IDENTIFICATION OF A MUTATION IN A VIETNAMESE FAMILY WITH EMERY-DREIFUSS MUSCULAR DYSTROPHY USING WHOLE EXOME SEQUENCING

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ABSTRACT

Emery-Dreifuss muscular dystrophy (EDMD) is a degenerative neuromuscular disease associated with at least nine genes, including *EMD, LMNA, FHL1, TMEM43, SUN1, SUN2, TTN, SYNE1,* and *SYNE2*. Herein, we identified a heterozygous missense *LMNA* mutation (NM_170707.4: c.1357C>T,p.R453W) in three members of a Vietnamese family using whole-exome sequencing (WES), in which the proband was an 11-year-old girl presenting humeroperoneal muscle weaknesses and generalized contracture. Her father and one other relative also exhibited multiple signs of muscular atrophy and contracture. Sanger sequencing in the extended family verified the causative nature of this mutation, establishing a confirmed diagnosis of autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD2). The clinical presentations of each patient in this study are different from each other, demonstrating the intrafamilial phenotypic variability of this mutation. Early identification of the underlying genetic course of the disease by sequencing, combined with clinical findings provides solid evidence to diagnosis process, genetic counseling and management strategy.

Keywords: EDMD, *LMNA*, Sanger, WES, Vietnamese.

INTRODUCTION

Emery-Dreifuss muscular dystrophy (EDMD) is a rare degenerative myopathy with a triad of clinical symptoms including joint contracture, muscle atrophy, and a cardiac defect. Joint contractures of the neck extensor, Achilles tendon, and elbow flexor typically appear during early childhood. Muscle weakness and wasting affect humeroperoneal muscles such as proximal upper limbs and distal lower limbs at first and progressively worsen (Rowland et al., 1979). The most serious complication is cardiac involvement, which develops around the third decade of life and might lead to sudden cardiac death. The overall prevalence rate is estimated to be 1.3-2:100000 (Bonne et al., 1993). Currently, there is no curative treatment but only palliative interventions to reduce muscle contracture and heart-related symptoms.

Establishing a diagnosis for EDMD is based on family history, clinical findings, muscle imaging, and molecular testing. As EDMD manifestations overlap with those of other muscular diseases, especially when cardiac involvements occur after the second decade of life, genetics approaches, including single-gene testing, gene panels or sequencings, become useful to point out the genetic basis. The Online Mendelian Inheritance in Man (OMIM) database classifies EDMD into seven sub-categories, with three main entities are X-linked EDMD (EDMD1, OMIM#310300), autosomal dominant EDMD (EDMD2, OMIM#181350), and autosomal recessive EDMD (EDMD3; OMIM#616516). While X-linked EDMD is caused by mutations on gene *EMD* and *FHL1* on the X chromosome, both autosomal recessive and dominant forms are often linked with various mutations on gene *LMNA*. Genetic mutations on *LMNA* and *EDM* genes can account for approximately 40% of reported cases (Bonne et al., 2003; Meinke et al., 2011).

Mapped on chromosome 1q.21.1-21.2, *LMNA* (OMIM# 150330) contains 12 exons and spans over a region of 24 kb. It encodes for two isoforms A and C via alternative splicing, with the first 566 amino acids are identical between the two types. Nuclear lamin A/C proteins are type V intermediate filament, providing structural integrity for nuclear envelope (Dittmer & Misteli, 2011). This protein is expressed in almost every cell, but its mutations only cause alterations in certain cell types, which can be explained by two hypotheses. The "mechanical stress" hypothesis proposes that mutations in *LMNA* lead to aberrancy in the nuclear lamina-envelope architecture, decreasing cellular tolerance to physical stress (Carmosino et al., 2014). Therefore, tissues regularly exposed to high physical tension are more vulnerable to *LMNA* defects. The second hypothesis suggests that disruption in the interaction among nuclear lamina proteins, chromatin modifiers and transcriptional factors during genome organization leads to alteration in gene expression (Storey et al., 2020). While these two models are independent of each other, growing evidence suggests that there is an interplay between these two as modifications in gene expression and differentiation can impact cell development in muscle tissues and vice versa.

The HGMD database reports 71.4% of mutations in this gene are nonsense or missense (Stenson et al., 2003). They are scattered along the nucleotide sequence, with very few mutational hotspots (Bonne et al., 2003). Since the first *LMNA* mutation was introduced in 1999, more than 700 different variants are listed; however, there is no available data from the Vietnamese population. Thanks to unceasing advanced breakthroughs in sequencing technology, the discovery rate of variants in the human genome is improved with high accuracy and in-deep coverage. In this study, we identified the mutation NM_170707.4:c.1357C>T,p.R453W in *LMNA* in 3 people of a 5-generation Vietnamese family by using whole-exome sequencing (WES). Sanger sequencing of affected individuals and healthy family members further validated the heterozygous variant was causative.

MATERIALS AND METHODS

Study subjects and genomic DNA extraction

This study received approval from the Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology (No: 2-2019/NCHG-HĐĐĐ). All participants agreed to donate blood samples for segregation analysis by written consent.

Genomic DNA was extracted and purified from peripheral blood using GeneJET Whole Blood Genomic DNA Purification Mini kit (ThermoFisher Scientific, USA), following the manufacturer's protocol. The quality of DNA samples was assessed by NanoDrop One/One machine. All DNA samples were stored in EDTA-containing tubes at -20 °C degree.

Whole-exome sequencing (WES)

WES was performed on the proband (V-4). DNA library was prepared using the SureSelectXT Human All Exon 50 Mb on the HiSeq Illumina platform (Illumina, USA). Short reads were mapped onto the human reference genome (UCSC hg19, NCBI build 37.1) using Novoalign (http://www.novocraft.com/products/novoalign/). Unmapped and low-quality reads were removed using Bamtools (https://bio.tools/BamTools). PCR duplications were filtered out by Picard version 2.18.7 (http://broadinstitute.github.io/picard/). Variant callings were performed following Genome Analysis Toolkit (GATK) Best Practices in GATK pipeline (https://www.broadinstitute.org/gatk/index.php), and the remaining variants including small indels and single nucleotide variants (SNV) were annotated via ANNOVAR (https://annovar.openbioinformatics.org/en/latest/).

PCR and Sanger sequencing

Validation of the causative variant was done on the proband (V-4) and ten family member samples (III-1, III-3, IV-5, IV-7, IV-9, IV-10, IV-11, V-1, V-2, V-5). The target site and flanking regions were amplified using our designed primers (primer sequences are available upon request) following this protocol: an initial denaturation of 95 °C for 5 min, then 40 cycles of 95 °C for 30 s, 68 °C for 30 s, 72 °C for 30 s and a final extension of 72 °C for 5 mins. Purified PCR products were sequenced using ABI Big Dye Terminator v3.1 Sequencing Standard Kit (Applied Biosystems, CA) on ABI 3500 Genetic Analyzer sequencer (Applied Biosystems). Sequencing results were viewed on SnapGene Viewer 4.3.10 (GSL Biotech, USA).

Prediction tools

The pathogenicity of the variant was evaluated by several *in silico* programs: SIFT (Sim et al., 2012), Polyphen-2 (Adzhubei et al., 2010), Mutation Taster (Schwarz et al., 2014), CADD (Rentzsch et al., 2021), and PROVEAN (Choi & Chan, 2015). In addition, protein sequence conservation at amino acid position 453 across different species was assessed using ClustalOmega (Madeira et al., 2019).

RESULTS

Case presentation

The proband (V-4) was an 11-year-old female born first child to nonconsanguineous parents of Vietnamese Kinh ethic. Pregnancy and delivery were uneventful. Around the age of 5, she started showing gait disturbance such as troubles standing up and climbing stairs (Fig. 1a). Scapular winging and contractures (head, neck, elbow, knees, and Achilles tendon) were noted simultaneously, while pelvic muscle involvement appeared later. Her spine still retained normal curvature. Creatine kinase (CK) levels were 2881.1 U/L (2015) and 1224 U/L (2019). MRI images displayed abnormal white matter in the left parietal lobe. No cardiac or respiratory defects were detected at the time of the study.

The proband's father, patient IV-10 had disease onset at 4 years old. Overall movements were strictly limited due to multiple contractures (head, neck, elbows,

spine, Achilles tendons) (Fig. 1b). Spine rigidity was gradually aggravated, causing lordosis, scoliosis, and scapular winging. Dystrophic features were shown on muscle biopsy. The patient lost ambulation completely and required full wheelchair access. In the follow-up, the patient reported having symptoms of lightheadedness, daytime drowsiness, somniloquy and low blood pressure. The neck extensor was tightly contracted, causing excessive neck pain.

Patient V-2 was a 26-year-old male, cousin of the proband. His initial signs of contracture appeared around the age of 4 when he had difficulty placing whole feet on the ground while moving and had to tiptoe. His motor skill was impaired to a mild degree. Kyphoscoliosis, contractures of spine, head, neck, and elbow were slowly progressive. The patient did not exhibit any sign of a cardiac problem. The main clinical phenotypes of the affected proband, her father and cousin are summarized in Table 1.

Family history evaluation also noted EDMD2 phenotypes in III-2, IV-1, IV-4, and V-3. The diagnosis could not be verified because of their deceased statuses, yet medical examination reported their causes of mortality involved cardiac complications at various degrees. Furthermore, V-3 had excessive muscle contracture, leading to restrictive pulmonary function. The rest of the extended family appeared to retain normal mobility, muscular strength and cardiac activities.
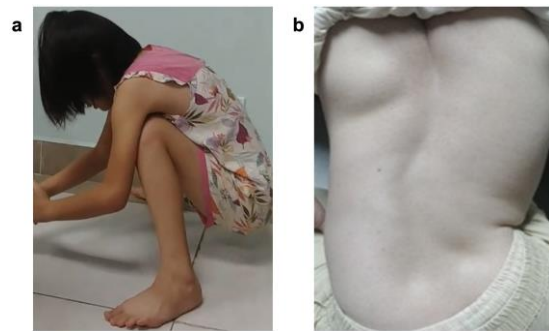

Figure 1. a) Contracture in Achilles tendon rendered the proband (V-4)'s ability to stand up and move around properly; b) Spinal deformities of IV-10 show lordosis and scoliosis

Table 1. Clinical features of affected individuals in our study

Patien ID	V-4	IV-10	V-2
Mutation	NM_170707.4: c.1357C>T, p.R453W		
Gender	F	M	M
Age of onset	5	4	4
Age of review	11	35	26
Muscle atrophy	Humeroperoneal weakness, calf hypertrophy.	Humeroperoneal weakness, scapular winging	Humeroperoneal weakness, scapular winging
Spine rigidity	Normal curvature	Scoliosis, lordosis	Kyphoscoliosis
Contractures	Heel cord, neck, elbow and ankle	Heel cord, neck, elbow, ankle	Heel cord, ankle, elbow
Cardiac involvement	Absent	Yes	Absent
CK level (U/L)	2881.1 (2015); 1224(2019)	Not available	Not available

Sequencing analysis

Heterozygous missense variant NM_170707.4: c.1357C>T, p.R453W was detected in the *LMNA* gene in the proband by WES and later identified by Sanger sequencing of sample IV-10 and V-2 (Figs. 2a, 2b). Healthy family members (III-1, III-3, IV-5, IV-7, IV-9, IV-11, V-1, V-5) did not possess this mutation, validating its genotype/phenotype correlation.
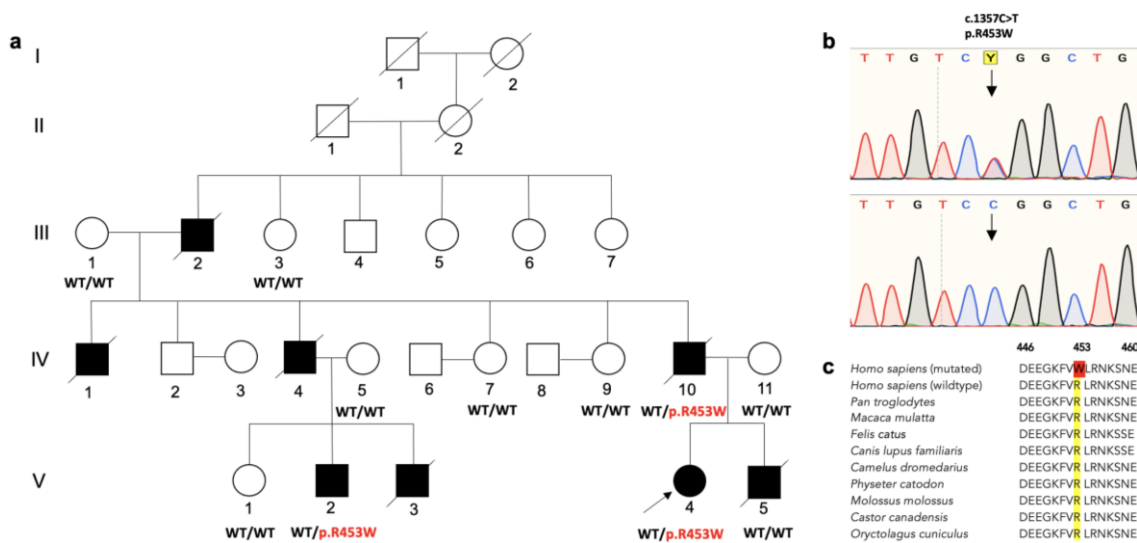
Figure 2. (a) Family pedigree of five generations in the studied family, in which V-4 is the proband and V-2, V-10 display EDMD phenotypes. (b) Sanger sequencing results at position c.1357 of exon 7 on gene LMNA: heterozygous missense mutation C>T and wildtype nucleotide C. (c) Conservation of p.R453 across different species

In silico analysis

The change from arginine to tryptophan at position 453 of the *LMNA* gene was predicted to be deleterious using *in silico* prediction tools (SIFT, Polyphen2, MutationTaster, CADD, PROVEAN). Cluster Omega indicated the conservative nature of this amino acid among humans and nine other species (Fig. 2c).

DISCUSSION

The heterozygous missense variant (c.C1357T; p.R453W) in *LMNA* is one of the very first mutations that are linked with AD-EDMD. It was initially described by Bonne in 1999 and reported in multiple literatures (Bonne et al., 1999; Fan et al., 2020; Park et al., 2017) . Being one of the most frequent EDMD-2 hotspots, the mutation accounts for 2.7% (66/2420) records on the UMD-*LMNA* database. The c.1357C>T exhibits a broad spectrum of inter- and intrafamily clinical manifestations. The difference in age of onset, phenotypic variability, and severity depends on a case-by-case basis. Besides displaying typical criteria, our proband (V-4) had the abnormal white matter in the left brain's parietal lobe that was not found in IV-10 and V-2. Though her cognitive function was unimpaired, it remained elusive whether this aberrance could have a potential harmful effect. A distinctive hallmark of EDMD - cardiac involvement, did not present in our proband or her cousin and two unrelated Han Chinese who also harbored c.1357C>T (Dai et al., 2015). Though no confirmed diagnosis has yet formed, patient IV-10 began to experience heart function decline, resulting in certain mental impairments mentioned earlier. Cardio conduction disturbance was observed in a Korean patient and four unrelated individuals with variable expressivity (Lee et al., 2017; Scharner et al., 2011). In a French family, four affected individuals demonstrated classic EDMD2 muscular phenotypes, but only two of them required ventricular assistance (Bonne et al., 2000). Because of the unpredictable penetrance of this variant, combined with a high sudden death rate (46%) in *LMNA*-gene mutation patients, carriers of c.1357C>T should be monitored closely for any indication of heart-related problems (van Berlo et al., 2005). The remarkable phenotypic diversity of this

mutation, accompanied by the late development of heart-related issues, indicates that standard clinical examination and imaging are not sufficient to establish a definitive diagnosis. With the rapid emergence of innovative technology, molecular testing becomes more affordable and efficient in detecting the underlying cause of a disease. Besides identifying the recurrent mutations, sequencing such as WES can screen for novel one at a high detection rate and the fast turnover, significantly reducing the rate of delayed and uncertain diagnosis.

As a component of the intermediate filament network lining up the inner nuclear membrane of almost every cell, Lamin A/C is a multifunctional protein. It stabilizes the cellular structure and maintains the integrity of the nuclear cytoskeleton under mechanical stress (Nmezi et al., 2019; Osmanagic-Myers et al., 2015). Interaction with chromatins and telomeres indicates its role in gene rearrangement and DNA repair process (Dittmer & Misteli, 2011). Various studies also demonstrated lamin A/C association with different types of gene promoters, enhancers and regulators during the replication and transcription process (Dittmer & Misteli, 2011; Ikegami et al., 2020; Shimi et al., 2010). Because of its peptide product association with many signaling pathways, mutations on the *LMNA* gene give rise to an extensive range of at least ten discrete disorders. This pleiotrophism could be partially understood by the fact that pathogenic variants are dispersed over different domains. Each of these domains performs a distinctive function. Our mutation, p.R453W, is located on exon 7 of the coding sequence, corresponding to the globular Ig-fold domain (residues 430-545) (Krimm et al., 2002). Made up of two β-sheets, the Ig fold is responsible for protein-ligand interaction (Dittmer & Misteli, 2011). As this domain remains relatively unchanged across species, a missense mutation resulting in amino acid modification is likely to have a detrimental effect. Crystallography of the Ig-fold C terminal showed that p.R453 is positioned on the surface of this highly conserved domain. In the tertiary formation, this residue is a part of an epitope that can interact with specific ligands, suggesting that the mutant form of p.R453 may have a dominant - negative effect or detrimental gain-of-function (Benedetti et al., 2007). This finding is in accordance with a structural analysis done by Krimm et al. (2002), in which p.R453W was observed to destabilize the conformation of the C-terminal, leading to the perturbation of lamin A/C interaction with binding partners.

Global genetic databases, in particular those on rare neuromuscular disorders, are mainly constituted of data generated from Caucasian ethnic groups. Only recently, there have been more attempts to conduct research on Asian populations, which are composed of a diverse array of ethnic groups. However, the attention is predominantly on certain countries such as Japan, Korea or China and with limited data on the others. This is the first report of a Vietnamese family carrying the c.C1357T variant and expressing non-cardiac EDMD2 phenotypes to the best of our knowledge. Besides clarifying the genetic root to the patients and their physician, this case study provides information to generate genotype-phenotype correlation and increase the populational diversity of the EDMD public database.

CONCLUSION

We present a known mutation, NM_170707.4: c.1357C>T, p.R453W, in the *LMNA* gene, inherited in a heterozygous dominant pattern. The segregation of the mutation was confirmed in the family using Sanger sequencing. Insight about genetic cause could be crucial to genetic counseling, helping patients and their family make an informed decision regarding a future reproductive decision and family planning. In EDMD, life-threatening cardiac problems often manifest long after the initial diagnosis; regular follow-up by a multidisciplinary team of neurologists, cardiologists and surgeons are highly recommended to provide the most optimized management plan to the patient.

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