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CASE REPORT

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CASQ1-related myopathy: The first report from China and the literature review

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Abstract

Calsequestrin 1 (CASQ1) is the most crucial Ca^{2+} binding protein localized in the sarcoplasmic reticulum (SR) of skeletal muscle. With high capacity and low affinity for Ca^{2+}, CASQ1 plays a significant role in maintaining a large amount of Ca^{2+} necessary for muscle contraction. However, only five mutations in *CASQ1* have been identified to date. Here, we report a 42-year-old Chinese female patient who presented with a 12 years history of slowly progressive upper limb weakness, predominantly affecting distal muscles, which was uncommon comparing to other CASQ1-related patients. Next-generation sequencing (NGS) analysis revealed a novel heterozygous mutation (c.766G > A, p.Val256Met) in *CASQ1*. Functional studies confirmed the likely pathogenicity of this variant. Muscle histopathology revealed rare optically empty vacuoles in myofibers and atypical eosinophilic granules in the cytoplasm, which has not been observed before. We also performed a literature review on all the pathogenic mutations in *CASQ1* and summarized their genetic and clinical characteristics. This is the first report on CASQ1-related myopathy from China, further expanding the mutation spectrum of *CASQ1* gene and provides new insights into the function of CASQ1.

KEYWORDS

CASQ1, eosinophilic granules, muscle pathology, myopathy, neuromuscular disorder

1 | INTRODUCTION

Calsequestrin (CASQ) is the most crucial Ca^{2+} binding protein localized in the sarcoplasmic reticulum (SR) of skeletal and cardiac muscle.[1] CASQ is present in all vertebrates and has two isoforms, CASQ1 and CASQ2. CASQ1 is only expressed in skeletal muscles, especially in fast-twitch fibers. CASQ2 is a component of slow-twitch skeletal muscle and is present in cardiomyocytes.[2]

With high capacity and low affinity for Ca^{2+}, CASQ1 plays a significant role in maintaining a large amount of Ca^{2+} necessary for muscle contraction. It is able to dual-directionally regulate the ryanodine receptors (RyRs) calcium release channels, likely through interactions with junctin and triadin in a complex quaternary structure to preserve the intracellular SR Ca^{2+} storage.[3] Also, CASQ1 can reversely regulate the store-operated Ca^{2+} entry (SOCE) pathway by inhibiting stromal interaction

molecule 1 (STIM1)/calcium release-activated calcium channel protein 1 (Orai1) interaction to reduce extracellular Ca^{2+} entries.[4] Depending on the intraluminal SR Ca^{2+} levels, CASQ1 monomers assemble to form large polymers,[5] which function as a pivotal factor in buffering Ca^{2+} in the SR terminal cisternae, thus keeping a large-capacity reserve that can be released on stimulus to evoke muscle contraction.

Mutations in *CASQ1* have been associated with two forms of myopathy. A founder mutation (c.731A > G, p.Asp244Gly) was first reported in a group of patients presented with vacuolar myopathy with accumulation of SR protein aggregates.[6] Other mutations were associated with tubular aggregate myopathy.[7–9] In the present case, we identified one novel mutant of *CASQ1* with peculiar clinical and ultrastructural features that had not been reported before, and also summarized previously reported *CASQ1* mutations in order to give researchers a better understanding of CASQ1 in the pathophysiological status of skeletal muscle.

2 | CASE REPORT

A 42-year-old Chinese female patient came to our neuromuscular center because of slowly progressive weakness for 12 years. Around 30 years of age, she began to notice difficulties opening hair claws, while other daily activities were not affected. The symptoms gradually progressed and after a year she had difficulties in fist clenching. By 36 years of age, she could not lift her arms overhead. She was born to non-consanguineous parents and without a remarkable family history of neuromuscular disease.

Physical examinations revealed mild right scapular winging, severe weakness (Medical Research Council, MRC grade 2/5) of distal upper limb muscles and relatively slight weakness (MRC 4−/5) of scapular and proximal upper muscles, while lower limb muscles were spared (Table S1). She had prominent atrophy of ulnar forearm muscles. Her tendon reflexes were normal. There was no myalgia, sensory disturbance, or muscle cramps. Serum creatine kinase (CK) level was within normal range (80.9 IU/L, normal range 40.0–200.0 IU/L).

Electromyography (EMG) analysis revealed numerous complex repetitive discharges and myotonic discharges in bilateral biceps brachii muscles. Compound motor action potentials of biceps brachii were of high mixed with low amplitude, long mixed with short duration. Nerve conduction studies and electrocardiogram were unremarkable. Muscle biopsy of the left biceps brachii showed myopathic features including increased fiber size variation, occasional necrotic fibers (Figure 1B), mild myofibrillar disarrays, and moth-eaten fibers (Figure 1C). Hematoxylin and eosin (HE) stain also demonstrated optically empty vacuoles and fine eosinophilic granules in myofibers (Figure 1A). There was type I fiber predominance with type II fiber atrophy (Figure 1D).

NGS analysis (YULONG DIAGNOSTICS) covering 139 genes associated with neuromuscular disorders identified a novel heterozygous mutation in the sixth exon of *CASQ1* (NM_001231.4:c.766G > A, p.Val256Met), causing a replacement of valine with methionine at amino acid position 256 of CASQ1 (Figure 2A). Sequence conservation analysis identified that this valine at position 256 was conserved across different species (Figure 2B). This residue constitutes a helical region starting from position 249

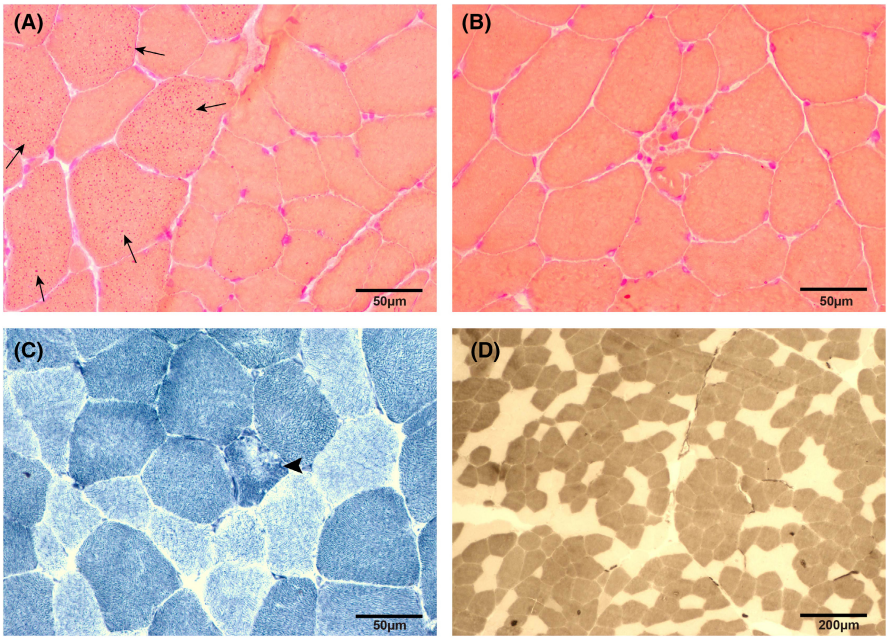

FIGURE 1 Myopathological findings of the present case. Representative light microscopy images of muscle biopsy (A,B) Hematoxylin and eosin (HE) staining showed eosinophilic granules (arrows) scattered in myofibers and necrotic fibers. (C) Nicotinamide adenine dinucleotide (NADH) staining showed moth-eaten fiber (arrowhead). (D) Cytochrome C oxidase, adenosine triphosphatase (ATPase) staining demonstrated a small grouping of type I fibers, with mild atrophy of type II fibers. Scale bars: 50 μm (A–C), 200 μm (D).

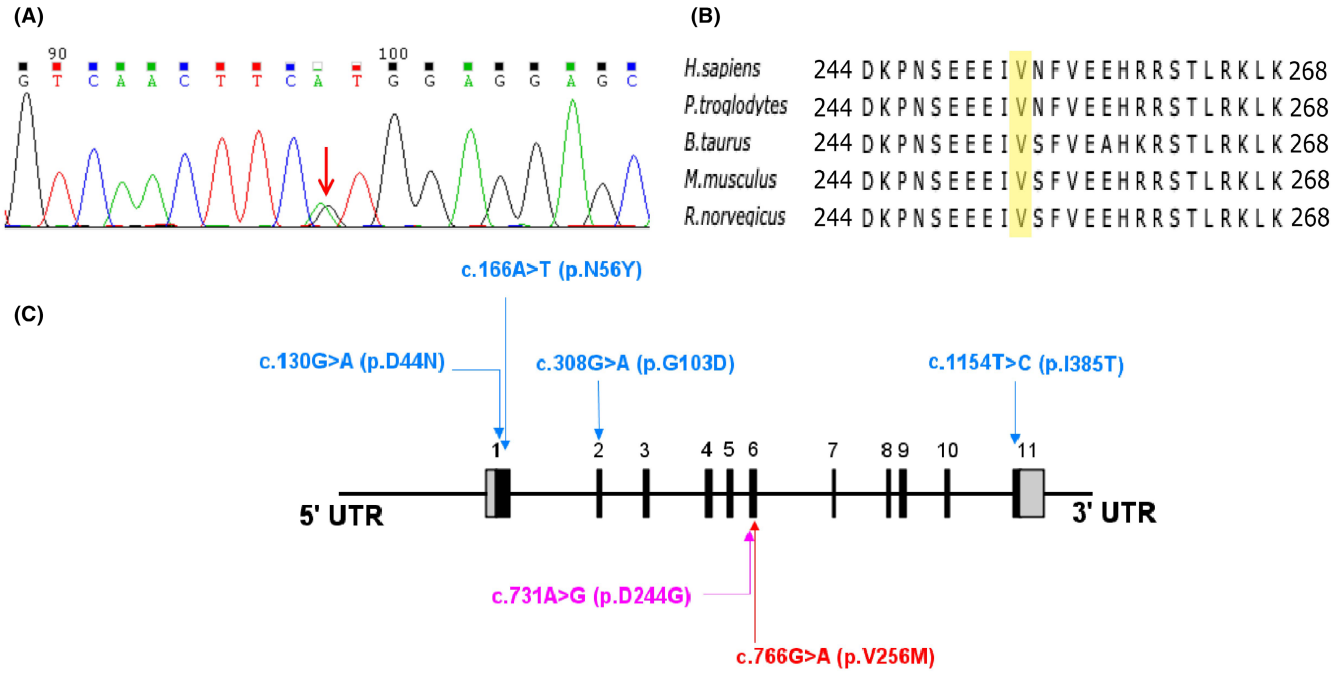

FIGURE 2 Reported myopathy-associated mutations in *CASQ1*. (A) DNA sequencing of *CASQ1* from the patient. A heterozygous missense mutation (c.766G > A, p.Val256Met) was identified (red arrow). (B) Multiple amino acid sequence alignment of CASQ1. The p.Val256Met mutation occurred at an valine (V) residue that was evolutionarily conserved. (C) Schematic structure of the *CASQ1* gene. Exons were shown as boxes, with translated regions shown in black and untranslated regions shown in light gray. The p.Val256Met mutation was found in exon 6. (Red label represented the novel mutation, purple labels represented protein aggregate myopathy (PAM) related mutations in *CASQ1*, and blue labels represented tubular aggregate myopathy (TAM) related mutations in *CASQ1*).

to 258 of CASQ1, which has not been identified with special function. Based on the predicted values of PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), and MutationTaster (http://mutpred.mutdb.org/), c.766G > A (p. Val256Met) was deemed pathogenic (Table S2). This result concurred with destabilizing effects predicted by I-Mutant3.0 (http://gpcr2.biocomp.unibo.it/) and Mupro (http://mupro.proteomics.ics.uci.edu/) (Table S3). Predictions of secondary structure using SOPMA (https://npsa-prabi.ibcp.fr/) showed the mutation to induce an increased tendency to form extended strand and beta turn, while conversely on alpha helix (Table S4).

3 | DISCUSSION

To date, only five *CASQ1* mutations associated with neuromuscular disorders have been reported (Table 1, Figure 2C). In 2014, Rossi et al.[5] reported the first *CASQ1* mutation, c.731A > G (p.Asp244Gly) in eight patients characterized by mild proximal weakness, fatigue, and large vacuoles containing aggregation of SR proteins. Subsequently, Lewis et al.,[10] D'Adamo et al.[11] and Semplicini et al.[9] identified the similar phenotype in patients with c.731A > G (p.Asp244Gly). In these patients, sarcoplasmic vacuolar aggregations and reduced Ca^{2+} release from the SR induced by abnormal CASQ1 aggregates were observed. They were thus diagnosed with protein aggregate myopathy (PAM). The pathogenesis of PAM was proposed as the the loss of the electric charge in aspartic acid at position 244. This location is not only a conserved high-affinity Ca^{2+} binding site but is also close to an interaction region for the adjacent CASQ1 monomers, thus the mutant leading to an increased propensity to form insoluble polymers with reduced Ca^{2+} binding ability.[10]

Additionally, several heterozygous missense mutations in *CASQ1* (c.130G > A, c.166A > T, c.308G > A, and c.1154T > C) were reported in patients with tubular aggregate myopathy (TAM).[7–9] Patients presented with muscle fatigue, exercise intolerance, post-exercise myalgia, and proximal muscle weakness. TAM was first attributed to mutations in *STIM1* and *ORAI1*,[12] along with the dysregulation of SOCE. Despite the similar phenotype, these cases showed pathological changes different from the ones with c.731A > G mutation. They were characterized by vacuoles filled with numerous SR proteins including sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA), STIM1, ORAI1, and CASQ1,[13] consistent with tubular aggregate pathology in muscle fibers. Functional studies by Barone et al.[7] showed an increase in Ca^{2+}-dependent aggregation for the CASQ1 protein with c.1154T > C mutation, and

TABLE 1 *CASQ1* mutations in previous studies associated with neuromuscular disorders

Mutation	Exon	Genetics	Disease classification	Clinicopathological features	Proposed mechanism	References
c.731A > G (p.Asp244Gly)	6	Heterozygous	PAM	i. Myalgia, exercise intolerance, mild proximal weakness, and muscle cramping, with elevated plasma creatine kinase (CK) levels ii. Optically empty vacuoles involving aggregation of sarcoplasmic reticulum (SR) proteins, almost exclusively in type II fibers	Loss of the electric charge in D244, which resulting in increased propensity to form insoluble polymers with reduced Ca^{2+} binding ability	5,9–11
c.130G > A (p.Asp44Asn)	1	Heterozygous	TAM	i. Muscle fatigue and diffuse exercise-induced myalgia ii.Cytoplasmic aggregates of swollen membranous tubules	Decreased Ca^{2+} dependent polymerization leading to decreased Ca^{2+} binding ability and a reduced inhibitary effect on SOCE	7
c.166A > T (p.Asn56Tyr)	1	Heterozygous	TAM	i. Progressive muscle weakness in proximal muscles of lower limbs ii. Tubular aggregates	Decreased Ca^{2+} dependent polymerization	8
c.308G > A (p.Gly103Asp)	2	Heterozygous	TAM	i. Exercise intolerance, post-exercise myalgia, stiffness, and early fatigue ii. Vacuoles filled with amorphous material, consistent with tubular aggregates, only in type II fibers	Decreased Ca^{2+} dependent polymerization leading to decreased Ca^{2+} binding ability	7–9
c.1154T > C (p.Ile385Thr)	11	Heterozygous	TAM	i. Myalgia and proximal muscle weakness ii. Tubular aggregates	Moderately increased Ca^{2+} dependent polymerization leading to decreased Ca^{2+} binding ability and a reduced inhibitary effect on SOCE	7

Abbreviations: PAM, Protein aggregate myopathy; TAM, Tubular aggregate myopathy.

a reduced Ca^{2+}-dependent aggregation for c.130G > A and c.308G > A mutants. Remarkably, c.130G > A and c.1154T > C mutants were shown to possess a reduced inhibition of SOCE comparable to the wild type of CASQ1, further corroborating the imbalance of Ca^{2+} homeostasis and SOCE both leading to the occurrence of TAM.

In terms of clinical manifestation, the majority of patients reported to date presented with progressive yet mild proximal weakness. In rare cases, hyperCKaemia was the sole overt manifestation.[6] By contrast, our patient displayed distal weakness that was confined to upper limbs throughout more than 10 years progression. Her CK levels remained constantly within normal range, which might partially explain the slow progression rate. Muscle atrophy was limited to the ulnar forearms, which was different from previously described patients with prominent atrophy involving the quadriceps, scapular muscles, or pectoral muscles.[9] This patient did not have the mild hypertrophy as reported in some cases.[6,7] Despite experimental evidence suggesting that CASQ1 deficiency is causally related to malignant hyperthermia-like arrhythmia, respiratory and cardiac insufficiency have rarely appeared in CASQ1-related cases.[14] Our patient did not show any evidence of respiratory or heart involvement either. It is important to underline that atypical granules stained red with HE were detected to discrete in myofibers in large quantities, which has not been observed in other patients with *CASQ1* mutations. The mechanism of these abnormal eosinophilic granules remained unknown, but it was believed to have connections with the decreased stability in CASQ1, and might be a new pathological phenotype of PAM. We also found a few optically empty vacuoles which was similar to the one observed in c.731A > G mutant, but far less than that in distribution.[9]

4 | CONCLUSION

In summary, we report the first patient from China with CASQ1-related myopathy due to a novel heterozygous mutation (c.766G > A, p.Val256Met). This is also the first CASQ1 case with predominant distal upper limb weakness, starting from distal compartment and later involving proximal extremities, and her serum CK was throughout within normal range. This patient is featured by atypical eosinophilic granules on muscle pathology, which might be a new pathological phenotype of PAM. Finally, we have reviewed all pathogenic mutations in *CASQ1* to date and summarized their genetic and clinical characteristics. Our study expands the phenotypic spectrum of CASQ1-related myopathy and further studies should be performed to explore the mechanisms operated by CASQ1 in maintaining skeletal muscle Ca^{2+} homeostasis.

AUTHOR CONTRIBUTIONS

HY and YL conceived the idea and revised the literature. KZ interpreted the results and wrote the manuscript. HD, QL, LX, and KH participated in the clinical management and data collection. GZ performed the data analysis. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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