Genes & Diseases (2018) 5, 331–334

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

SHORT COMMUNICATION

Identification of a novel nonsense mutation in kyphoscoliosis peptidase gene in an Iranian patient with myofibrillar myopathy

Reza Ebrahimzadeh-Vesal [a], Atieh Teymoori [b], Ali Mohammad Dourandish [c], Mohsen Azimi-Nezhad [d,*]

[a] *Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran*
[b] *Department of Medical Genetics, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*
[c] *Medical Genetics Counseling Center, Neyshabur, Iran*
[d] *Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Received 11 August 2018; accepted 28 September 2018
Available online 2 October 2018

KEYWORDS
Kyphoscoliosis peptidase gene;
Myofibrillar myopathy;
Next generation sequencing;
Novel mutation;
Rare genetic neuromuscular disorders

Abstract Myofibrillar myopathies (MFMs) are rare genetic and slowly progressive neuromuscular disorders. Several pathogenic mutations have been reported in MFM-related genes including *DES*, *CRYAB*, *MYOT*, *LDB3* or *ZASP*, *FLNC*, *BAG3*, *FHL1* and *DNAJB6*. Although MFMs is commonly inherited in an autosomal dominant manner, the inheritance pattern and novel mutated genes are not thoroughly elucidated in some cases. Here, we report discovery of a novel nonsense mutation in a 29-year-old Iranian male patient with motor disorders and deformity in his lower limbs. His parents are second cousins. Hereditary Motor Sensory Neuropathy as initial genetic diagnosis was ruled out. Whole exome sequencing using NGS on Illumina HiSeq4000 platform was performed to identify the disease and possible mutated gene(s). Our data analysis identified a homozygous nonsense unreported c.C415T (p.R139X) variant on kyphoscoliosis peptidase (*KY*) gene (NM_178554: exon4). Sanger sequencing of this mutation has been performed for his other related family members. Sequencing and segregation analysis was confirmed the NGS results and autosomal recessive inheritance pattern of the disease.

* Corresponding author.
E-mail address: aziminm@mums.ac.ir (M. Azimi-Nezhad).
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2018.09.004

Introduction

Myofibrillar myopathies (MFMs) are among rare genetic neuromuscular disorders which are characterized by slowly progressive weakness in both proximal (near to the center of the body) and distal (the hands and feet) skeletal muscles and smooth muscular dysfunctions.[1] According to the genetic background of affected individuals, MFM could be associated with diverse clinical manifestations.[2] Although MFM may be diagnosed during childhood, the clinical features of the disease often appear in mid-adulthood (Ferrer & Olivé, 2008). In addition to the major skeletal muscle disorders, facial muscles weakness usually leads to different problems in speech and swallowing.[3] Moreover, overt cardiomyopathy with arrhythmia, conduction defects and/or congestive heart failure has been reported in some cases. Sensory symptoms, muscle stiffness, aching and cramps are uncommon complications in MFM patients.[4]

The diagnosis of MFM is usually based on clinical symptoms, electromyogram (EMG), nerve conduction studies and muscle biopsy.[5] Several mutations have been reported across exons and exon-intron boundaries of MFMs related genes. They are mostly associated with filament proteins of muscle fibers, including *DES* (desmin),[6] *CRYAB* (alpha-crystallin B chain),[7,8] *MYOT* (myotilin),[9] *LDB3* or *ZASP*(LIM domain-binding protein 3),[10] *FLNC* (filamin-C),[11] *BAG3* (BAG family molecular chaperone regulator 3),[11] *FHL1* (four and a half LIM domains protein 1)[2] and *DNAJB6* (DnaJ homolog subfamily B member 6).[12] MFMs usually inherited in an autosomal dominant manner. Although, *FHL1* gene inherited in X-linked recessive pattern.[13] However, the inheritance pattern in some families is not thoroughly elucidated which needs the characterization of pathogenic variants and segregation analysis in the family. Regarding the complications in screening the mutations of MFM-related genes by conventional methods, molecular genetics approaches using next generation sequencing (NGS) can be more precise and helpful in identification of affected patients. In this article, whole exome sequencing has been performed to identify the possible causing mutations for a male patient with foot deformities and muscular weakness.

Material and methods

Case presentation

A 29-year-old male patient with motor disorders and deformities in his lower limbs was referred to Hakim hospital (Neyshabour, Iran) from the welfare administration. He was unable to normal movement and has walking difficulties. His muscular weakness symptoms appeared at the age of about 3. His lower limbs were completely affected with weakness and muscle atrophy. His both legs have equinovarus foot deformity. He has mild scoliosis and joint contracture. His facial appearance and upper limbs muscles were normal. His IQ was normal.

His parents are second cousins. His affected sister has muscle weakness. His aunt (mother-side) was died with similar clinical symptoms. His lactate dehydrogenase (LDH = 575 IU/L) and creatine phosphokinase (CPK = 308 IU/L) showed abnormally high level in the serum. He did not perform electromyogram (EMG) and muscle biopsy. Written informed consent form was obtained from the all of the participant from this study.

Hereditary motor sensory neuropathy NGS panel

Molecular diagnosis of hereditary motor sensory neuropathy was performed using targeted next generation sequencing panel including 37 genes to identify mutations; *AARS*, *DNM2*, *DYNC1H1*, *EGR2*, *FGD4*, *FIG4*, *GARS*, *GDAP1*, *GJB1*, *HSPB1*, *HSPB8*, *KARS*, *KIF1B*, *LITAF*, *LMNA*, *LRSAM1*, *MED25*, *MFN2*, *MPZ*, *MTMR2*, *NDRG1*, *NEFL*, *PMP22*, *PRPS1*,*PRX*, *RAB7A*, *SBF2*, *SBF1*, *SH3TC2*, *TRPV4*, *YARS*, *BSCL2*, *INF2*, *AIFM1*, *DHTKD1*, *PDK3*, *GNB4)*.This panel was selected per request and diagnosis of his neurologist. The panel was performed using a custom designed Nimblegen chip capturing the genes of interest followed by Next Generation Sequencing (NGS). A heterozygous c.3681C > T (p. Ala1227=) variant with uncertain clinical significance on *SH3TC2* gene was detected. After segregation analysis and pedigree validation, clinical significance of this variant was excluded.

Whole exome sequencing (WES)

Whole exome paired-end sequencing analysis with 100X coverage was performed on extracted genomic DNA from peripheral blood of the proband to identify the disease causing mutations. Human whole exome enrichment was performed using Agilent Sure Select V6 Target Enrichment Kit, followed by highly specific and sensitive NGS method using Illumina HiSeq4000 platform. This method allows detection of point mutations and small indels (≤20 bp). Sequencing of all human exons and flanking 10 bp sequence plus UTR regions of >23000 genes was accomplished.

Sanger sequencing

The genomic DNA was extracted from peripheral blood using high pure PCR template preparation kit (Roche) according to the manufacturer's protocol. The specific forward (5′-GCCATGGTTCCCAAACTAAC-3′) and reverse (5′-CTGCCTAGTGCCTTCTGTCC-3′) primers were designed to validate the c.C415T (p. R139X) mutation on *KY* gene using Sanger sequencing. Each PCR cycle consisted of denaturation at 94 °C for 20 s, primer annealing at 58 °C and extension at 72 °C for 30 s for each one, followed by a final extension at 72 °C for 5 min to amplify 449 bp PCR products. The PCR reactions were performed using ready to use 2X master mix as manufacturer's protocol (Promega). The purified PCR products were sequenced by ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Results

WES results indicated a homozygous nonsense c.C415T (p. R139X) variant on kyphoscoliosis peptidase (*KY*) gene. This variant has not been reported for its pathogenicity. Its clinical significance is not thoroughly elucidated. Its frequency in normal population is very low (0.00008 in the NHLBI Exome Sequencing Project, and it is not found in

GnomAD). It is predicted to be harmful because the nonsense variant which resulted in the conversion of Arg-139 amino acid to termination codon most likely leads to activate nonsense-mediated mRNA-decay pathway. Also, this abnormal nonsense variant may leads to produce a truncated polypeptide and loss of normal function of protein.

The *KY* gene is located on long arm of the chromosome 3 (3q22.2) and has 11 exons. This variant there is in exon 4. The *KY* gene is associated with autosomal recessive myopathy myofibrillar7 (MFM7) (Hedberg-Oldfors et al, 2016). Sanger sequencing of c.C415T variant has been performed for his parents (II.2 and II.3), his normal sister (III.1) and his affected sister (III.2) to validate its clinical significance. Also, carrier testing using Sanger sequencing has been performed for his wife (III.4) (Fig. 1). His aunt most likely had same mutation on *KY* gene. Although she may be had another type of neuromuscular disease since it is not possible to determine genetic status for her.

Sanger sequencing results indicated heterozygous genotype for his parents and homozygous T/T mutant genotype for his affected sister. His clinically normal sister and his wife showed homozygous C/C normal genotype (Fig. 2).

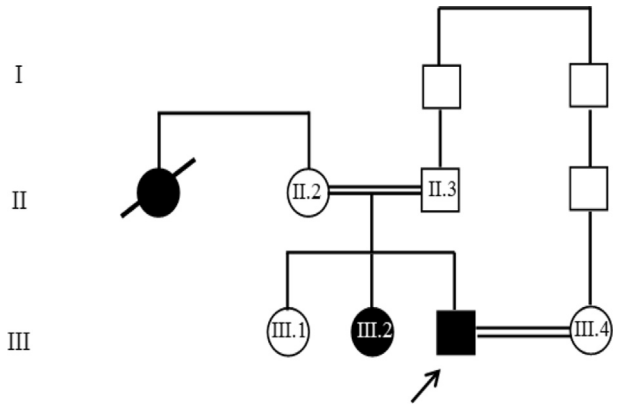

Figure 1 Pedigree of this family was shown. The arrow indicates the proband. His parents are second cousins.

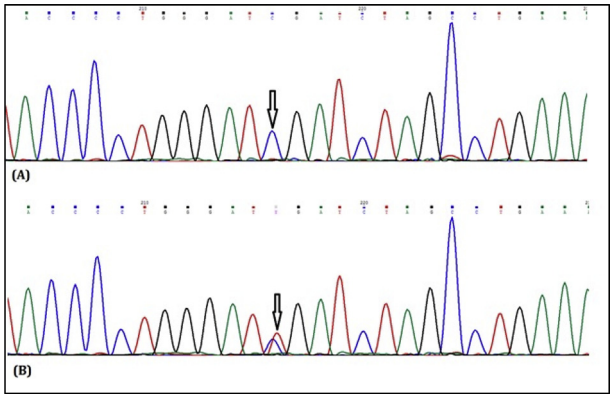

Figure 2 Sanger sequencing results of detected variant for related family members of the patient; Patient's sister has homozygous normal genotype (C/C) for c.C415T (p.R139X) variant on *KY* gene **(A).** Patient's father has heterozygous genotype (C/T) for c.C415T variant (p.R139X) on *KY* gene **(B)**.

Discussion

Kyphoscoliosis peptidase enzyme is involved in the function, maturation and stabilization of neuromuscular junctions. It is required for normal muscle growth.[14] *KY* gene mutations are associated with the same spectrum of autosomal recessive MFM7 disorder.[15] MFM7 (MIM: 617114) is a muscular disorder which is characterized by disintegration of the sarcomeric Z-disc and myofibrils. MFM7 is clinically characterized by early childhood onset of slowly progressive muscle weakness and mild atrophy primarily affecting the lower limbs which is associated with joint contractures. We have reported a novel homozygous nonsense c.C415T (p. R139X) variant on *KY* gene. Mutations and variants in *KY* gene have been also reported in the similar symptoms' congenital myopathy and neuromuscular disorders.[16,17] The patient of this study has equinovarus foot deformity in his two legs and muscular weakness in lower limbs and also mild scoliosis and joint contracture that all of this phenotypes were described as the symptoms of KY mutations in MFM7 and spastic paraplegia.[15,16]

Molecular diagnosis to identify pathogenic mutations in affected patients with MFMs should be considered. Therefore, genetic counseling could be recommended for all individuals with MFMs and their related family members. The carrier couples with identified mutated genes should performed prenatal diagnosis to prevent of born new affected child.

Conclusions

A rare pathogenic mutation in *KY* gene was identified in a 29-year-old patient with MFM disorder which supports the link between *KY* gene mutations and neuromuscular disorders of patients with *MFM*. Our report can be helpful in finding an appropriate genetic counseling and molecular diagnosis for affected individuals with *MFM*.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Selcen D. Myofibrillar myopathies. *Neuromuscul Disord NMD*. 2011;21(3):161—171. https://doi.org/10.1016/j.nmd.2010.12.007.
2. Schoser B, Goebel HH, Janisch I, et al. Consequences of mutations within the C terminus of the FHL1 gene. *Neurology*. 2009;73(7):543—551. https://doi.org/10.1212/WNL.0b013e3181b2a4b3.
3. Claeys KG, Fardeau M. Myofibrillar myopathies. *Handb Clin Neurol*. 2013;113:1337—1342. https://doi.org/10.1016/B978-0-444-59565-2.00005-8.
4. Selcen D. Myofibrillar myopathies. *Curr Opin Neurol*. 2008;21(5):585—589. https://doi.org/10.1097/WCO.0b013e32830a752b.
5. Schroder R, Schoser B. Myofibrillar myopathies: a clinical and myopathological guide. *Brain Pathol (Zurich, Switzerland)*. 2009;19(3):483—492. https://doi.org/10.1111/j.1750-3639.2009.00289.x.
6. Dalakas MC, Park KY, Semino-Mora C, Lee HS, Sivakumar K, Goldfarb LG. Desmin myopathy, a skeletal myopathy with

cardiomyopathy caused by mutations in the desmin gene. *N Engl J Med*. 2000;342(11):770—780. https://doi.org/10.1056/NEJM200003163421104.
7. Forrest KML, Al-Sarraj S, Sewry C, et al. Infantile onset myofibrillar myopathy due to recessive CRYAB mutations. *Neuromuscul Disord NMD*. 2011;21(1):37—40. https://doi.org/10.1016/j.nmd.2010.11.003.
8. Reilich P, Schoser B, Schramm N, et al. The p.G154S mutation of the alpha-B crystallin gene (CRYAB) causes late-onset distal myopathy. *Neuromuscul Disord NMD*. 2010;20(4):255—259. https://doi.org/10.1016/j.nmd.2010.01.012.
9. Selcen D, Engel AG. Mutations in myotilin cause myofibrillar myopathy. *Neurology*. 2004;62(8):1363—1371.
10. Selcen D, Engel AG. Mutations in ZASP define a novel form of muscular dystrophy in humans. *Ann Neurol*. 2005;57(2):269—276. https://doi.org/10.1002/ana.20376.
11. Shatunov A, Olive M, Odgerel Z, et al. In-frame deletion in the seventh immunoglobulin-like repeat of filamin C in a family with myofibrillar myopathy. *Eur J Human Genet EJHG*. 2009;17(5):656—663. https://doi.org/10.1038/ejhg.2008.226.
12. Sarparanta J, Jonson PH, Golzio C, et al. Mutations affecting the cytoplasmic functions of the co-chaperone DNAJB6 cause limb-girdle muscular dystrophy. *Nat Genet*. 2012;44(4):450—455. https://doi.org/10.1038/ng.1103. S1-2.
13. Selcen D, Engel AG. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Myofibrillar Myopathy*. 1993. Seattle (WA).
14. Blanco G, Coulton GR, Biggin A, et al. The kyphoscoliosis (ky) mouse is deficient in hypertrophic responses and is caused by a mutation in a novel muscle-specific protein. *Hum Mol Genet*. 2001;10(1):9—16.
15. Yogev Y, Perez Y, Noyman I, et al. Progressive hereditary spastic paraplegia caused by a homozygous KY mutation. *Eur J Hum Genet*. 2017;25(8):966.
16. Hedberg-Oldfors C, Darin N, Engman MO, et al. A new early-onset neuromuscular disorder associated with kyphoscoliosis peptidase (KY) deficiency. *Eur J Hum Genet*. 2016;24(12):1771.
17. Straussberg R, Schottmann G, Sadeh M, et al. Kyphoscoliosis peptidase (KY) mutation causes a novel congenital myopathy with core targetoid defects. *Acta Neuropathol*. 2016;132(3):475—478. https://doi.org/10.1007/s00401-016-1602-9.