginine at these sites into neutral amino acids would result in alterations in α-pore current and facilitate the formation of abnormal ω-currents, mechanistically responsible for periodic paralysis.[17] The p.Arg1242Gly variant involved a glycine substitution at the third charged R residue within the S4 segment of the voltage sensor in structural domain IV. Functional studies conducted previously have demonstrated a significant reduction in the inward current of the central alpha pore, accompanied by the presence of abnormal outward current.[11] During depolarization, the voltage sensor forms an ion-conducting channel, allowing positive ions to flow outward. The ω-currents reduce the amplitude and slope of action potentials, leading to decreased muscle excitability.[18] This mechanism leads to permanent muscle weakness and myopathic damage over time. When hyperpolarized, the mutant voltage sensor creates an ion-conducting pathway that excites positive ions.[19] The inward ion flow can induce calcium release without action potentials, resulting in muscle cramps, edema, and pain.[20] The mutation decreases calcium inward current density through the central pore and shifts the steady-state inactivation curve to more negative potentials.[21] These changes further contribute to the hypoexcitability of muscle fibers. In addition, depolarization induced by omega currents activates compensatory outward potassium currents, maintaining normal serum potassium levels during attacks, and explaining the normokalemic phenotype.[11] Together, these mechanisms result in muscle cramps, permanent weakness, normokalemic periodic paralysis, and related symptoms.[11] Our cases exhibited symptoms similar to the five previously reported cases carrying the p.Arg1242Gly mutation, including myalgia, muscle weakness, and hyperCKemia. At the triad structure, a close electrophysiological interaction exists between the Cav1.1 and RyR1 channels. Studies have shown that the cytoplasmic domain of RyR1 binds to the II–III loops of CaV1.1.[22] And cryo-electron microscopy reveals that CaV1.1 forms tetrads, precisely aligned with the structure of RyR1.[22] Moreover, mutations in the α1S II1242I–IV loops influence DHPR gating transitions important both for excitation-contraction coupling and activation of L-type conductance.[23] The p.Arg1242Gly variant is located in sensor S4 and does not physically interact with Ryr1, but the mutation may cause the conformational change of Cav1.1 allowing the

interaction with Cav1.1 and calcium release. Meanwhile, variants in RyR1 have been associated with a phenotype of exertional myalgia, muscle cramp, and muscle stiffness.[24] Consequently, we proposed a hypothesis, albeit needing experimental support, that mutations in the amino acid sites affected by the Cav1.1-RyR1 complex might underlie a group of clinically similar subtypes of ion channelopathies characterized by myalgia-weakness phenotype.

In summary, this study described an autosomal-dominant family with *CACNA1S* mutation, in which patients presented with a triad of symptoms: myalgia, muscle weakness, and asymptomatic hyperckemia. The phenotype of exertional myalgia and weakness broadened the spectrum of *CACNA1S*-associated myopathy, serving as a bridge between periodic paralysis and congenital myopathies. The similarities between *CACNA1S*-associated myalgia-weakness and RyR1-associated myalgia-weakness underscored a shared pathogenesis of excitatory-contractile coupling at the triad of skeletal muscle.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of First Affiliated Hospital of Nanchang University. The approval number given by the ethical board is IITS2024796.

Consent for publication

The patients or their parent(s) provided a written informed consent for the publication of this article.

Author contributions

Si Luo: Conceptualization; Writing – original draft.

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Dandan Tan: Data curation.

Yusen Qiu: Project administration.

Meihong Zhou: Data curation; Investigation.

Daojun Hong: Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

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