Identification of a compound heterozygous missense mutation in LAMA2 gene from a patient with merosin-deficient congenital muscular dystrophy type 1A

Afshin Khorrami1 | Pouya Goleij2 | Vahidreza Karamad3 | Elham Taheri4 | Behrouz Shadman3 | Parisa Emami5 | Gholamreza Jahangirzadeh6 | Saba Hajazimian7 | Alireza Isazadeh7 | Behzad Baradaran7 | Mansour Heidari8

Abstract

Background: Merosin-deficient congenital muscular dystrophy type 1A (MDC1A) is occurred by mutations in LAMA2 gene that encodes the laminin α2 chain (merosin). MDC1A is a predominant subtype of congenital muscular dystrophy. Herein, we identified two missense mutations in LAMA2 gene in compound heterozygous status in an Iranian patient with MDC1A using whole-exome sequencing (WES).

Methods: In the present study, we evaluated genetic alterations in an Iranian 35-month-old boy with MDC1A and his healthy family using WES method. The identified mutations further confirmed by Sanger sequencing method. Finally, in silico analysis was conducted to further evaluation of molecular function of the identified genetic variants.

Results: We identified two potentially pathogenic missense mutations in compound heterozygous state (c.7681G>A p.Gly2561Ser and c.4840A>G p.Asn1614Asp) in LAMA2 gene as contributing to the MDC1A phenotype. The healthy parents of our proband are single heterozygous for identified mutations. These variants were found to be pathogenic by in silico analysis.

Conclusions: In general, we successfully identified LAMA2 gene mutations in an Iranian patient with MDC1A using WES. The identified mutations in LAMA2 gene can be useful in genetic counseling, prenatal diagnosis, and predicting prognosis of MDC1A.

Keywords
congenital muscular dystrophy, LAMA2 gene, mutation, whole-exome sequencing
1 | INTRODUCTION

The merosin-deficient congenital muscular dystrophy type 1A (MDC1A) with autosomal recessive inheritance affects the peripheral and central nervous system in children. This disorder is characterized by increased levels of creatine kinase (CK) in serum, hypotonia, abnormalities of white matter, poor cry and suck, failure to thrive, and muscle weakness. The prevalence of MDC1A is 1–9 per 1,000,000 children and constitutes 1–6% of all congenital muscular dystrophy cases. Furthermore, this disorder is rarer in Asian population and more common in European countries and Caucasians race. The various mutations in the LAMA2 gene (with 65 exons) are the main cause of MDC1A. Other major factors involved in congenital muscular dystrophies are presented in Figure 1.

LAMA2 gene, on chromosome 6q22, encodes the laminin-α2 chain, which connects with laminin-γ1 and laminin-β1 chains and forms the heterotrimeric laminin-211 protein. The laminin-211 protein is a main component of the extracellular matrix and the skeletal muscle membrane. The interaction of this protein with various matrix macromolecules plays an important role in tissue phenotypes, cell movement, and cell differentiation. Previous studies reported that the genetic variations of MDC1A are compound heterozygous or homozygous mutations. Moreover, de novo mutations are the rare events and a few have been reported in MDC1A patients.

The early diagnosis of MDC1A is based on high serum concentrations of CK, deficiency of merosin in skin or muscle biopsy, alterations in white matter on brain, and clinical examination. Previous studies reported the efficiency of whole-exome sequencing (WES) for the molecular diagnosis of the congenital muscular dystrophy. However, use of WES method is not cost-effective in patients with clinical overlap. A previous study on an Iranian patient with congenital muscular dystrophy revealed an improved diagnostic yield of WES method.

In the present study, potentially pathogenic mutations of LAMA2 gene were evaluated in an Iranian patient with MDC1A using WES along with Sanger sequencing. We identified two mutations in the compound heterozygous state on LAMA2 gene. Furthermore, in silico analysis suggests that these mutations can cause production of a defective protein by LAMA2 gene.

2 | MATERIALS AND METHODS

2.1 | Case presentation

This patient is a 35-month-old male referred Aria Gene Medical Genetics Laboratory, Qom, Iran. He was the second child in a healthy family without any neuromuscular diseases history. This patient is the offspring of a non-consanguineous marriage. His only older brother is healthy without any problems (Figure 2). He was born normally at 36rd weeks of pregnancy through a spontaneous vaginal delivery. He did not show any abnormalities in neonatal period and was discharged from the hospital on third day. He was breastfeeding, and he had no problems for the first year of his life. After one year, physical developmental and motor milestones delays were observed (sat at 10 months, crawled at 16 months, stood unaided at 25 months, and started walking at 31 months). His family worries started at 25 months, because he cannot stand unsupported and always had difficulty running. The preliminary examinations revealed a tightness of ankles and mild proximal muscle weakness. Moreover, this patient was with bilateral clubfoot, cataract, vermis hypoplasia, and microphthalmia. However, development of the speech and intellectual was normal. There was no vision or hearing problems. According to the ethical standards of Helsinki Declaration, the studied patient and his parents were informed about the aim of present study and signed an informed consent. The present study was approved by the Institutional Review Board (IRB), Qom University of Medical Sciences, Qom, Iran.

2.2 | Genomic DNA extraction

The peripheral blood lymphocytes (5 ml) were received from the studied patient and his healthy parents. Extraction of the genomic DNA was conducted using a standard DNA purification kit (Roche, Switzerland). The purity and quantity of the genomic DNA samples were evaluated using NanoDrop instrument (Thermos Fisher Scientific, USA). The genomic DNA samples with appropriate OD 260/280 ratio (1.7 to 1.9) were further evaluated for quality. The quality of the genomic DNA samples was evaluated using electrophoresis on 1% agarose gel. Finally, the genomic DNA samples without smear or diffuse and with a sharp band were stored at −20°C and then used for molecular analysis.

2.3 | Whole-exome sequencing (WES)

The WES was used for the proband, and his healthy mother and father. The capture of the exome sequence was conducted using the SureSelect Human All Exon V5 Kit (Agilent Technologies, United States). The capture library was sequenced via 2x150 paired-end sequencing on a Hiseq2000 Sequencer (Illumina, United States). The sequence reads were aligned to human reference genome by Burrows-Wheeler Aligner algorithm, and then, processing was performed using SAMtools. All small deletions–insertions (indels) and single nucleotide polymorphisms (SNPs) were analyzed using Genome Analysis Toolkit (GATK) and VarScan software. The variants annotate was conducted using the ANNOVAR software. The variants in homozygous condition were excluded, and frameshift, missense, and nonsense mutations were considered as pathogenic. The pathogenic potential of the missense mutations was analyzed using the MutationTaster, FATHMM, Polyphen-2, M-CAP, PROVEAN,
FIGURE 1  The major proteins involved in congenital muscular dystrophies: location and interaction
SIFT, REVEL, MetaLR, and MetaSVM software. All variants with autosomal recessive, dominant, and X-linked inheritance models were assumed for the analysis. The mutations passed these filtering were considered as pathogenic.\textsuperscript{18}

2.5 \textbf{Sanger sequencing}

The Sanger sequencing was performed to validate the candidate mutations in proband and his parents. The target exons containing mutations of LAMA2 gene were amplified using polymerase chain reaction (PCR) and designed primers. The products of PCR were sequenced using ABI 3130 automated sequencer (Applied Biosystems, Forster City, CA, USA). The obtained sequences were analyzed using Mutation Surveyor software.\textsuperscript{19}

\section{RESULTS}

\subsection{Clinical findings}

In this study, an Iranian family member with congenital muscular dystrophy was evaluated. The clinical experiments were all normal for gland function, renal function, hepatic function, lipoproteins, triglyceride, cholesterol, glucose, alkaline phosphatase, electrolyte, thyroid, ammonia, lactic acid. The karyotype analysis was normal in the proband. However, CK level was at 812 IU/l (normal <200 IU/l). The electromyography revealed a myopathic process. The magnetic resonance imaging (MRI) or brain revealed an agyria area in occipital cortex. The T2-weighted images were detected swelling and widening of gyri, extensive white matter abnormalities, mainly frontal. The cardiac function has decreased and the dilated cardiomyopathy with dysfunction of left ventricle contractility was detected. Therefore, we suggested MDC1A as a possible diagnosis (Table 1).

\subsection{Detection of LAMA2 mutation using WES}

The obtained results of WES revealed a heterozygous missense mutation c.7681G>A p. Gly2561Ser (exon 55) in the LAMA2 gene. Moreover, another heterozygous missense mutation c.4840A>G p. Asn1614Asp (exon 33) was detected in the LAMA2 gene. These indicated that the compound heterozygous variants (c.7681G>A and c.4840A>G) co-segregated with this disease in this family.

\subsection{Confirmation of detected LAMA2 mutation using Sanger sequencing}

The two identified mutations (c.7681G>A and c.4840A>G) in the LAMA2 gene were confirmed using Sanger sequencing. We found that the two mutations of LAMA2 gene were in the compound

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{No.} & \textbf{Clinical features} & \textbf{Characteristic} & \textbf{No.} & \textbf{Clinical features} & \textbf{Characteristic} \\
\hline
1 & Age of onset & Birth & 14 & Gland function & Normal \\
2 & Consanguineous marriage & Yes & 15 & Renal function & Normal \\
3 & Karyotype analysis & Normal & 16 & Hepatic function & Normal \\
4 & Current age (month) & 35 & 17 & Lipoproteins & Normal \\
5 & Serum CK & 812 IU/l & 18 & Triglyceride & Normal \\
6 & Max. motor milestone & Sat unsupported & 19 & Cholesterol & Normal \\
7 & Contractures & Yes & 20 & Glucose & Normal \\
8 & Mental Retardation & No & 21 & Alkaline phosphatase & Normal \\
9 & White Matter Changes & Yes & 22 & Electrolyte & Normal \\
10 & Eye involvement & Myopia & 23 & Thyroid & Normal \\
11 & Cardiac function & Mild hypertrophy & 24 & Ammonia & Normal \\
12 & Scoliosis & No & 25 & Lactic acid & Normal \\
13 & Facial dysmorphism & No & 26 & Respiratory function & Normal \\
\hline
\end{tabular}
\caption{The clinical features of the studied patient with MDC1A}
\end{table}
heterozygous state in the studied patient. However, parents of the proband were in heterozygous state for c.4840A>G (mother) and c.7681G>A (father) mutations.

4 | DISCUSSION

MDC1A is an autosomal recessive disease which occur by mutations in LAMA2 gene and represents the predominant subtype of congenital muscular dystrophy. Important presentations of MDC1A are increased white matter abnormalities, increased levels of CK, and absence of laminin-α2 chain around muscle fibers. However, due to genetic and clinical heterogeneity, precise molecular diagnosis of MDC1A is still a challenge for clinicians. Recently, targeted WES method has emerged as a powerful molecular diagnosis tool which widely used to identify causal genes in genetic diseases.

In this study, we used WES method combined with Sanger sequencing to identify genetic causes of MDC1A in an Iranian patient. Our study identified two mutations in LAMA2 gene in an Iranian patient with MDC1A in compound heterozygous status. Further in silico analysis demonstrated that these mutations are possible pathogenic in our proband. Other family members with heterozygous mutations of LAMA2 gene (c.7681G>A or c.4840A>G) were healthy, which may be due to preserving a partly normal LAMA2 gene-encoded protein. This evidence demonstrated that identified compound heterozygous mutations were the cause of MDC1A phenotype in our proband.

Previously, diagnosis of MDC1A was performed according to the clinical presentations, such as white matter alternations, high levels of serum CK, severe congenital hypotonia, and deficiency of merosin expression in biopsied muscle. The muscle biopsy seems to be an essential method to confirm the diagnosis of congenital muscular dystrophy. However, the molecular genetic diagnosis may be an alternative method if the clinical phenotypes support the diagnosis of congenital muscular dystrophy. Our proband was suspected to have congenital muscular dystrophy due to white matter abnormalities, high serum CK levels, and appendicular hypotonia. Therefore, we used molecular genetic analysis to identification of possible mutations of LAMA2 gene which is responsible for the symptoms of MDC1A.

Interaction of merosin with various matrix macromolecules and skeletal muscle membrane plays an important role in tissue phenotypes, cell movement, and cell differentiation. To date, approximately 90 mutations have been described in LAMA2 gene. In present patient, we identified two missense mutations in heterozygous status which is located in exons 33 and 55. The G domain at the C terminus of merosin (exons 46–64) is responsible in the connection between the dystrophin-glycoprotein and the extracellular matrix. Deficiency of this domain disrupts the link between subsarcolemmal cytoskeleton and extracellular matrix, which causes muscle degeneration. The severe phenotype of our proband may explain by this evidence.

Limited evidence has been reported for cardiac defects related to the laminin-α2 deficiency in patients with MDC1A. A previous study specifically addressed involvement of the cardiac defects in patients with laminin-α2 deficiency. The reported cardiac abnormalities in patients with MDC1A included borderline changes in cardiac function, dilated cardiomyopathy, and right bundle branch block. Moreover, cerebral white matter abnormalities are commonly reported in patients with MDC1A. However, underlying mechanisms responsible for white matter abnormalities in patients with MDC1A remain elusive and thus cause abnormal signal intensity of white matter. In our proband, we observed white matter abnormalities of parietal and occipital lobes, whereas the corpus callosum and cerebellum were normal. The white matter abnormalities are a typical feature of patients with MDC1A compared with other congenital muscular dystrophy subtypes.

Generally, we identified two potentially pathogenic mutations in a compound heterozygous state (c.7681G>A p. Gly2561Ser and c.4840A>G p. Asn1614Asp) in LAMA2 gene responsible for MDC1A phenotype in an Iranian patient. These results can refine prenatal diagnosis, genetic counseling, and treatments of patients with LAMA2 gene-caused MDC1A.

ACKNOWLEDGMENTS
The authors thank the participants for being involved in this study.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

ETHICAL APPROVAL
All procedures performed in the studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of Qom University of Medical Sciences and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from patient and parents.

DATA AVAILABILITY STATEMENT
The raw data analyzed during the current study are not publicly available due to the aim to protect the confidentiality of the patients but are available from the corresponding author on reasonable request.

ORCID
Alirea Isazadeh https://orcid.org/0000-0002-8781-1177
Behzad Baradaran https://orcid.org/0000-0002-8642-6795

REFERENCES
1. Ip JJ, Hui PK, Chau MT, Lam WW. Merosin-deficient congenital muscular dystrophy (MDCMD): a case report with MRI, MRS and DTI findings. J Radiol Case Rep. 2012;6(8):1-7.
2. Graziano A, Bianco F, D’Amico A, et al. Prevalence of congenital muscular dystrophy in Italy: a population study. Neurology. 2015;84(9):904-911.
3. Bönemann CG, Wang CH, Quijano-Roy S, et al. Members of international standard of care committee for congenital muscular dystrophies; diagnostic approach to the congenital muscular dystrophies. Neuromuscul Disord. 2014;24(4):289-311.
4. Durbeej M. Laminin-α2 chain-deficient congenital muscular dystrophy: pathophysiology and development of treatment. Curr Top Membr. 2015;76:31-60.
5. Tao J, Duan J, Pi X, Wang H, Li S. A splicing LMNA mutation causing laminopathies accompanied by aortic valve malformation. J Clin Lab Anal. 2021;35(4):e23736.
6. Xiong H, Tan D, Wang S, et al. Genotype/phenotype analysis in Chinese laminin-α2 deficient congenital muscular dystrophy patients. Clin Genet. 2015;87(3):233-243.
7. Turner C, Mein R, Sharpe C, Love DR. Merosin-deficient congenital muscular dystrophy: A novel homozygous mutation in the laminin-2 gene. J Clin Neurosci. 2015;22(12):1983-1985.
8. Colognato H. The extracellular matrix protein laminin and function: the laminin family. Membr. 2000;218(2):213-234.
9. Holmberg H, Yurchenco PD. Form and function: the laminin family. Dev Dyn. 2000;218(2):213-234.
10. Oliveira J, Gruber A, Cardoso M, et al. LAMA2 gene mutation update: Toward a more comprehensive picture of the laminin-α2 variome and its related phenotypes. Hum Mutat. 2018;39(10):1314-1337.
11. Zhou J, Tan J, Ma D, et al. Identification of two novel LAMA2 mutations in a Chinese patient with congenital muscular dystrophy. Front Genet. 2018;9:43.
12. Yu M, Zheng Y, Jin S, et al. Mutational spectrum of Chinese LGMD patients by targeted next-generation sequencing. PLoS One. 2017;12(4):e0175343.
13. Das Bhomik A, Dalal A, Matta D, Sundaram C, Aggarwal S. Targeted next generation sequencing identifies a novel deletion in LAMA2 gene in a merosin deficient congenital muscular dystrophy patient. Indian J Pediatr. 2016;83(4):354-355.
14. Fattahi Z, Kalhor Z, Fadaee M, et al. Improved diagnostic yield of neuromuscular disorders applying clinical exome sequencing in patients arising from a consanguineous population. Clin Genet. 2017;91(3):386-402.
15. Hashemi-Gorji F, Yassaee VR, Dashti P, Miryounesi M. Novel LAMA2 gene mutations associated with merosin-deficient congenital muscular dystrophy. Iran Biomed J. 2018;22(6):408.
16. Ahmadi M, Dehghanifard A, Isazadeh A, et al. A novel homozygous MYO7A mutation: case report. Acta Med Iran. 2018;56(5):348-350.
17. Heidari M, Soleyman-Nejad M, Isazadeh A, et al. Identification of a novel homozygous mutation in the DDR2 gene from a patient with spondylo-meta-epiphyseal dysplasia by whole exome sequencing. Iran J Basic Med Sci. 2020;23(11):1-8.
18. Heidari M, Soleyman-Nejad M, Taskhiri MH, et al. A heterozygous STXBP1 gene de novo in mutation in an Iranian child with epileptic encephalopathy: case report. Acta Med Iran. 2019;57(8):518-521.
19. Heidari M, Soleyman-Nejad M, Taskhiri MH, et al. Identification of two novel mutations in the ATM gene from patients with ataxia-telangiectasia by whole exome sequencing. Curr Genom. 2019;20(7):531-534.

How to cite this article: Khorrami A, Goleij P, Karamad V, et al. Identification of a compound heterozygous missense mutation in LAMA2 gene from a patient with merosin-deficient congenital muscular dystrophy type 1A. J Clin Lab Anal. 2021;35:e23930. https://doi.org/10.1002/jcla.23930