A Novel Mutation in Lamin A/C Causing Familial Dilated Cardiomyopathy Associated With Sudden Cardiac Death

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ABSTRACT

Background: Dilated cardiomyopathy (DCM), a cardiac heterogeneous pathology characterized by left ventricular or biventricular dilatation, is a leading cause of heart failure and heart transplantation. The genetic origin of DCM remains unknown in most cases, but >50 genes have been associated with DCM. We sought to identify the genetic implication and perform a genetic analysis in a Spanish family affected by DCM and sudden cardiac death.

Methods and Results: Clinical assessment and genetic screening were performed in the index case as well as family members. Of all relatives clinically assessed, nine patients showed clinical symptoms related to the pathology. Genetic screening identified 20 family members who carried a novel mutation in *LMNA* (c.871 G>A, p.E291K). Family segregation analysis indicated that all clinically affected patients carried this novel mutation. Clinical assessment of genetic carriers showed that electrical dysfunction was present previous to mechanical and structural abnormalities.

Conclusions: Our results report a novel pathogenic mutation associated with DCM, supporting the benefits of comprehensive genetic studies of families affected by this pathology. (*J Cardiac Fail 2015;21:217–225*)

Key Words: Dilated cardiomyopathy, lamin A/C, novel mutation, sudden cardiac death.

Dilated cardiomyopathy (DCM) is characterized by cardiac left ventricular (LV) dilation and subsequent abnormal contraction; dilation of both ventricles may occur. The pathology can manifest at any age but occurs more frequently after middle age. A leading cause of sudden cardiac death (SCD) in developed countries, cases can also range in symptoms from severe heart failure to asymptomatic.[1]

Genetic factors play a role in the pathogenesis of DCM, but the genetic basis remains unknown in most cases.[2] Currently, more than 60 genes have been reported to cause monogenic DCM, most of them encoding for sarcomeric, cytoskeletal, and desmosomal proteins.[3] To date, 20% of mutations associated with DCM are linked to *TTN*, the gene encoding titin protein; 6% are related to *LMNA*, the gene encoding lamin A/C protein.[4] Lamin A/C maintains the structural integrity of the nuclear envelope and organizes chromatin within the nucleus, thereby influencing DNA transcription.[5] Some reports of genetic studies in families affected by DCM and carrying mutations in *LMNA* discuss the severity of clinical findings associated with mutations in this gene compared with other DCM-related genes.[6,7] A recent report indicated that subjects with *LMNA* mutations show prolonged PR interval in electrocardiography (ECG), myocardial septal fibrosis, and ventricular arrhythmias; all these symptoms are indicative of DCM.[8]

We have identified a large family affected by DCM with a high incidence of SCD. We have performed genetic analyses both to identify the causative mutation and to provide phenotype-genotype correlation, providing further insight on genetic contributions to DCM pathology.

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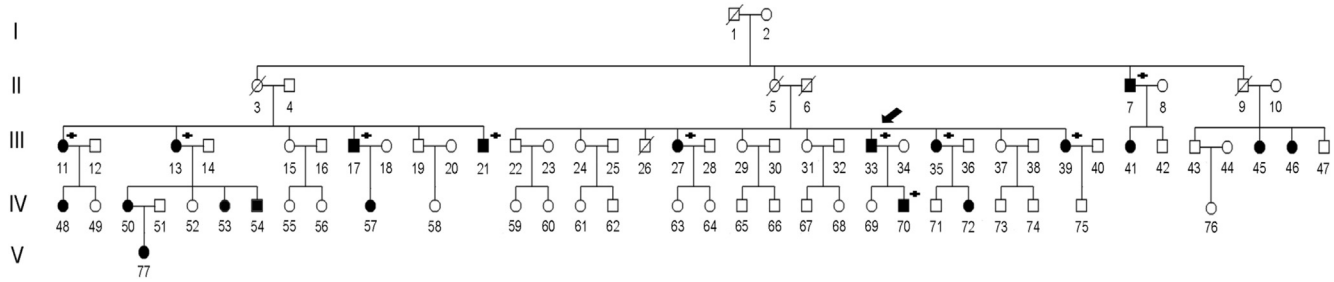

Fig. 1. Family pedigree. The generations (I−V) are indicated at the left, and each individual is identified with a pedigree number. The index patient is indicated with an arrow (III-33). Mutation carriers are shown in black, and relatives without mutation are white. Slashes indicate individuals who are deceased. Clinically diagnosed patients are indicated with a plus sign.

Methods

Clinical Assessment

Written informed consents were obtained from all family members included in our study. Detailed clinical information was obtained from each subject, comprising family history, age of presentation, initial symptoms of heart failure, physical examination, serum creatine kinase, ECG, transthoracic echocardiography (TTE), Holter ECG monitoring, treadmill testing, and, when appropriate, cardiac magnetic resonance imaging (MRI). Routine TTE with the use of Doppler and tissue Doppler imaging (TDI) was performed according to the standards of the American Society of Echocardiography.[9,10] The diagnosis of DCM was based on symptoms, physical signs, ECG, and chest X-ray findings of ventricular dysfunction, supported by TTE or angiographic confirmation of global ventricular dilatation.[11] In this study all patients underwent clinical history, ECG, TTE with TDI, Holter ECG and exercise stress testing. Only those who showed any impairment in the TTE underwent MRI.

Genetic Analysis

Genomic DNA was extracted with the use of the Puregene DNA purification kit (Qiagen). DNA quality was assessed by means of spectrophotometry (optical absorbance 260/280:260/230 of a minimum of 1.8:2.2), and quantity was determined by means of fluorometry (Qubit; Life Technologies) before processing to obtain the 3 μg needed for sequencing. DNA integrity was assessed on a 0.8% agarose gel.

The index patient (III-33, see Fig. 1) was analyzed with the use of NGS technology. Library preparation was performed according to the manufacturer's instructions (Sureselect XT Custom 0.5−2.9 Mb library; Agilent Technologies). Sequencing was performed on the MiSeq system (Illumina) with the use of 2 × 150 bp read lengths. We evaluated the 55 genes most commonly involved in SCD-related pathologies according to the literature (Table 1).

The secondary bioinformatic analysis of the data obtained included a first-step trimming of the FAST-Q files. The trimmed reads were then mapped with the use of GEM II, output was joined and sorted, and uniquely and properly mapping read pairs were selected. Finally, variant calls over the cleaned binary sequence alignment/map file were performed with the use of SAM tools v1.18 and GATK v2.4 to generate the first raw variant call format files. Variants were annotated with dbSNP IDs, Exome Variant Server (EVS), the 1000 Genomes browser, in-house database IDs, and Ensembl information, if available. Regions with poor coverage were sequenced with the use of Sanger sequencing.

Tertiary analysis was then performed. For each genetic variation identified, alignment between different species was performed with the use of the Universal Protein Resource database.[12] The possible pathogenicity of challenged variants was consulted in silico, with the use of the consensus deleteriousness score of the missense single-nucleotide variants (SNVs) database (CONDEL)[13] and the Protein Variation Effect Analyzer database (PROVEAN).[14] Allelic frequency was determined in the Exome Variant Server,[15] dbSNP,[16] and the 1000 Genomes genetic variants database.[17]

Noncommon genetic variants (minor allele frequency [MAF] $<1\%$) were confirmed by means of Sanger sequencing. Genetic variants identified in the index case were analyzed in all other family members with the use of Sanger technology.

Results

Clinical Data

The index patient (III-33; Fig. 1) was a 37-year-old man with a history of palpitations and skipped beats that were later documented as premature ventricular contractions (PVCs) and nonsustained ventricular tachycardia (Fig. 2). PVCs were noted both at rest and during exercise. Exercise stress testing also showed ventricular extrasystoles with nonsustained ventricular tachycardia. He never experienced syncope. His LV size was mildly dilated and sphere shaped (57 mm); systolic function was depressed (47%), and the mitral inflow E-wave was higher than the A-wave, consistent with a pseudonormalization diastolic pattern and confirmed with the use of TDI. Accordingly, cardiac MRI was performed, which showed discrete LV dilation (Fig. 3). He had neither signs nor symptoms of heart failure. He became progressively more symptomatic from premature ventricular contractions, and received an implantable cardioverter-defibrillator (ICD).

The index patient had 2 children. One of them (IV-70), a 5-year-old boy, occasionally experienced supraventricular extrasystoles and showed a long PR interval (244 ms). His echocardiographic evaluation was normal. The index patient had 2 brothers and 7 sisters. One of the brothers (III-26) passed away suddenly at age 31 years while he was sleeping. No clinical report or autopsy information was available. One sister (III-27), age 44 years, presented at the time of this study with several episodes of both