Gene xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

New intronic splicing mutation in the *LMNA* gene causing progressive cardiac conduction defects and variable myopathy☆

Y. Rogozhina [a,*], S. Mironovich [a], A. Shestak [a], T. Adyan [b,d], A. Polyakov [b], D. Podolyak [a], A. Bakulina [c], S. Dzemeshkevich [a], E. Zaklyazminskaya [a,d]

[a] Petrovsky Russian Research Center of Surgery, Abricosovsky pereulok, 119991 Moscow, Russia
[b] Research Centre of Medical Genetics, Moskvorechie street, 115478 Moscow, Russia
[c] Novosibirsk State University, Pirogova str. 2, Novosibirsk, Russia
[d] Pirogov Russian National Research Medical University, Ostrovityanova str. 1, Moscow 117997, Russia

ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 29 August 2016
Accepted 3 October 2016
Available online xxxx

Keywords:
Lamin
Lamin A/C-dependent limb-girdle muscular dystrophy type 1B
Defect splice site mutation
Deep intronic mutation

ABSTRACT

Background: Most of mutations in the *LMNA* gene are unique and have been found in only a few unrelated families. The clinical interpretation of new genetic variants, especially beyond the coding area and canonical splice sites, is proving to be difficult and requires advanced investigation.
Methods: This study included patients with progressive cardiac conduction defects with neuromuscular involvement. The clinical evaluation included medical history and 24-h Holter monitoring. The genetic evaluation included mutation screening in the *LMNA* gene by the Sanger sequence. Sanger sequencing was followed by RT-PCR of the target fragment of cDNA. *In silico* modeling was performed with CCBulder and Modeller software.
Results: The diagnosis of limb-girdle muscular dystrophy type 1B (LGMD1B) was established. The new intronic variant c.513 + 45 T > G was found in the *LMNA* gene in the proband and affected daughter. The insertion of 45 bp was confirmed in the proband's cDNA. The structural and possible functional effects of the aberrant protein were predicted.
Conclusions: Variant c.513 + 45 T > G in the *LMNA* gene likely translates into the longer lamin A/C proteins with additional 15 amino acids. This variant is thought to be pathogenic. Intronic variants in the *LMNA* gene located beside canonic splice sites may be responsible for some genotype-negative cases with clinical phenotype of laminopathies.

1. Introduction

More than 460 mutations in the *LMNA* gene are known to be causative for at least 10 different allelic disorders, including lamin A/C-dependent limb-girdle muscular dystrophy type 1B (LGMD1B) (http://www.umd.be/LMNA/) (Worman and Bonne, 2007; Bertrand et al., 2011). Most of these mutations are unique and have been found in only a few unrelated families.

The genetic screening for mutations in the *LMNA* gene of patients with cardiomyopathies accompanied with progressive cardiac conduction defects is recommended (Priori et al., 2015). The screening plays an important role in clinical decision-making (Priori et al., 2015). Genetic testing using classical Sanger sequencing and Next generation sequencing (NGS) is being used with increasing frequency. The molecular confirmation of LGMD and the subsequent genetic counseling of family members, however, are still proving to be very challenging. There is a subset of clinically evident LGMD patients where no mutation can be found, even after screening all known candidate genes. There are variants of unknown clinical significance (VUCS) in some families, which require additional studies in order to determine if they are pathogenic mutations or benign variants. Several open bioinformatic tools (PolyPhen-2, SIFT, PROVEAN) are widely use to predict the pathogenicity of missense variants. Most nonsense and frame-shift variants, as well as splice mutations within 1–12 bp of adjacent intronic areas, are pathogenic (Richards et al., 2015). There are also many known splice mutations located besides the coding areas and well-recognized canonic sequences. For example, the most common Taiwanese mutation c.936 + 919G > A in the *XGAL* gene is deep in the intronic area, and is known to be responsible for the cardiac form of Fabry disease (Chien et al., 2012). The pathogenic role of two other intronic variants, c.8051 + 373G > T and c.6872-961 A > G, in the *FBN1* gene was confirmed by functional studies in Marfan syndrome patients (Gillis et al.,

Abbreviations: EDMD, Emery-Dreifuss Muscular Dystrophy; CCD, cardiac conduction defect; VUCS, variant of unknown clinical significance; CK, creatine phosphokinase; AVB, atrioventricular block; PM, pacemaker.

☆ The nucleotide sequence data reported and available in the ClinVar database under the variation ID SCV000268124.1.

* Corresponding author at: Medical Genetics Laboratory, Petrovsky Russian Research Center of Surgery, Abricosovsky pereulok, 119991 Moscow, Russia.
E-mail address: julia.rogozhina@gmail.com (Y. Rogozhina).

http://dx.doi.org/10.1016/j.gene.2016.10.001
0378-1119/

2014; Guo et al., 2008). There are several intronic variants in the *LMNA* gene that affect splicing sites and cause laminopathies (Renou et al., 2008; Carboni et al., 2011). The analysis of new genetic mutations in the introns is often difficult and requires further investigation. This could explain why such a findings are often "filtered" and discarded from the final report.

Here we present the results of clinical, genetic, and *In silico* analyses of the novel intronic variant c.513 + 45 T > G in the *LMNA* gene found in a family with a progressive cardiac conduction defect, variable neuromuscular involvement, and plasma creatine phosphokinase (CK) elevations.

2. Methods

2.1. Clinical evaluation

This study was performed in accordance with the Helsinki declaration and local ethics committee. Data obtained from each individual in the study included personal and familial medical history, creatine phosphokinase (CK) measurement, 12-lead resting ECG, 24-h ECG Holter monitoring and transthoracic echocardiogram.

2.2. Genetic analysis

Genomic DNA was extracted from the white blood cells of four family members. Mutation screening in the *LMNA* gene was performed by PCR-based bidirectional Sanger sequencing with a set of original intronic oligoprimers flanking all exons with about 200 bp. Primer sequences are available upon request. Mutation analysis in off-springs was performed by the PCR-based restriction fragment length polymorphism (RFLP) method with BstENI restriction enzymes (SibEnzyme, Russia). The prevalence of new genetic variants in healthy controls (200 DNA samples) and an Emery-Dreifuss muscular dystrophy cohort (91 probands) was determined by MLPA.

2.3. Extraction of RNA and reverse transcription-polymerase chain reaction (RT-PCR)

The RNA samples were extracted from fresh blood samples by MagNA Pure Compact RNA Isolation Kit (Roche, USA). The cDNA was synthesized by Transcriptor First Strand cDNA Synthesis Kit (Roche, USA). PCR with cDNA and Sanger sequencing was performed with the primers in the junction between exons 1–2 (1-2F: 5´- CTGAAAGCGCG CAATACCAAG-3´), 3–4 (3-4R: 5´- TACAGTGAGGAGCTGCGTGAGA-3´) and 6–7 (6-7R: 5´- GGAGGAGAGGCTACGCCTGT-3´).

2.4. In silico bioinformatics analysis

The potential influence of a novel genetic variant on splicing sites was performed in accordance with previously published guidelines (Richards et al., 2015). Open software Netgene2 (http://www.cbs.dtu.dk/services/NetGene2/) and NNSPLICE (http://www.fruitfly.org/seq_tools/splice.html) were used. In order to predict the presence of coiled coil structures several methods were used: Coils, Ncoils, MARCOIL (Lupas et al., 1991; Lupas, 1996; Delorenzi and Speed, 2002). The prediction was performed on Genesilico Metaserver (https://genesilico.pl/meta2/). A model of coiled coil Coil1B of WT lamin A/C was made by CCBuilder V1.0 web-server (http://coiledcoils.chm.bris.ac.uk/app/cc_builder/building/) with default parameters. This model was used as a template for homology modeling of Coil1B of mutated lamin A/C with Modeller software.

3. Results

3.1. Clinical case

A 42 years old male proband of Azeri origin presented with symptoms that included difficulty walking, muscular weakness in his feet and pelvic girdle, progressive physical activity intolerance, palpitations, and one stress-induced pre-syncopal episode, which had occurred a year earlier. The first clinical evaluation was performed in 2012, the follow-up period comes up to 5 years. The proband presented with bradyarrhythmia (heart rate 40–50 bpm), progressive atrioventricular block (AVB) of II degree, and premature ventricular contractions (PVCs). Cardiac chambers size, valvular structure and function, ejection fraction (63–65%), and contractility were all normal. Total CK in blood tests was consistently increased (485–500 IU/L). At the first evaluation he had a pronounced lumbar hyperlordosis and pseudo-hypertrophy of calf muscles, but no joints contractures were noted. He was diagnosed with limb-girdle muscular dystrophy (LGMD) with cardiac involvement. Physical examinations were performed on his three children (14, 13, and 6 years old). The cardiac conduction defect was treated by implantation of the dual-chambered PM. The two sons (13 and 14 years old) were apparently healthy at the time of clinical evaluation. The 6 years old daughter had lumbar spine hyperlordosis, gait instability and mild muscle weakness, but no arrhythmias or conduction defects were noted.

The proband's pedigree is shown in Fig. 1. The familial tree reconstructed based on verbal testimony contains 51 people in 4 generations. Several family members were affected with cardiac and/or

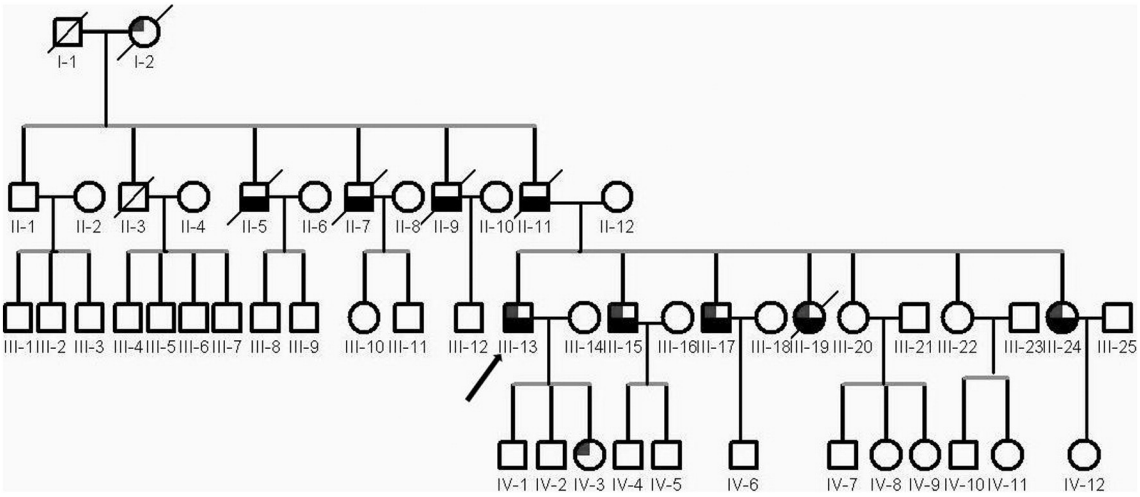

Fig. 1. Proband's pedigree. The arrow indicates the proband. Squares and circles indicate males and females, respectively. Precise medical records were available only for proband (III-13) and for his offsprings (IV-1, IV-2, IV-3). The phenotypes of relatives is denoted based on proband's verbal report. Open symbols indicate healthy family members, half-black symbols patients who had only cardiac complains. Upper left-quarter grey symbols indicate the presence of myopathy.

Please cite this article as: Rogozhina, Y., et al., New intronic splicing mutation in the *LMNA* gene causing progressive cardiac conduction defects and variable myopathy, Gene (2016), http://dx.doi.org/10.1016/j.gene.2016.10.001