Neurology Asia 2021; 26(2) : 301 – 305

A novel LDB3 (c.1720G>A) mutation causes myofibrillar myopathy

[1]Xue Li, [2]Haitao Yang, [1]Jiaze Tan, [1]Yulan Fan, [3]Jingchuan Fan, [1]Fei Xiao

[1]Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Neurology, Chongqing; [2]Department of Radiology, The First Affiliated Hospital of Chongqing Medical University, Chongqing; [3]Department of Medical Laboratory Technology, Institute of Life Sciences of Chongqing Medical University, Chongqing, China

Abstract

Z-disc-associated, alternatively spliced, PDZ motif-containing protein-related myofibrillar myopathy (ZASP-MFM) is a rare autosomal dominant and late-onset distal myopathy with partial cardiomyopathy, which occurs because of lim domain-binding 3/Z band alternatively spliced PDZ-containing protein (*LDB3/ZASP*) mutations. We describe the clinical, pathological, genetic findings, and muscle magnetic resonance imaging (MRI) changes in a Chinese pedigree with ZASP-MFM. Muscle biopsies, muscle MRI, and *LDB3* gene sequence analysis were carried out. Muscle biopsies revealed the presence of muscular dystrophy-like changes and myogenic changes in the proband, and the MR images of lower limbs showed the symmetrical involvement of anterior compartment muscles of the thigh and posterior compartment muscles of the calf. Gene analysis for *LDB3* revealed a novel heterozygous mutation c.1720G>A in the pedigree.
Conclusion: A novel point mutation in *LDB3* was detected, expanding the spectrum of *LDB3* mutations known to be associated with ZASP-MFM.

Keywords: Lim domain-binding 3 (*LDB3*), muscle biopsy, myofibrillar myopathies, muscle MRI, Z band alternatively spliced PDZ-containing protein (ZASP)

INTRODUCTION

Myofibrillar myopathies (MFM) are a group of genetic neuromuscular disorders with a late-onset distal myopathy with or without cardiomyopathy, resulting from histological abnormalities of the Z-disc, causing gradual disorganization of the intermyofibrillar network that results in accumulation of abnormal protein and rimmed vacuoles within the muscle sarcoplasm.[1] In the past 20 years, several types of MFMs have been identified. The Z-disc-associated, alternatively spliced, PDZ motif-containing protein-related myofibrillar myopathy (ZASP-MFM), also termed zaspopathy, exhibits an autosomal dominant inheritance. Patients with ZASP-MFM become symptomatic in the fourth to seventh decades of life with proximal and distal muscle weakness of lower limbs, partially accompanied by cardiomyopathy.[2] The LIM domain-binding 3 (*LDB3*) gene encodes ZASP,[3] which is present in the skeletal muscle and myocardium.[4] Myopathological changes are characterized by abnormal protein accumulation in muscle fibers. The mutations in the *LDB3* gene cause decrease in connections between Z-disc and thin filament, which are associated with MFM and cardiomyopathy.[5]

In this study, we identified a Chinese family with an autosomal dominant inherited ZASP-MFM and the presence of a novel *LDB3* point mutation within the family.

METHODS

There were two members (the proband and his brother, II-3 and II-1) with ZASP-MFM in this particular Chinese family, which was confirmed with the muscle MRI and muscle biopsy and heterozygous mutation in the Department of Neurology at the First Affiliated Hospital of Chongqing Medical University, Chongqing, China. A detailed medical history was obtained for all family members. The members of the ZASP-MFM pedigree were all of Chinese Han origin and settled in China. All family members underwent clinical physical examination, serum creatine

Address correspondence to: Fei Xiao, Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Neurology, 1st You Yi Road, Chongqing 400016, China. E-mail address: feixiao81@126.com

Date of Submission: 17 October 2020; Date of Acceptance: 24 February 2021

kinase, electromyogram and echocardiography examination. Muscle MRI and genetic test were conducted for the affected pedigree members. After obtaining informed consent from the subjects included in this study, the Medical Ethics Committee of the First Affiliated Hospital of Chongqing Medical University approved the study protocol.

Muscle MRI

Axial plane images of the thighs and lower leg muscles (II-1 and II-3) were taken using a 3.0-Tesla MRI scanner (Signa Excite HD, GE Medical System, Milwaukee, WI, USA) as described earlier.[6] Assessment of the fatty infiltration, edema, and muscle bulk for 17 muscles from the proband (II-3) and his brother (II-1) was according to the Laminin-Mercuri classification: 0, normal muscle; 1, punctate hyperintense lesions; 2, scattered hyperintense lesions, which accounted for less than 30% of the muscle volume; 3, scattered fused hyperintense lesions, which accounted for 30–60% of the muscle volume; 4, large sheets of fused hyperintense lesions, which accounted for over 60% of the muscle volume; and 5, all muscle tissue being replaced by fat tissue.[7]

Muscle biopsy

Open muscle biopsy was performed from the index case and the muscle specimen was from the left musculi tibialis anterior. Further immunohistochemical staining was performed by using an antibody against ZASP at a dilution of 1:200 (Abcam, Cambridge, UK).[6] The ultrastructure of the muscle specimens was examined by standard procedures.

Genetic test and in silico analysis

Whole exome sequencing was carried out for the proband. Mutation identification was by Sanger sequencing. The sequence of *LDB3* was obtained from the GenBank human genome database (https://www.ncbi.nlm.nih.gov/). Multiple *LDB3* protein sequence alignment was compared across the species. Polymorphism Phenotyping 2 (PolyPhen-2) software (http://geneics.bwh.harvard.edu/pph2/), and MutationTaster (http://www.mutationtaster.org/) was applied to predict the potential functional impact of *LDB3* mutations.

RESULTS

Clinical findings

The proband (II-3), a forty-year-old man presented with progressive bilateral calf muscle atrophy for 27 years. The proband had difficulty running and jumping from 13 years of age with gradual progression and exhibited bilateral thigh muscle atrophy since the past five years. He had trouble walking, climbing stairs with frequent falls. He did not complain of myalgia or numbness. Physical examination showed symmetrical bilateral muscle atrophy in lower limbs with prominence in the distal region (Figure 1B). The muscle strength was 4+/5 (MRC Rating, the UK Medical Research Council Rating) in cervical muscle and shoulder girdle muscle, and 4/5 in pelvic girdle muscle and proximal and distal muscle of lower limbs. Serum creatine kinase (CK) elevated to 788 U/L (normal range 40-200 U/L). An electromyogram showed myogenic damage inaffected muscles. An electrocardiogram was intact. Echocardiography examination showed healthy cardiac structure with left ventricular diastolic dysfunction.

The proband reported that his mother (I-2) had developed lower limb atrophy and weakness starting from 20 years of age.

The elder brother (II-1) of the proband, a 44-year-old man, presented with bilateral calf muscle weakness and atrophy since age 15, and has progressed gradually for 10 years. Muscle weakness and atrophy progressed to the proximal muscle of lower extremities, and he had difficulty climbing stairs, squatting, and standing. Physical examination showed bilateral hip, thigh, and calf muscle atrophy with prominence in the distal region (Figure 1C). The muscle strength was 3/5 in quadriceps femoris muscle, 4/5 in other proximal muscle of lower limbs, and 5-/5 in the distal muscle of lower limbs. Electrocardiogram and echocardiography examinations were normal.

Muscle MRI

Lower limb MRIs showed similar involvement pattern for the proband (Figure 2A-B) and his brother (Figure 2C-D). Extensive symmetrical involvement of lower extremity muscles was observed (Figure 2A-D). The brother (II-1) had a more severe phenotype than the proband (II-3). The anterior and posterior muscles of the thighs were involved almost symmetrically, of which the semitendinosus, semimembranosus, vastus lateralis, vastus intermedius, vastus medialis, rectus femoris adductor magnus of the proband and

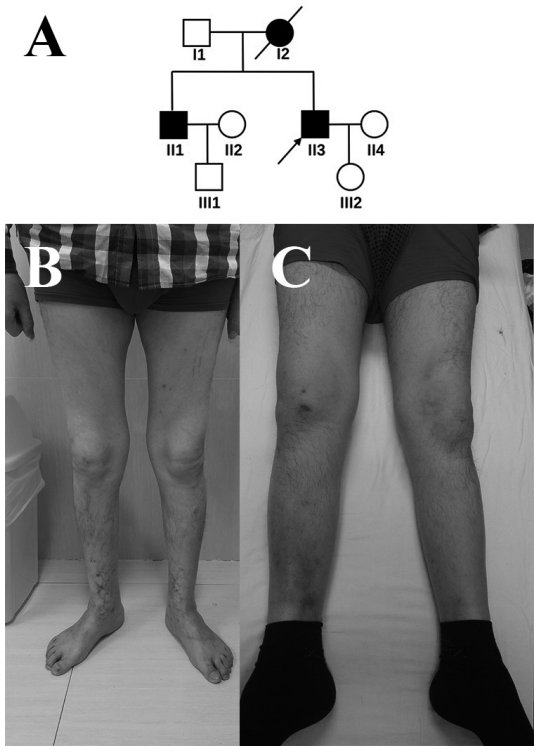

Figure 1. Pedigree diagram and clinical images in the siblings. The affected siblings showed muscle atrophy in the lower extremities, with involvement of both the thigh and calf muscles, and more pronounced involvement of the calf muscle. (A) Chinese family pedigree with Z-disc-associated, alternatively spliced, PDZ motif-containing protein (ZASP)-related myofibrillar myopathy (ZASP-MFM). Squares = males, circles = females, black symbols = affected patients, arrow = proband, oblique line = the deceased identity. (B) The proband (II-3). (C) The proband's brother (II-1).

his brother showed extensive fatty degeneration. Interestingly, the sartorius, gracilis, and adductor longus was slightly affected and relatively spared in both cases. Posterior muscle groups of the calf, including soleus and gastrocnemius, were mainly affected, while the anterior muscle groups were spared relatively.

Muscle biopsy

In the proband, myopathological changes showed myogenic changes or muscular dystrophy-like changes (Figure 2E). Muscle fibers showed moderate immunoreactivity to ZASP by immunohistochemical staining (Figure 2F). Electron microscopy demonstrated myofibril disorganization (Figure 2G).

Genetic test

A novel heterozygous mutation c.1720G>A in the *LDB3* gene was identified in the proband, which results in the substitution of glutamic acid (E) with lysine at residue 574 (p.E574K) in the protein. An identical heterozygous mutation was also detected in the elder brother of the proband (II-3). The c.1720G>A mutation was absent in other affected individuals in the literature, Human Gene Mutation Database (HGMD), or ClinVar. The amino acid p.E574K substitution was "probably damaging" and may disrupt the function of *LDB3* as predicted by the PolyPhen-2 software. Mutation Taster predicted the c.1720G>A in the *LDB3* gene is a functional "disease-causing" mutation.

DISCUSSION

MFM was characterized by myofibrillar-associated protein aggregation as described by De Bleecker *et al*. in 1996.[8] Selcen and Engel first described ZASP-MFM in 2005 in a series of 11 patients with an autosomal dominant family history[9], on an average, onset was in the sixth decade and presented mainly with distal myopathy. *LDB3* is located on chromosome 10 and binds to α-actinin to maintain the integrity of myofibrils. ZASP-MFM is autosomal dominant, which is due to the substitution of a single amino acid in the ZASP, causing late-onset distal myopathy.

Several *LDB3* mutations have been reported in Caucasians, in which p.A165V mutation was the most frequent.[2] However, there were just two Asian ZASP-MFM cases reported so far, one in a Japanese patient[10] and the other in a Chinese family.[11] It has been reported that patients with zaspopathy are late-onset, slow-progressive, with symmetrical weakness in both the proximal and distal lower limbs, and without upper limb involvement and respiratory insufficiency.[2] The cardiac manifestation was rare and usually mild. Interestingly, the onset age in our pedigree was before the second decade, and therefore earlier than previously reported cases. Upper limbs, and cardiac and respiratory systems were not involved. Abnormal protein aggregates, as evidenced by immunoreactivity to ZASP, were present within the muscle fibers in the proband.

In our patients, muscle MRI findings resembled those from previous patients with ZASP-MFM.[12] The severity of fatty infiltration was higher in the elder brother. We hypothesized that the severity of fatty infiltration might be associated with the disease course, and requires confirmation by long-term follow-up and dynamic MRI scan.

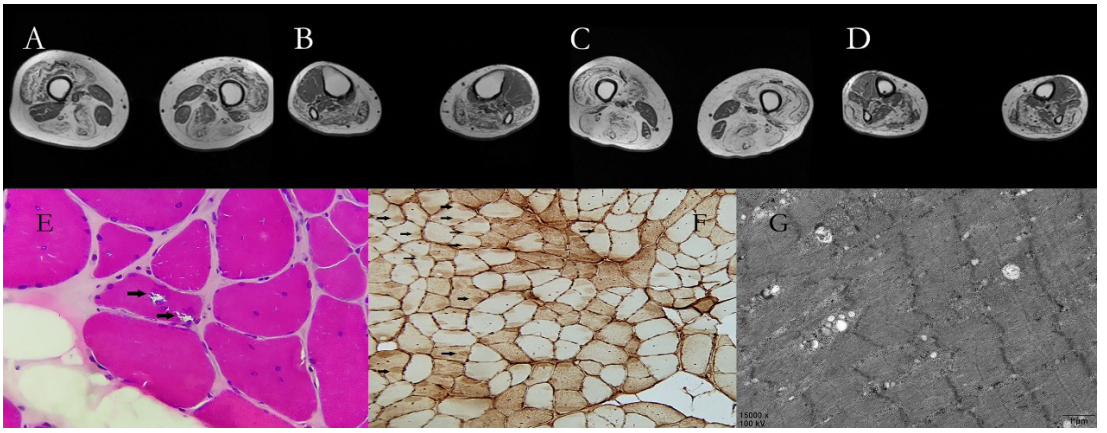

Figure 2. Muscle MRI findings in the siblings and histochemical characterization of the proband. The muscle MRI showed similar involvement pattern in the proband and his brother, in which involvement of the anterior and posterior muscles of the thighs was almost symmetrical and showed extensive fatty degeneration. (A) Thigh of the proband. (B) Calf of the proband. (C) Thigh of the proband's brother. (D) Calf of the proband's brother. (E) Hematoxylin-eosin staining showed different sizes of muscle fibers, nuclei internalization, moderate connective tissue hyperplasia, and rimmed vacuoles (arrows) in the muscle fibers; magnification: ×400. (F) ZASP staining showed moderately immunoreactive amorphous deposits in the muscle fibers; magnification: ×100. (G) Electron microscopy showed disorganization of the structure of Z-disks in some areas (arrow); magnification: ×15,000.

Combined with the typical clinical, muscle MRI manifestations, and abnormal ZASP accumulation in the muscle of the proband, and missense mutation in *LDB3*, we confirmed the diagnosis. Thus, the novel heterozygous mutation was regarded as pathogenic; however, further functional studies will aid to clarify the exact mechanism of the pathogenesis in the future.

In conclusion, we demonstrated a pedigree diagnosed clinically with ZASP-MFM and genetically with a novel c.1720G>A mutation in the *LDB3* gene and suggest that their young onset of the family in the 2nd decade different from the patients sharing a same mutation.

ACKNOWLEDGEMENTS

We thank the patients for their willingness to take part in this study. This work was supported by Scientific and Technological Research Projects from Chongqing Education Commission of China, Grant number: KJQN201800420, Joint project of Chongqing Health Committee and science and Technology Bureau (2019MSXM075), and the Fifth Senior Medical Talents Program of Chongqing for Yong and Middle-aged.

DISCLOSURE

Conflicts of interest: None

REFERENCES

1. Béhin A, Salort-Campana E, Wahbi K, *et al*. Myofibrillar myopathies: state of the art, present and future challenges. *Revue Neurologique* 2015; 171(10): 715-29.
2. Griggs R, Vihola A, Hackman P, *et al*. Zaspopathy in a large classic late-onset distal myopathy family. *Brain* 2007; 130(6):1477-84.
3. Jani K, Schock F. Zasp is required for the assembly of functional integrin adhesion sites. *J Cell Biol* 2007, 179(7):1583-97.
4. Faulkner G, Pallavicini A, Formentin E, *et al*. ZASP: A New Z-band alternatively spliced PDZ-motif protein. *J Cell Biology* 1999; 146(2):465-75.
5. Lin X, Ruiz J, Bajraktari I, Ohman R, *et al*. Z-disc-associated, Alternatively Spliced, PDZ Motif-containing Protein (ZASP) Mutations in the actin-binding domain cause disruption of skeletal muscle actin filaments in myofibrillar myopathy. *J Biol Chem* 2014; 289(19):13615-26.
6. Tan J, Yang H, Fan J, *et al*. Patients with neutral lipid storage disease with myopathy (NLSDM) in Southwestern China. *Clin Neurol Neurosurg* 2018: S0303846718300969.
7. Li W, Zheng Y, Zhang W, Xiao J, Yuan Y. Progression and variation of fatty infiltration of the thigh muscles in Duchenne muscular dystrophy, a muscle magnetic resonance imaging study. *Neuromuscul Disord* 2015; 25(5):375-80.
8. Nakano S, Satoshi, Engel, *et al*. Myofibrillar myopathy with abnormal foci of desmin positivity. *J Neuropath Exp Neurol* 1996; 55(5):549-62.
9. Selcen D, Engel AG. (2005). Mutations in ZASP define a novel form of muscular dystrophy in humans. *Ann Neurol* 2005, 57(2):269-76.

10. Cai H, Yabe I, Sato K, *et al*. Clinical, pathological, and genetic mutation analysis of sporadic inclusion body myositis in Japanese people. *J Neurol* 2012; 259(9):1913-22.
11. Zheng J, Chen S, Chen Y, Zhu M, Hong D. A novel mutation in the PDZ-like motif of ZASP causes distal ZASP-related myofibrillar myopathy. *Neuropathology* 2017;37(1):45-51.
12. Olivé M, Odgerel Z, Martínez A*, et al*. Clinical and myopathological evaluation of early- and late-onset subtypes of myofibrillar myopathy. *Neuromuscul Disord* 2011, 21(8):533-42.