A novel mutation in N-terminal actin-binding domain of DMD presenting recurrent exertional rhabdomyolysis: a case report

Jong-Mok Lee

Department of Neurology, Kyungpook National University Hospital Korea

Case Report

Keywords: Dystrophinopathy, actin-binding domain, novel mutation, rhabdomyolysis, metabolic myopathy, in-silico analysis

Posted Date: December 13th, 2018

DOI: https://doi.org/10.21203/rs.2.97/v1

Abstract

Background: Rhabdomyolysis associated with inherited conditions refers to recurrent episodes evoked by minimal exercise, history of malignant hyperthermia, familial history, or hyperCKemia between attacks.

Case presentation: We report a 17-year-old male presented recurrent exertional rhabdomyolysis with myoglobinuria and hyperCKemia between attacks mimicking metabolic myopathies. The immunoreactivity to C-terminal of dystrophin from the muscle biopsy was absent. Genetic analysis revealed a novel hemizygous *DMD* c.119T>A (p.Leu40His) nonsense mutation in the N-terminal actin-binding domain. In-silico test for the structure of dystrophin predicted to destabilize interaction in the vicinity by losing hydrophobicity at residue 40.

Conclusion: Muscular dystrophies including dystrophinopathies should not be overlooked in the case with recurrent rhabdomyolysis.

Keywords: Dystrophinopathy; actin-binding domain; novel mutation; rhabdomyolysis; metabolic myopathy; in-silico analysis

Background

Rhabdomyolysis refers to the acute muscle fiber necrosis, the breakdown of striated muscle [1]. Clinically, it is manifested as acute muscle weakness associated with myalgia. Dark, black colored urine may be accompanied owing to the breakdown of striated muscle, which can lead to acute renal insufficiency [1]. The major causes of rhabdomyolysis are acquired etiology, namely: physical exertion, crush injury, tissue ischemia, drugs and toxins [1]. However, underlying inherited conditions should be considered in the following situations: (1) recurrent rhabdomyolysis evoked by minimal exercise, fever, heat or cold exposure, or fasting, (2) previous history of malignant hyperthermia, (3) family history of rhabdomyolysis, malignant hyperthermia or myopathy, and (4) patients with hyperCKemia between attacks [2].

Repeated exertional rhabdomyolysis is a remarkable feature of inherited metabolic myopathies, however one of uncommon symptoms in muscular dystrophies [2]. Therefore, muscular dystrophies have a lower priority and can be overlooked during making a diagnosis. Here, we reported a patient with a novel mutation in *DMD* who presented as recurrent exertional rhabdomyolysis.

Case Presentation

A 17-year-old boy was referred to the department of neurology with recurrent myalgia following exercise. He had suffered an easy fatigability and myalgia several minutes after exercise such as running or tracking the mountain every time since childhood. These symptoms had recovered spontaneously within a day. The second wind phenomenon was not seen. Incidentally, he was found to have an elevated aspartate aminotransferase (73 U/L, normal range < 40 U/L, AST) and alanine aminotransferase (65 U/L,

normal range < 41 U/L) at age thirteen years old. Spontaneous recover had left him undiagnosed. A year later, he experienced a dark urine for a day after 500-meter running. In seventeen years old, he presented a myalgia on bilateral thigh with a dark urine after 10-minute walking, which led to visit our hospital. The laboratory performed on a same day in referring hospital showed an elevated level of serum creatine kinase (73,529 U/L, normal range 39-308 U/L, CK), AST (88 U/L), myoglobin (>1,000 ng/mL, normal range 0-110 ng/mL), and myoglobinuria. These laboratory disarrangements brought the patient to our hospital.

In neurological examination at our hospital, the muscle strengths of bilateral upper and lower extremities were normal. The deep tendon reflexes were mildly reduced. The atrophy or arthrogryposis was not identified.

The routine laboratory showed an elevated level of serum CK (1,484 U/L), AST (45 U/L), myoglobin (430 ng/mL), and aldolase (185 U/L, normal range < 7.6 U/L). Complete blood count, thyroid stimulating hormone, alanine aminotransferase, and urine myoglobin were normal. The ischemic forearm exercise test was normal, showing an elevated serum level of lactic acid and ammonia after exercise (Fig. 1A).

The electrocardiogram showed a normal sinus rhythm. The findings of nerve conduction studies were normal. The needle electromyography demonstrated positive sharp waves with small amplitude and short duration motor unit action potentials on right first dorsal interosseous, biceps, and tibialis anterior muscles, indicating active myopathic changes.

Left biceps brachii biopsy was performed. Routine stain including hematoxylin and eosin, nicotinamide adenine dinucleotide tetrazolium reductase, and modified Gomori trichrome could not be evaluated due to the cryostat artifact. However, the immunohistochemistry (IHC) of muscle biopsy in paraffin block revealed an absent expression of dystrophin using the antibody against the C-terminal region (Thermofisher scientific, PA5-16734, USA, 1:200 dilution, Fig 1B) and has been repeated to confirm.

Whole exome sequencing revealed a novel hemizygotic missense mutation c.119T>A (p.Leu40His, exon 3, NM_004006.2, NG_012232.1, Fig. 1C) in *DMD* by investigating variants which affect protein function, show a depth of more than 30, and filtered by allele frequency of PopFreqMax less than 0.0001 consisting of the Genome Aggregation Database (gnomAD), the Exome Aggregation Consortium (ExAC), and 1000 Genome (1000genome). This variant is not present in Human Gene Mutation Database (HGMD) or Leiden Open Variation Database (https://databases.lovd.nl/shared/genes/DMD) and predicted to be pathogenic by using SIFT/PROVEAN and Mutation taster system. Structure of mutated dystrophin protein was predicted to be destabilizing using SDM web server (ΔΔG = 0.88 kcal/mol) and FoldX (ΔΔG = 19.5414 kcal/mol) [3, 4]. Both wild type (Leu40) and mutated (His40) residues were expected not to be part of aggregation-prone regions by an Aggrescan3D server [5], although the mutated residue (His40) can become solvent exposed by JPred4 server [6].

Discussion

Initially, we had suspected metabolic myopathies for the cause of recurrent exertional rhabdomyolysis. However, we could diagnose dystrophinopathies based on the absent immunoreactivity to dystrophin C-terminal, elevated resting CK levels, and a novel mutation *DMD* c.119T>A (p.Leu40His) in genetic analysis, which is predicted to be pathogenic by in-silico test.

Patients with muscular dystrophies have been reported with exercise intolerance, namely; anoctaminopathy, caveolinopathy, dysferlinopathy, dystrophinopathy, fukutin-related proteinopathy, and sarcoglycanopathy [2]. In cases of dystrophinopathies with exertional rhabdomyolysis or myalgia [2, 7-11], genetic analysis has identified the in-frame deletion in the most cases [2, 7-9], and missense mutations of the rod domain rarely [10, 11]. The mutant p.Leu40His found in our case is located on N-terminal actin-binding domain (N-ABD) of dystrophin, which has not been reported previously to have a pathogenicity for exertional rhabdomyolysis or myalgia. Recently reported case harboring p.Asn76Ile in dystrophin also supports the mutation in N-ABD can be pathogenic for Becker muscular dystrophy [12].

Concerning the immunoblotting, IHC to dystrophin rod-domain was absent or markedly reduced in patients with in-frame deletions [2, 7], whereas IHC to dystrophin C-terminal and rod domain was normal in patients with missense mutations [11]. However, absent immunostaining to dystrophin C-terminal has been reported to be identified in the patient with a missense mutation in N-ABD [12], which is compatible with our patient.

In terms of pathomechanism, the loss of hydrophobicity in the actin binding domain has been suggested in the previous study [13]. Amino acid substitution from leucine to arginine at the position 54 is well formulated, which is associated with Duchenne muscular dystrophy [13]. Similarly in our case, the leucine at the position 40 is surrounded by the residue with a hydrophobic side chain at the position Phe21, Val25, Phe41, and Ile111 (Fig. 2A). The amino acid change from leucine to histidine at the position 40 is expected to disrupt the interaction in the vicinity by losing hydrophobicity (Fig. 2B). Stability analysis and secondary structure prediction also showed destabilizing and solvent exposed nature of p.Leu40His mutation, respectively, although the predicted value of energy transfer differs among prediction systems and the mutant was predicted not to be an aggregation prone region. However, the downstream mechanism leading to phenotypical severity remains unknown in our case.

Conclcusions

We reported the case showing that a novel missense mutation of N-ABD in *DMD* is associated with recurrent symptoms, which is supported by in-silico analysis predicting pathogenicity in protein structure and IHC. Maintaining a high degree of clinical suspicion of muscular dystrophies is advised when patients present recurrent exertional rhabdomyolysis.

Abbreviations

AST: aminotransferase

CK: creatine kinase

IHC: immunohistochemistry

N-ABD: N-terminal actin-binding domain

Declarations

Ethical approval and consent to participate

The author declares that ethics approval was not required for this case report. Written informed consent to participate was obtained from the patient and the parent.

Consent for publication

Written informed consent was obtained from the patient and the parent for the publication of this case report. A copy of the written consent is available for review by the series editor of this journal.

Availability of data and material

All data containing relevant information to support the study findings are included in the manuscript.

Competing interest

The author declares that he has no competing interests.

Funding

None.

Author's contributions

JL collected and interpreted the data, wrote the manuscript, and prepared the figures.

Acknowledgements

Not applicable.

References

1. Warren JD, Blumbergs PC, Thompson PD. Rhabdomyolysis: a review. Muscle Nerve. 2002;25 3:332-47.
2. Liewluck T, Tian X, Wong LJ, Pestronk A. Dystrophinopathy mimicking metabolic myopathies. Neuromuscul Disord. 2015;25 8:653-7; doi: 10.1016/j.nmd.2015.04.001.

3. Pandurangan AP, Ochoa-Montano B, Ascher DB, Blundell TL. SDM: a server for predicting effects of mutations on protein stability. Nucleic Acids Res. 2017;45 1:229-35; doi: 10.1093/nar/gkx439.
4. Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L. The FoldX web server: an online force field. Nucleic Acids Res. 2005;33:382-8; doi: 10.1093/nar/gki387.
5. Zambrano R, Jamroz M, Szczasiuk A, Pujols J, Kmiecik S, Ventura S. AGGRESCAN3D (A3D): server for prediction of aggregation properties of protein structures. Nucleic Acids Res. 2015;43 1:306-13; doi: 10.1093/nar/gkv359.
6. Drozdetskiy A, Cole C, Procter J, Barton GJ. JPred4: a protein secondary structure prediction server. Nucleic Acids Res. 2015;43 1:389-94; doi: 10.1093/nar/gkv332.
7. Minetti C, Tanji K, Chang HW, Medori R, Cordone G, DiMauro S, et al. Dystrophinopathy in two young boys with exercise-induced cramps and myoglobinuria. Eur J Pediatr. 1993;152 10:848-51.
8. Lahoria R, Milone M. Rhabdomyolysis featuring muscular dystrophies. J Neurol Sci. 2016;361:29-33; doi: 10.1016/j.jns.2015.12.013.
9. Figarella-Branger D, Baeta Machado AM, Putzu GA, Malzac P, Voelckel MA, Pellissier JF. Exertional rhabdomyolysis and exercise intolerance revealing dystrophinopathies. Acta Neuropathol. 1997;94 1:48-53.
10. Saad FA, Vita G, Toffolatti L, Danieli GA. A possible missense mutation detected in the dystrophin gene by Double-Strand Conformation Analysis (DSCA). Neuromuscul Disord. 1994;4 4:335-41.
11. Veerapandiyan A, Shashi V, Jiang YH, Gallentine WB, Schoch K, Smith EC. Pseudometabolic presentation of dystrophinopathy due to a missense mutation. Muscle Nerve. 2010;42 6:975-9; doi: 10.1002/mus.21823.
12. Koczok K, Mero G, Szabo GP, Madar L, Gombos E, Ajzner E, et al. A novel point mutation affecting Asn76 of dystrophin protein leads to dystrophinopathy. Neuromuscul Disord. 2018;28 2:129-36; doi: 10.1016/j.nmd.2017.12.003.
13. Norwood FL, Sutherland-Smith AJ, Keep NH, Kendrick-Jones J. The structure of the N-terminal actin-binding domain of human dystrophin and how mutations in this domain may cause Duchenne or Becker muscular dystrophy. Structure. 2000;8 5:481-91.

Figures

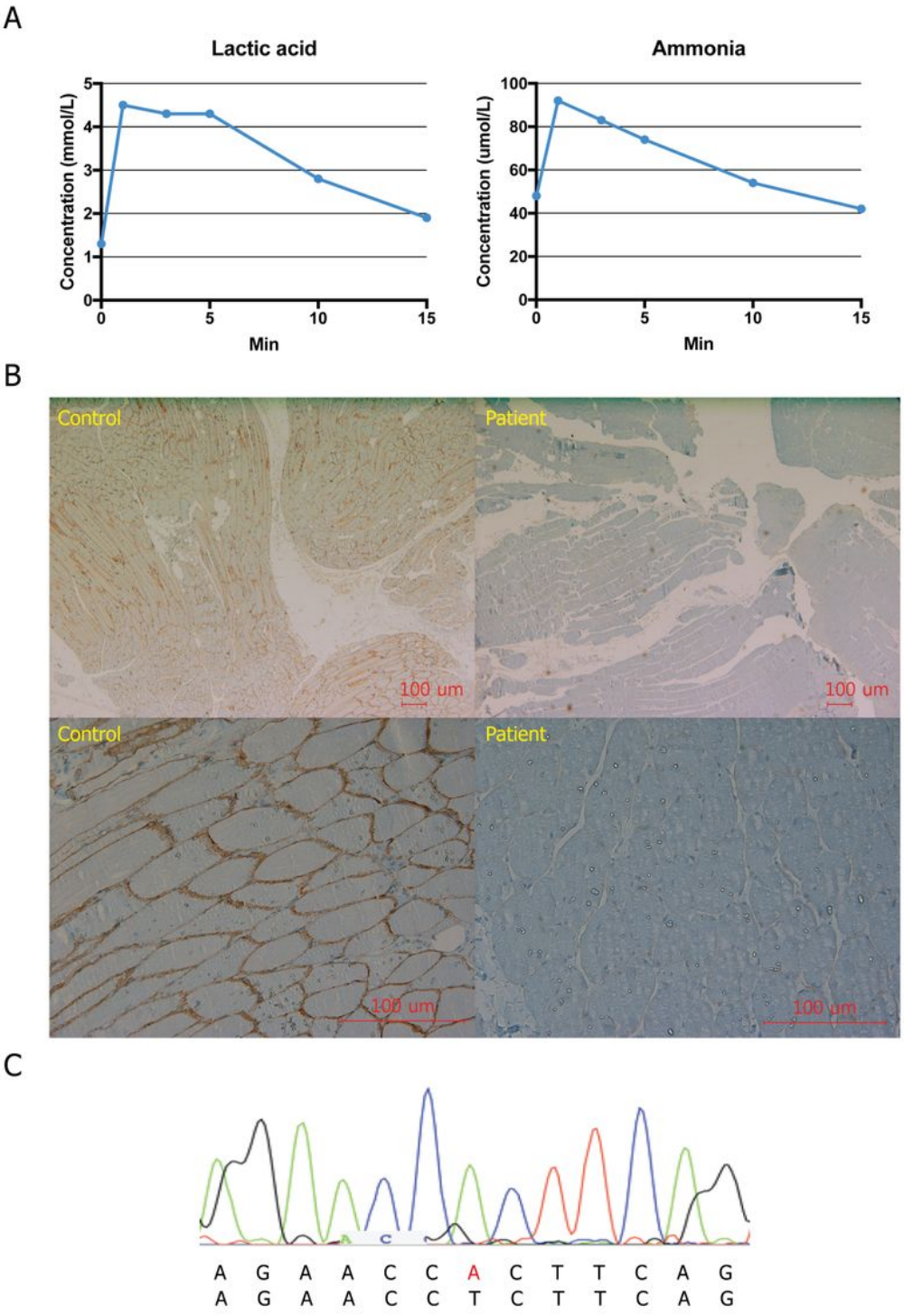

Figure 1

The results of ischemic forearm exercise test, immunohistochemistry using dystrophin C-terminal antibody, and genetic analysis. (A) Exercise-associated lactate and ammonia production was identified. (B) Absent immune activity against to dystrophin C-terminal antibody. (C) The mutation c.119T>A (p.Leu40His, NM_004006.2) in DMD was confirmed by Sanger sequence.

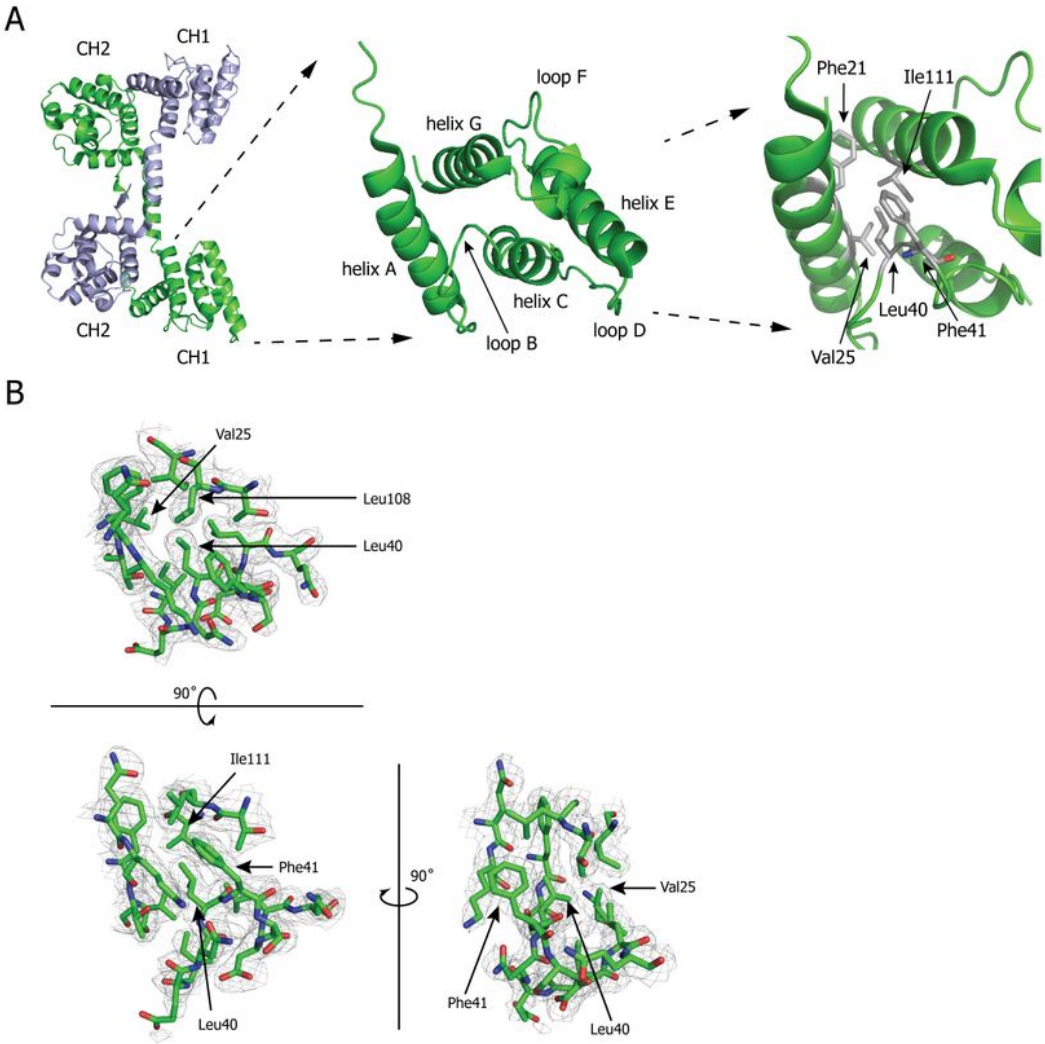

Figure 2

Structure of human dystrophin and pathogenic missense mutation in the dystrophin N-terminal actin-binding domain. (A) Left panel: dimer of human dystrophin N-terminal actin-binding domains, Middle panel: structure of the CH1 subdomain, Right panel: Leucine residue at the position 40 and its vicinities. (B) Stereoview of the 2Fo-Fc electron-density map in the vicinity of leucine at residue 40. The electron-density map is defined at 1.0σ.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.pdf