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Novel LDB3 Mutation in a Patient With Autosomal Dominant Myofibrillar Myopathy

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Abstract

Introduction: Myofibrillar myopathy (MFM) is a rare human disease, characterized by a distinct histopathological pattern of myofibrillar degeneration and protein aggregates. LDB3 protein encoded by this gene is a key Z-disk protein that interacts with α-actinin and protein kinase C.

Case Presentation: In this paper, we identified the novel heterozygous, and hence, dominant mutation in the LIM domain-binding protein 3 gene (LDB3) in a patient affected by myofibrillar myopathy (MFM). We performed direct sequencing in an Iranian patient with autosomal-dominant inheritance of MFM characterized by clinical features, and we identified a heterozygous missense mutation in exon 10, c.1687A > G (p.Ile563Val) in the LDB3 gene on chromosome 10:88476524.

Conclusions: Bioinformatics analyses using SIFT, Mutation Taster and Polyphen-2 indicated that p.Ile563Val was predicted to be damaging, disease causing, and probably damaging to and causing LDB3 dysfunction. As such, this mutation produces novel protein coding transcripts, which might explain the MFM phenotype in the patient.

Keywords: Myofibrillar Myopathy, LDB3 Protein, Human, Sequence Analysis, DNA

1. Introduction

Myofibrillar myopathies (MFM, [MIM 601419]) are a group of clinically and genetically heterogeneous neuromuscular disorders defined by ectopic expression of proteins, such as desmin, and myofibrillar disorganization starting at the Z-disk and protein aggregates in muscle fibers (1). The clinical phenotypes of myofibrillar myopathies are widely heterogeneous. Patients usually present with progressive muscle weakness that can involve both proximal and distal muscles, with the age of onset ranging from infancy to late in adulthood, but in most cases the symptoms appear in the fourth and fifth decades. However, other features are extremely variable. The diagnosis of MFM is frequently difficult because of the substantial phenotypic and pathomorphological variability (2, 3). In recent years, an increasing number of genes have been recognized to be involved in MFM pathogenesis, causing subgroups of the disease. Until now, mutations in nine genes have been identified to cause MFM: titin (TTN), DNA J homolog subfamily B member 6 (DNAJB6), bcl-2 associated athanogene protein 3 (BAG3), four and a half LIM domain protein 1 (FHL1), Z-band alternatively spliced PDZ containing protein (ZASP, also LDB3), αB-crystallin (CRYAB), desmin (DES), filamin C (FLNC) and myotilin (MYOT, also TTID) (4-11). More than 50% of cases are caused by unresolved gene defects. In this report, we describe the first dominant acting heterozygous mutation in the LDB3 gene, which affects a family with severe myofibrillar myopathy. This mutation produces novel protein coding transcripts that might explain the MFM phenotype in the patient.

2. Case Presentation

The patient was a 21-year-old man, and the last child of consanguineous parents of southwest Iran. He was admitted to the neurologist for investigation of slowly progressive walking difficulties that began two years previously. He also had slowly progressive hand and foot weakness (distal muscles) and occasional stiffness and cramping of the leg muscles after exercise. He had severe weakness of toe extensor, anterior tibial and peroneal muscles, and mild weakness of iliopsoas, quadriceps, hamstring and finger extensor muscles. His parents were relative (his father died at age 70 and had suffered from Parkinson and his mother was 60 years old and was reported to be unaffected).

Genomic DNA was extracted from peripheral leukocytes of the patient and controls, using standard procedures (12), and PCR sequencing was performed to investigate the possible cause of muscular dystrophies, including analysis of the mutations in LDB3 (ZASP) in the patient with MFM phenotypes.

PCR was conducted under the following conditions: 200 μM deoxyribonucleotide triphosphates (dNTPs), 100 ng genomic DNA, 2.5 units supertaq polymerase, 1.5 mm

MgCl, and 25 pmol each primer (Table 1) (13). Amplification was carried out in 25 μL volumes and 35 cycles: 94°C for one minute, 65°C for 35 seconds and 72°C for one minute. The sequencing reactions were carried out and the sequences were compared to the reported gene sequence using the BLASTN program.

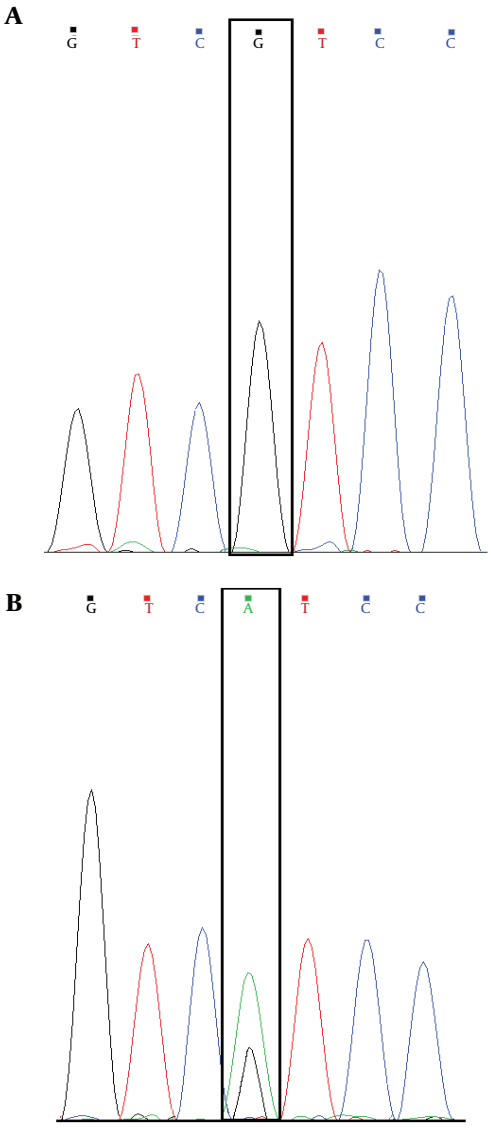
Table 1. Primers Used for the Screening of the LDB3 Gene

Exon/Primer	Primer Sequence (5 - 3) T	PCR Product Size, bp
1		221
ZASP 1F	GTGCCCTCTCACTCAACCCT	
ZASP 1R	ACACATGCCCTCCTCCAAGC	
2		335
ZASP 2F	TGGCCTTTCCTCAGGACCAC	
ZASP 2R	TCCTGCACAGTTTTGTAGCC	
3		230
ZASP 3F	TGACTCTGGCTCTCTCTTGCT	
ZASP 3R	TCCAGGAACCAGGGCTGAGT	
4		506
ZASP 4F	GGCTCGCGCTAACACATCTG	
ZASP 4R	GCCACCTGTGGAGAGCTGTA	
5		266
ZASP 5F	CACTCCTTGCTCTCCTCACC	
ZASP 5R	CTCTATCCACGCCAGACACA	
6		380
ZASP 6F	TGTAACCGCCACCTGTTGCC	
ZASP 6R	TCCAGGAGGTCCAACGTGAG	
7		353
ZASP 7F	CCACCAATGGGCATGGAGCA	
ZASP 7R	AGCAGGACTCCCTGGCTTCT	
8		178
ZASP 8F	TTGCTGTGTCTCCCGTGAGT	
ZASP 8R	GAGGTCCCTTCCATGAGTGA	
9		317
ZASP 9F	GGTGAACACATTCCCTAACC	
ZASP 9R	CCCAGCAGAGTTATACATTG	
10		331
ZASP 10F	GCTCCCTTGACCTGTTGTCT	
ZASP 10R	GCCCTAACTACCTTGGACAC	
11		257
ZASP 11F	GGCTGTCCTTCTGGGTGTAA	
ZASP 11R	TCTTGGCTCTTGTGGCTCCT	
12A		348
ZASP 12AF	CATTTCTCTGGCTAGGAGTG	
ZASP 12AR	CTGGGAGAAGCTATCATCTG	
12B		352
ZASP 12BF	TGCACCCTCGGTGGCCTACA	
ZASP 12BR	CTCCCAACCAGGGCTCAGAC	
13		267
ZASP 13F	GTTCTGGGAGCTGCCTTACT	
ZASP 13R	GGAAGAGACATGGGTCAGAG	
14		200
ZASP 14F	AGTCAAGCCCGCTCCCTCTC	
ZASP 14R	CACATGCCATCGAAGTGTTC	
15		290
ZASP 15F	TGATTTGGGGTTTGTCTTGG	
ZASP 15R	CTAGCGTGGCAAGGTATGTA	
16		229
ZASP 16F	GTCTCACGCAGGTCTGTTCT	
ZASP 16R	GCTTCCTCTCTCTCCCCATT	

The search for rare variants (MAF, 1%) that were specifically found in the affected man was carried out with different open access Web tools. The effect of the candidate variant in protein structure and phylogenetic conservation was predicted by using bioinformatics tools, such as PolyPhen-2 (Polymorphism Phenotyping v2), SIFT (Sort Intolerant from Tolerant) and Mutation Taster, to estimate the pathogenicity risk for the variant.

Analysis of DNA sequences in coding exons of the LDB3 gene represents a novel heterozygous missense. So far, the substitution (the c.1687A > G, p.Ile563Val mutation in exon 10 of LDB3 (Figure 1)) has neither been observed as a polymorphism in the latest 1000 Genomes Project databases, nor as a causative mutation in any accessible disease mutation database (for example, HGMD: Human Gene Mutation Database) (Table 2).

Figure 1. The Result of DNA Sequencing



A, Normal Control; B, a Patient; c.1687ATC > GTC heterozygous missense mutation (p.Ile563Val) in exon 10 of LDB3 gene was detected in the patient.

Table 2. Missense Mutations Available of the LDB3 Gene in the HGMD

Codon Change	Amino Acid Change	Codon Number	Phenotype	Reference
GAC/AAC	Asp/Asn	117	Cardiomyopathy, dilated	(13)
AAG/ATG	Lys/Met	136	Cardiomyopathy, dilated	(13)
GCC/ACC	Ala/Thr	147	Myofibrillar myopathy	(8)
GCC/GTC	Ala/Val	165	Myofibrillar myopathy	(8)
GCC/ACC	Ala/Thr	174	Myofibrillar myopathy	(14)
CGC/TGC	Arg/Cys	268	Myofibrillar myopathy	(8)
ATC/GTC	Ile/Val	563	Myofibrillar myopathy	Present study. 2015

Table 3. Various in Silico Bioinformatics Tools Have Been Developed That Predict the Novel Mutation

LDB3/Software	SIFT Score	PolyPhen Score	Mutation Taster
ENST00000429277, I563V	0.01 (DAMAGING)	0.993 (PROBABLY DAMAGING)	Disease causing

LIM domain binding 3 (LDB3 [ENSG00000122367]) is at position chr10:88476524 A.G. In humans, LDB3 has nine annotated protein coding transcripts. The novel mutation produces an amino acid alter (Ile to Val) that modifies four of the nine coding isoforms predicted in the Ensembl database, and the function of this mutation is unknown.

Bioinformatics analyses indicate that the I563V mutation most probably causes LDB3 dysfunction, leading to the MFM clinical phenotype. Furthermore, analysis using various programs, e.g., SIFT (deleterious, score 0.00), Mutation Taster (disease causing, P value 1.0) and Polyphen-2 (probably damaging, score 1.00) indicated p.Ile563Val was predicted to be disease causing and probably damaging (Table 3). Unfixed muscle tissue of this patient was not accessible and no muscle biopsy could be obtained in order to conduct supplementary proteomic analyses for further clarification. Furthermore, family members were not accessible for segregation analysis to clarify the pathogenicity.

3. Discussion

In the present study, using direct sequencing, we looked for mutations in this patient in the LDB3 gene previously associated with MFM or muscular dystrophy. We identified the c.1687A > G (p.Ile563Val) mutation in exon 10 of this gene in members of a consanguineous family with an autosomal dominant mode of inheritance with severe MFM. So far, this mutation has not been reported in any of the information banks. All bioinformatics tools applied classified the identified substitution as pathogenic.

The LDB3 gene (OMIM: 605906) has 16 exons and spans approximately 70 kb (15). There are three isoforms of LDB3 in human skeletal muscle, which are produced by alternative splicing of exons 9 and 10. The prenatal long isoform (ZASP-L) contains exon 10 and the postnatal long isoform (ZASP-Ldelex10) lacks exon 10. Both long isoforms include a PDZ domain, ZASP-like motif encoded by exon 6 and 3 LIM domains. The short isoform (ZASP-S) lacks the LIM domains, because it has a stop codon in exon 9 (15). PDZ domain-containing proteins interact with a number of proteins involved in clustering and targeting of membrane proteins or with each other in cytoskeletal assembly. LIM domain-binding protein 3 encoded by this gene is a key Z-disk protein that interacts with α-actinin and protein kinase C. LDB3 protein also interacts with all members (MYOZ1, MYOZ2, MYOZ3) of the myozenin family (16, 17).

The three different missense mutations in the LDB3 gene (A147T, A165V, and R268C), recently published by Selcen and Engel, were found in patients in a heterozygous form, causing myofibrillar myopathy. The first two mutations occurred in exon 6, but R268C occurred in exon 9 (8). Since both mutations were detected on the same gene in LDB3, a similar phenotype might be expected in the patients of both families such as progressive proximal and/or distal weakness, cardiac involvement and peripheral neuropathy. Mutant LIM domain-binding protein 3 (ZASP) is predicted to weaken the linkage of Z-disk filaments to thin filaments. This novel mutation (c.1687A > G (p.Ile563Val) mutation in exon 10) may change the function of the ZASP-L and LIM domains.

In summary, in this study we identified by direct sequencing the first dominant and heterozygous mutation in the LDB3 gene causing MFM and the first in a non-European patient. Consequently, defects in this protein could destabilize the muscle cell membrane and, at the same time, weaken myofibrils. This may eventually result in clinical and molecular features resembling MFM. Our finding confirms previous reports that the muscle phenotype associated with LDB3 mutations is consistent.

References

1. Olive M, Kley RA, Goldfarb LG. Myofibrillar myopathies: new developments. *Curr Opin Neurol.* 2013;**26**(5):527–35. doi: 10.1097/WCO.0b013e328364d6b1. [PubMed: 23995273]
2. Schroder R, Schoser B. Myofibrillar myopathies: a clinical and

myopathological guide. *Brain Pathol.* 2009;**19**(3):483–92. doi: 10.1111/j.1750-3639.2009.00289.x. [PubMed: 19563540]
3. Ferrer I, Olivé M. Molecular pathology of myofibrillar myopathies. *Expert Rev Mol Med.* 2008;**10**:e25.
4. Goldfarb LG, Park KY, Cervenakova L, Gorokhova S, Lee HS, Vasconcelos O, et al. Missense mutations in desmin associated with familial cardiac and skeletal myopathy. *Nat Genet.* 1998;**19**(4):402–3. doi: 10.1038/1300. [PubMed: 9697706]
5. Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A, et al. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. *Nat Genet.* 1998;**20**(1):92–5. doi: 10.1038/1765. [PubMed: 9731540]
6. Selcen D, Engel AG. Mutations in myotilin cause myofibrillar myopathy. *Neurology.* 2004;**62**(8):1363–71. [PubMed: 15111675]
7. Vorgerd M, van der Ven PF, Bruchertseifer V, Lowe T, Kley RA, Schroder R, et al. A mutation in the dimerization domain of filamin c causes a novel type of autosomal dominant myofibrillar myopathy. *Am J Hum Genet.* 2005;**77**(2):297–304. doi: 10.1086/431959. [PubMed: 15929027]
8. Selcen D, Engel AG. Mutations in ZASP define a novel form of muscular dystrophy in humans. *Ann Neurol.* 2005;**57**(2):269–76. doi: 10.1002/ana.20376. [PubMed: 15668942]
9. Selcen D, Bromberg MB, Chin SS, Engel AG. Reducing bodies and myofibrillar myopathy features in FHL1 muscular dystrophy. *Neurology.* 2011;**77**(22):1951–9. doi: 10.1212/WNL.0b013e31823a0ebe. [PubMed: 22094483]
10. Selcen D, Muntoni F, Burton BK, Pegoraro E, Sewry C, Bite AV, et al. Mutation in BAG3 causes severe dominant childhood muscular dystrophy. *Ann Neurol.* 2009;**65**(1):83–9. doi: 10.1002/ana.21553. [PubMed: 19085932]
11. Sato T, Hayashi YK, Oya Y, Kondo T, Sugie K, Kaneda D, et al. DNAJB6 myopathy in an Asian cohort and cytoplasmic/nuclear inclusions. *Neuromuscul Disord.* 2013;**23**(3):269–76. doi: 10.1016/j.nmd.2012.12.010. [PubMed: 23394708]
12. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;**16**(3):1215. [PubMed: 3344216]
13. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z, et al. Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. *J Am Coll Cardiol.* 2003;**42**(11):2014–27. [PubMed: 14662268]
14. Olive M, Odgerel Z, Martinez A, Poza JJ, Bragado FG, Zabalza RJ, et al. Clinical and myopathological evaluation of early- and late-onset subtypes of myofibrillar myopathy. *Neuromuscul Disord.* 2011;**21**(8):533–42. doi: 10.1016/j.nmd.2011.05.002. [PubMed: 21676617]
15. Lin X, Ruiz J, Bajraktari I, Ohman R, Banerjee S, Gribble K, 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. doi: 10.1074/jbc.M114.550418. [PubMed: 24668811]
16. Leung MC, Hitchen PG, Ward DG, Messer AE, Marston SB. Z-band alternatively spliced PDZ motif protein (ZASP) is the major O-linked beta-N-acetylglucosamine-substituted protein in human heart myofibrils. *J Biol Chem.* 2013;**288**(7):4891–8. doi: 10.1074/jbc.M112.410316. [PubMed: 23271734]
17. Faulkner G, Pallavicini A, Formentin E, Comelli A, Ievolella C, Trevisan S, et al. ZASP: a new Z-band alternatively spliced PDZ-motif protein. *J Cell Biol.* 1999;**146**(2):465–75. [PubMed: 10427098]