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Titin Mutation in Familial Restrictive Cardiomyopathy ☆

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ABSTRACT

Background: Familial restrictive cardiomyopathy (RCM) caused by a single gene mutation is the least common of the inherited cardiomyopathies. Only a few RCM-causing mutations have been described. Most mutations causing RCM are located in sarcomere protein genes which also cause hypertrophic cardiomyopathy (HCM). Other genes associated with RCM include the desmin and familial amyloidosis genes. In the present study we describe familial RCM with severe heart failure triggered by a de novo mutation in *TTN*, encoding the huge muscle filament protein titin.

Methods and results: Family members underwent physical examination, ECG and Doppler echocardiogram studies. The family comprised 6 affected individuals aged 12–35 years. Linkage to candidate loci was performed, followed by gene sequencing. Candidate loci/gene analysis excluded 18 candidate genes but showed segregation with a common haplotype surrounding the *TTN* locus. Sequence analysis identified a de novo mutation within exon 266 of the *TTN* gene, resulting in the replacement of tyrosine by cysteine. p.Y7621C affects a highly conserved region in the protein within a fibronectin-3 domain, belonging to the A/I junction region of titin. No other disease-causing mutation was identified in cardiomyopathy genes by whole exome sequencing.

Conclusions: Our study shows, for the first time, that mutations in *TTN* can cause restrictive cardiomyopathy. The giant filament titin is considered to be a determinant of a resting tension of the sarcomere and this report provides genetic evidence of its crucial role in diastolic function.

Introduction

Restrictive cardiomyopathy (RCM) is a primary disease of the myocardium characterized by restrictive ventricular physiology and increased filling pressures that cause progressive left- and right-sided heart failure. The main features are marked atrial enlargement, normal-to-reduced ventricular diastolic volumes and normal ventricular wall thickness [1–3]. By definition, systolic function is supposed to be normal or preserved in RCM, but contractility is often abnormal [3]. The overall prognosis is poor, especially with childhood onset, and patients often require cardiac transplantation. RCM may be found in systemic disorders causing abnormal substrate deposition, infiltration and fibrosis; it may also result from metastatic cancer, drug or radiation toxicity [1]. However, even after comprehensive work-up, including endomyocardial biopsy, many cases remain idiopathic. Some of these cases have a familial, genetically determined, etiology.

Familial RCM caused by a single gene defect is the least common of the heritable cardiomyopathies encountered in clinical practice. The mode of inheritance is often autosomal dominant, but may also be autosomal recessive and X-linked. In some families the disease is caused by mutations in sarcomeric proteins such as troponin I, β-myosin heavy chain, troponin T and α-cardiac actin [3–6]. Other genes associated with RCM include the desmin and familial amyloidosis genes (primarily *TTR*). Mutations in desmin account for some of the dominantly or recessively inherited cases [7], and are characterized by a concomitant conduction

☆ "The authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation".

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system disease and may present without myopathy [8]. The giant protein titin plays a fundamental role in sarcomere structure and regulating resting tension. Recent studies defined *TTN* mutations in a considerable portion of genetically determined dilated cardiomyopathy [9]. In the present study we implicate for the first time titin in causing familial restrictive cardiomyopathy.

Methods

Clinical evaluation

The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board, Sheba Medical Center and the Genetic Committee of the Ministry of Health, Israel. We identified a Jewish woman of Sephardic origin with paroxysmal atrial fibrillation and heart failure suffering from familial RCM (kindred R1VM). The diagnostic workup of the proband (II-4 in Fig. 1A) started with ruling out alternative causing diseases, and included MRI and endomyocardial biopsy. Informed consent was obtained from all participants or their legal representatives prior to inclusion in the study.

Patients and family members were examined on site by a dedicated team from the Heart Failure Service and underwent echo Doppler, 12 lead ECG, and blood testing for troponin I, creatine kinase and NT-BNP. All Doppler echocardiogram studies were performed with Vivid 7 or mobile Vivid I devices (General Electric, Tirat Carmel, Israel), using a 2/5 MHz transducer, and were recorded digitally for further off-line analysis. Affection status was defined by restrictive filling pattern on Doppler, concomitant with supportive evidence such as increased filling pressure or clinical history of congestive heart failure [3]. Serum levels of NT-proBNP were determined by electrochemiluminescence immunoassay (Elecsys proBNP kit cat. 03121640, Roche Diagnostics, Indianapolis, IN, USA).

Genetic studies

DNA was extracted from peripheral venous blood using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minnesota) and diluted to 200 ng/μl. For candidate loci analysis we performed linkage studies to genes previously implicated in RCM and other cardiomyopathies by using polymorphic short tandem repeat markers adjacent to the locus of interest. Nucleic acid sequences were obtained from the national center for biotechnology information (NCBI) (www.ncbi.nlm.nih.gov) and the ENSEMBL (www.ensembl.org) databases. Primers were generated with Primer-3 (http://frodo.wi.mit.edu/) and are available upon request. Allele lengths were determined by electrophoresis with ABI-3100 Genetic Analyzer and Genotyper software (ABI PRISM® 3100 Genetic Analyzer, Applied Biosystems, California). Linkage to candidate loci was studied by testing the segregation of polymorphic markers with disease phenotype. We excluded a locus when there was no haplotype sharing by 2 or more affected individuals.

After finding a segregation to the locus of the *TTN* gene, all 363 titin exons (NM_001267550) and three additional Novex exons were PCR-amplified and directly sequenced using a Big Dye Terminator DNA sequencing kit on a 3100 Avant Genetic Analyzer (PE Applied Biosystems, California) as previously described [10]. Family members and controls were genotyped for c.22862A > G *TTN* mutation (NM003319; N2-B = cardiac expressed isoform) by HpyCH4V restriction digestion and/or direct sequencing. Because genotyping results suggested a germ line mosaicism, LOD score was calculated according to the model of Weissbrod and Geiger [11].

In addition, whole exome sequencing of patient III-1 was performed using a LifeTech 5500xl sequencer (Life Technologies, Grand Island, NY) following sequence enrichment using the Agilent SureSelectXT2 Human All Exon V4 capture kit (Agilent Technologies, Santa Clara, CA). Raw colorspace sequences were error-corrected and aligned to the human reference genome version hg19 (http://genome.ucsc.edu/FAQ/FAQreleases.html) with version 2.5 of the 5500xl manufacturer's LifeScope software, using the "targeted.resequncing.pe" workflow. The aligned sequences were deduplicated, realigned and genotypes called using multiple third party programs, using the validated workflow described in [12]. The mean deduplicated coverage in the enriched regions was 128-fold, with >92% of exome bases covered at >10×. Resulting sequence variants were annotated with the maximum minor allele frequency values found in an internal database (to exclude sequencing platform artifacts), version 137 of the dbSNP database [13], or the large public personal genome datasets from the 1000 Genomes Project [14] and NHLBI Exome Sequencing Project version ESP6500SI (http://evs.gs.washington.edu/EVS/).

Results

Clinical data

The family with RCM family comprised 6 affected individuals aged 12–35 years (Fig. 1A and Table 1). The pedigree and clinical data are compatible with autosomal dominant inheritance with early onset disease. The proband, a 35-year-old female (II-4), was referred for evaluation with clinical presentation of heart failure, atrial fibrillation and thromboembolic phenomena. Echocardiography revealed preserved systolic function, severe diastolic dysfunction and signs of elevated filling pressure with no valvular dysfunction (Table 2, Fig. 2). Cardiac MRI ruled out constrictive pericarditis and demonstrated enlarged atria, mild contractile dysfunction (RVEF 35%) and dilatation (RVEDV 76 ml/m^2) of the right ventricle, with regions of excess trabeculation and extensive left ventricular late gadolinium enhancement in epicardial, mid-wall and subendocardial areas (Fig. 2). Endomyocardial biopsy obtained from the right ventricular septum of the proband showed myocyte hypertrophy with enlarged irregular nuclei, myocyte degeneration and extensive fibrosis. There was no fatty infiltration or evidence of amyloidosis, storage disease, or other infiltrative process (Fig. 3).

The proband's two daughters (III-2 and III-4 in Fig. 1A and Table 1) suffered from advanced heart failure and had echo findings of RCM, while her son qualified as RCM but was asymptomatic (III-3 in Fig. 1A

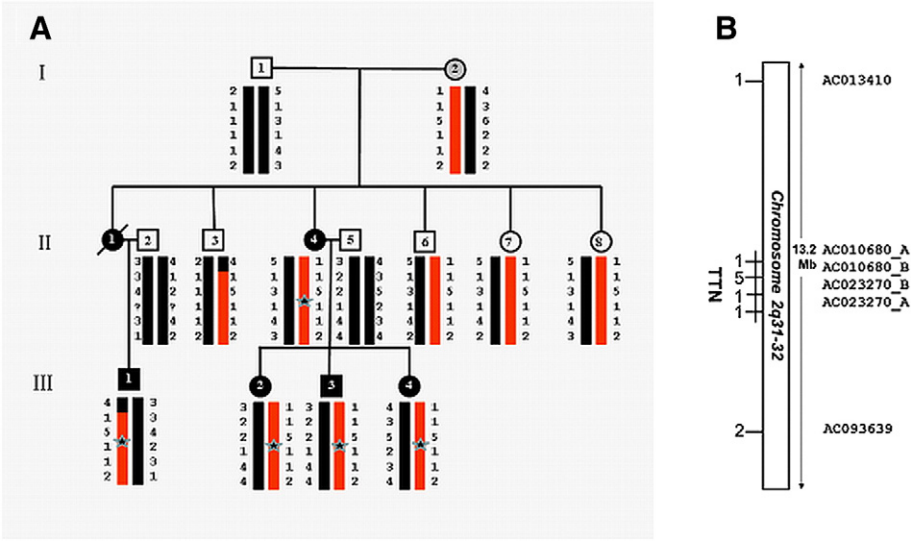

Fig. 1. RCM kindred and disease haplotype. A). Pedigree of the kindred R1VM with autosomal dominant RCM. II-4 is the proband. Filled symbols denote clinically affected status; Open symbols, unaffected; Gray, unclear clinical status. The polymorphic markers surrounding the *TTN* locus are indicated by numbers and a common haplotype is shown in red. Star, Y7261C mutation; B) The relative position of the polymorphic markers on chromosome 2q31. The disease haplotype was defined by 1-1-5-1-1-2 for the markers located at AC013410, AC010680 (A and B), AC023270 (B and A) and AC093639, respectively.