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ARTICLE

Doubly heterozygous *LMNA* and *TTN* mutations revealed by exome sequencing in a severe form of dilated cardiomyopathy

Roberta Roncarati[1,2], Chiara Viviani Anselmi[2], Peter Krawitz[3,4,5], Giovanna Lattanzi[6], Yskert von Kodolitsch[7], Andreas Perrot[8,9], Elisa di Pasquale[2,10], Laura Papa[2], Paola Portararo[2], Marta Columbaro[6], Alberto Forni[11], Giuseppe Faggian*[,11], Gianluigi Condorelli*[,1,12] and Peter N Robinson*[,3,4,5]

Familial dilated cardiomyopathy (DCM) is a heterogeneous disease; although 30 disease genes have been discovered, they explain only no more than half of all cases; in addition, the causes of intra-familial variability in DCM have remained largely unknown. In this study, we exploited the use of whole-exome sequencing (WES) to investigate the causes of clinical variability in an extended family with 14 affected subjects, four of whom showed particular severe manifestations of cardiomyopathy requiring heart transplantation in early adulthood. This analysis, followed by confirmative conventional sequencing, identified the mutation p.K219T in the lamin A/C gene in all 14 affected patients. An additional variant in the gene for titin, p.L4855F, was identified in the severely affected patients. The age for heart transplantation was substantially less for LMNA:p.K219T/TTN:p.L4855F double heterozygotes than that for LMNA:p.K219T single heterozygotes. Myocardial specimens of doubly heterozygote individuals showed increased nuclear length, sarcomeric disorganization, and myonuclear clustering compared with samples from single heterozygotes. In conclusion, our results show that WES can be used for the identification of causal and modifier variants in families with variable manifestations of DCM. In addition, they not only indicate that *LMNA* and *TTN* mutational status may be useful in this family for risk stratification in individuals at risk for DCM but also suggest titin as a modifier for DCM.
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Keywords: familial dilated cardiomyopathy; lamin A/C; titin; whole-exome sequencing; modifying variant

INTRODUCTION

Dilated cardiomyopathy (DCM) is characterized by cardiac chamber enlargement and impaired contraction of the left ventricle; approximately 30–50% of affected individuals have a familial form of DCM. The genetics of familial DCM is complex and includes over 30 disease-causing genes. With the exception of *TTN*, truncating mutations of which have been found in up to 27% of individuals with DCM,[1] a single DCM-causing gene accounts for no more than 6–8% of all cases. The known DCM disease genes include those encoding for proteins of the sarcomere, the Z-disk, the cytoskeleton, the mitochondria, RNA binding proteins, the sarcoplasmic reticulum, and the nuclear envelope.[2–4] Familial DCM exhibits a remarkable degree of clinical variability with respect to severity, penetrance, and age of onset.[5] Like familial hypertrophic cardiomyopathy (HCM), familial DCM is often characterized by incomplete penetrance, a high degree of variable expressivity even among family members, and by highly variable age of onset and rate of disease progression. The molecular correlates of these observations have remained largely unknown, which can make medical management difficult. Recently, two or more sequence alterations present either in the same or in different genes were demonstrated to occur in 3–5% of cases of familial HCM, associated with a greater clinical severity of HCM.[6–8] Similarly, compound and digenic heterozygosity were identified in patients with arrhythmogenic right ventricular cardiomyopathy, whereby several desmosome and cell-junction genes were found to carry more than one mutation likely associated with low penetrance.[9] It is to be expected that similar modifiers exist for DCM; even though a single case of a 14-year-old boy with manifestations of DCM and mutations in both *MYH7* and *TNNT2* has been published,[10] little is known about the genes and the molecular mechanisms involved in modifying the phenotype of familial DCM.

[1]Biomedical and Genetic Research Institute (IRGB), Milan Unit, National Research Council of Italy, Milan, Italy; [2]Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Milan, Italy; [3]Institute of Medical Genetics and Human Genetics, Charité Universitätsmedizin, Berlin, Germany; [4]Berlin-Brandenburg Center for Regenerative Therapies, Charité Universitätsmedizin, Berlin, Germany; [5]Max Planck Institute for Molecular Genetics, Berlin, Germany; [6]National Research Council of Italy, Institute of Molecular Genetics, Laboratory of Musculoskeletal Cell Biology, Rizzoli Orthopedic Institute, Bologna, Italy; [7]Centre of Cardiology and Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany; [8]Department of Cardiology, Campus Virchow-Klinikum, Charité-Universitätsmedizin Berlin, Berlin, Germany; [9]Experimental and Clinical Research Center (ECRC), Charité and the Max Delbrück Center for Molecular Medicine, Berlin, Germany; [10]Institute of Genetic and Biomedical Research (IRGB), National Research Council (CNR), Milan, Italy; [11]Division of Cardiac Surgery, University of Verona, Verona, Italy; [12]Humanitas Clinical and Research Center, Milan, Italy

*Correspondence: Professor G Faggian, Department of Cardiac Surgery, University of Verona, Piazzale Stefani 1, Verona 37126, Italy. Tel: +39 04 58121227; Fax: +39 04 58123308; E-mail: giuseppe.faggian@univr.it

or G Condorelli, Humanitas Clinical and Research Institute, Via Manzoni 56, Milan 20089, Italy. Tel: +39 02 82245201; E-mail: gianluigi.condorelli@humanitasresearch.it

or Professor PN Robinson, Institute of Medical Genetics and Human Genetics, Charité Universitätsmedizin, Augustenburger Platz 1 Berlin 13353, Germany. Tel: +49 30 450566006; Fax: +49 30 450569915; E-mail: peter.robinson@charite.de

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Whole-exome sequencing (WES), in which capture methods are used to enrich the sequences of the coding regions of genes from fragmented total genomic DNA, followed by massively parallel, 'next-generation' sequencing of the captured fragments, has led to a paradigm shift in diagnostics and disease-gene discovery projects in human genetics.[11] WES thus offers an interesting modality for the search for the causes of variable expressivity and non-penetrance in familial DCM. In this work, we investigated an extended Italian family diagnosed with DCM and cardiac conduction defects (CCM) using WES followed by targeted Sanger resequencing. In 14 out of the 41 family members enrolled in the study, a mutation in the gene for lamin A/C (*LMNA*) was identified. Five of them, clearly showing DCM features, were doubly heterozygous for variants in *LMNA* and in the titin gene (*TTN*). Four of these patients (all four older than 20 years of age) displayed a particularly severe clinical involvement at early ages.

MATERIALS AND METHODS

Patient recruitment

From May 1994 to September 2011, 10 patients affected by DCM underwent orthotopic heart transplantation at the University Hospital of Verona. Subsequently, these patients were found to belong to the same extended family, whose ancestors had moved from their native Verona to other regions of Italy and Northern Europe. In the course of this study, 42 living and 41 deceased family members were identified on the basis of data from parish church registries and hospital records. A total of 41 subjects were enrolled in the study, including 18 females and 23 males aged 5–77 years (Supplementary Figure S3). Fourteen persons were affected by a range of cardiological symptoms, ranging from mild dyspnea on exertion to severe congestive heart failure (CHF), leading to emergent heart transplantation. Twenty-seven persons showed no signs of cardiomyopathy. Written informed consent was obtained from all participating probands according to the study protocols approved by hospital ethics committees.

Study protocol

All persons were investigated using the same study protocol, comprising full medical history and physical examination, chest radiograph, electrocardiogram and echocardiography, coronary artery angiography, right ventricular catheterization, measurement of oxygen consumption, Holter monitoring, and endomyocardial biopsy (Supplementary Tables S1–S3 and Supplementary Figures S1A-S1B). The diagnosis of DCM was evaluated according to published guidelines.[12] The main diagnostic criteria were: left ventricular ejection fraction $<45\%$ and left ventricular end-diastolic dimensions $>117\%$ of the predicted value corrected for age and body surface area. Clinical data were extracted from medical records when direct examination at Verona Hospital was not feasible. Peripheral whole-blood samples of patients and family members were collected during examination or enrollment phase. DNA was extracted using the DNA Isolation Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions, for all samples. According to the pedigree structure and clinical data, patients V.13, V.15, and V.17 were chosen as severe DCM cases and VI.7 as a healthy control. DNA samples from these four patients were subjected to WES.

DNA samples, targeted exome capture, and massively parallel, next-generation sequencing (NGS)

An amount of 5 μg of DNA extracted from DCM (V.13, V.15, V.17) and non-DCM patients (VI.7) was sheared by nebulization. Adapter-ligated libraries were prepared with the Paired-End Sample Prep kit V1.0.1 (Illumina, San Diego, CA, USA), except that the gel-size selection step was replaced with a purification using magnetic bead-based solid-phase reversible immobilization beads (Agencourt, Beckman Coulter, Brea, CA, USA). Exome capture was performed with the SureSelect Human All Exon kit v 2.0 (Agilent Technologies, Palo Alto, CA, USA), which targets about 45 Mb. Pair-end sequencing was performed on the Illumina Genome Analyzer IIx (GAIIx), generating 100-bp end reads.

Read mapping and variant analysis

Pair-end reads were quality trimmed and aligned with the human genome reference sequence (NCBI build 37/UCSC hg19) by using CLC Bio Genomics Workbench (CLC Bio, Aarhus, Denmark). Single-nucleotide polymorphisms (SNPs) and short indels were called with CLC Bio, filtering out calls with a read coverage $<8\times$ and a Phred SNP quality of <20. Variants were filtered against NCBI dbSNP132, 1000 Human Genomes Project catalog, and the AVSIFT database, and their functional annotation was performed using Annovar.[13] Data were filtered for genes showing heterozygous variants in all three sequenced relatives (V.13, V.15, and V.17) and a homozygous reference sequence in the unaffected relative (VI.7).

PCR and Sanger sequencing validation of LMNA and TTN variants

The mutations LMNA:p.K219T (NM_170708:c.A656C) and TTN:p.L4855F (NM_133378:c.C14563T) were validated using Sanger methods on the four subjects (three affected and one clinically healthy) that were sequenced using WES. Following this, all 41 participating family members were tested for the presence of these sequence variants by Sanger sequencing (Supplementary Figures S2A and B).

Primer design and PCR set up

For each target, primers were designed with Primer 3 plus Software[14] starting from the sequences NM_170708 (*LMNA* exon 4) and NM_133378 (*TTN* exon 59). The primers are shown in Table 1. PCR was performed with an automated liquid handler (Tecan Freedom EVO; Tecan, Männedorf, Switzerland) in a final volume of 7.5 μl with $1\times$ GoTaq Hot Start Master Mix (Promega, Madison, WI, USA), 0.5 μM of each primer, and 1.5 μl of DNA previously normalized at a concentration of $\sim$5 ng/μl. Amplification was performed on Eppendorf mastercycler ep gradient with the following amplification protocol: initial denaturation at 95 °C for 2 min, 35 cycles at 95 °C for 30 min, 58 °C for 45 min, 72 °C for 1 min, and final extension at 72° C for 10 min. The amplification protocol was the same for each target gene.

All PCR reactions were purified enzymatically (ExoSAP-IT PCR Clean-up Kit; GE Healthcare) following the manufacturer's protocol; purified DNA was used as a template for sequencing analysis. The sequencing reaction was performed with an automated liquid handler (Tecan Freedom EVO) in a final volume of 10 μl with Big Dye Terminator kit v 3.1 Chemistry (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Capillary electrophoresis was performed on an ABI 3730 DNA Analyzers (Applied Biosystems).

Immunohistochemical staining

Heart samples were fixed in 10% formaldehyde, and paraffin-embedded sections (7 μm) were stained with hematoxylin and eosin. Lamin A/C staining was performed on paraffin-embedded sections from wild-type (WT), *LMNA*-, or *LMNA*/*TTN*-mutated myocardium after antigen retrieval at pH 9. Undiluted antibody was applied overnight at 4 °C. Lamin A/C was labeled using anti-lamin A/C polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; Sc-6215), which was applied for 1 h at room temperature.[15] Bound antibody was detected with a horseradish peroxidase-conjugated anti-goat Ig, using diaminobenzidine as a substrate. Samples were counterstained with hematoxylin.

Nuclear size was measured using the NIS elements software (Nikon, El Segundo, CA, USA), and counts obtained from triplicate examinations were plotted as mean ± SE. Samples from two controls, two *LMNA*

Table 1 PCR primers for validation of the *LMNA* and *TTN* sequence variants

Gene	*Primer sequence*
LMNA (NM_170708, exon 4)	F: 5′-AGCACTCAGCTCCCAGGTTA-3′
	R: 5′-CTGATCCCCAGAAGGCATAG-3′
TTN (NM_133378, exon 59)	F: 5′-TCAGTTTGGAAGGATGACACC-3′
	R: 5′-TGCCTGTTATTTGGCATTCA-3′

Abbreviations: F, forward primer; R, reverse primer.

single-heterozygote individuals, and two *LMNA*/*TTN* double-heterozygote individuals were counted.

RESULTS

Targeted WES

Forty-one subjects from an extended Italian family with DCM were investigated (Supplementary Tables S1–S4 and Supplementary Material), including four patients with unusually severe manifestations requiring HTX by the age of 35 (see excerpted pedigree in Figure 1a). We hypothesized that all family members affected by DCM would segregate an identical mutation in a DCM gene, but the severely affected persons could have an additional variant/s in the same or other genes. To test this hypothesis, we performed WES on three severely affected persons and one unaffected relative (Figure 1) as described in detail in Materials and methods.

Under the assumption that rare, heterozygous sequence variants are the best candidates for the etiology of familial DCM, which generally is transmitted as an autosomal-dominant trait, we filtered variants for those that were rare, predicted to be pathogenic and heterozygous in each of the three affected relatives, and not present in the unaffected relative (Table 2). Only 28 such variants were found. Annotation data from the Human Phenotype Ontology project[16] were used to filter these variants and the associated genes, revealing eight genes in which variation has been associated with human Mendelian disease (Supplementary Table S5 and Supplementary Material). Only two these genes were found to be associated with abnormalities in the cardiovascular system, *LMNA* (lamin A/C) and *TTN* (titin), both of which are known to be associated with familial DCM.[1,2,17–31] A missense mutation was identified in the gene for lamin A/C (*LMNA*:c.656A>C; p.K219T), previously reported in an unrelated

Table 2 Summary of computational variant filtering

	Sample 1	*Sample 2*	*Sample 3*	*Sample 4*	*Total*
SNV	25 392	26 099	23 843	24 916	41 821
SNV not in dbSNP/ThG	661	704	596	762	1982
Rare syn	98	125	80	128	325
Rare nonsyn	114	119	114	133	349
Indel	1233	1259	1179	1252	2051
Indel not in dbSNP/TG	422	449	419	433	865
Rare ex. Indel	49	50	40	48	92
Rare splicing	3	2	2	8	11
NSISS	166	171	156	189	452
Compatible	–	–	–	–	**28**

The total number of single-nucleotide variants (SNVs) is shown in the first row. These were further filtered to SNVs not present in dbSNP or in the 1000 genomes project (ThG), and finally, rare synonymous and rare nonsynonmous variants that are predicted to be pathogenic by AVSIFT[51] are shown. 'Indel' shows small insertions and deletions, which were also filtered against dbSNP and the 1000 Genomes project (ThG). 'Rare ex. Indel' shows rare frameshift or non-frameshift insertions or deletions within the coding sequence. Rare splicing shows mutations of the canonical splice site sequences. NSISS shows total rare nonsynonymous (missense) variants, indels, and splicing mutations. These variants were then filtered for being heterozygous in the three samples from affected persons and not present in the one sample from a healthy sibling (**compatible**). See Supplementary Tables S5 and S6 for a list of these variants.

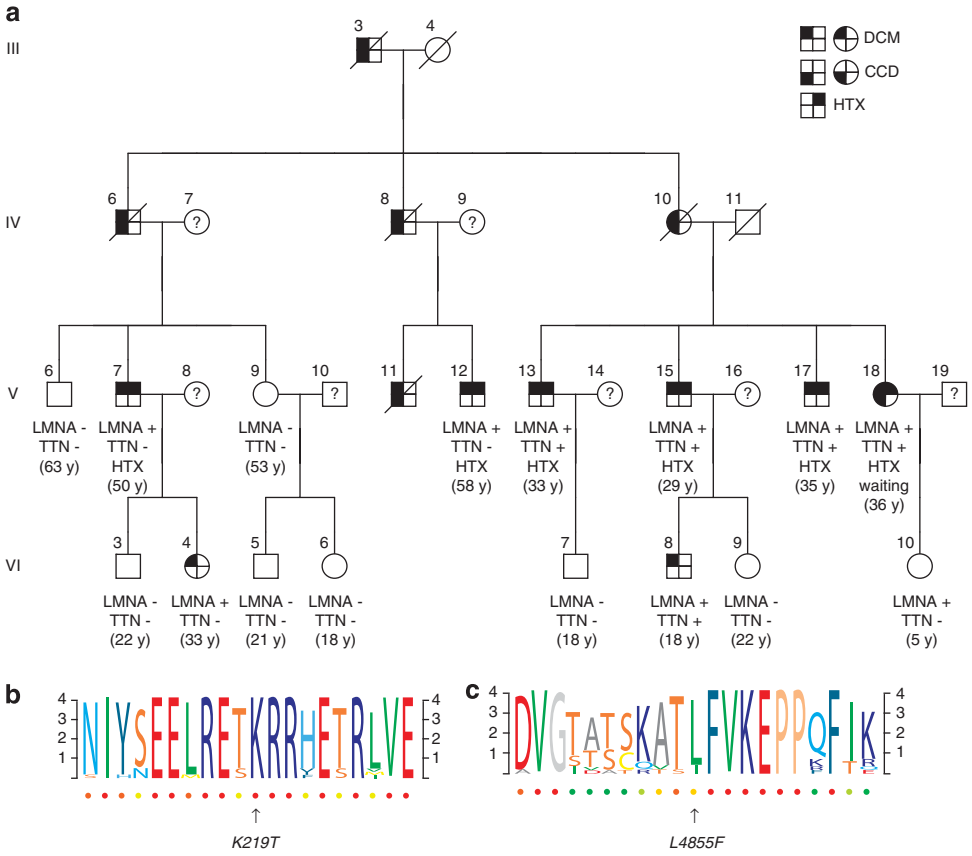

Figure 1 (**a**) Excerpt of pedigree. LMNA+: presence of *LMNA*:c.656A>C; TTN+: presence of *TTN*:c.14563C>T. HTX shows the age of heart transplantation in years. Four individuals were investigated by exome sequencing, including three with DCM (V.13, V.15, and V.17) and one healthy control (VI.7). (**b**) Sequence logo created with lamin A/C sequences from 21 species, ranging from human to zebrafish. (**c**) Sequence logo made with titin sequences from 17 species, ranging from human to zebrafish. The mutation p.L4855F affects a highly conserved leucine residue that is conserved in all analyzed species, except for mouse and rat, in which the corresponding residue is isoleucine.

individual with DCM,[17] and a previously unreported variant was found in the gene for titin (*TTN*:c.14563C>T; p.L4855F). There were no other variants in genes associated with cardiological phenotypes. Sanger sequencing was used to investigate all 41 probands. In all, 14 subjects were found to carry *LMNA*:c.656A>C, and five carried *TTN*:c.14563C>T. No family members were found to carry just the *TTN* variant but not the *LMNA* variant. The two family members from whom the variant could have come (III.4 and IV.11 in Figure 1a) had lived to over 70 years with no signs of cardiac disease, suggesting the possibility that *TTN*:c.14563C>T does not in itself cause DCM. However, because the third family member who could have conceivably introduced the mutation into the family, III.3, died suddenly at the age of 33 years, this possibility is far from certain. Individual III.3 must have carried the LMNA mutation. We note that *LMNA* mutations themselves may portend an increased risk of sudden death,[32] and therefore, sudden death at the age of 33 years is not necessarily an indication of the presence of an additional *TTN* mutation. Further clinical information on potential clinical signs of DCM in III.3 family was not available. The *TTN* sequence variant was not found in 410 unrelated healthy Italian subjects, and, in addition, was not present in the Exome Variant Server data set,[33] in which the sequence neighborhood surrounding the position of the mutation was covered at an average depth of 85 reads in 4845 samples. This suggests that TTN:p.L4855F (NM_133378:c.C14563T) is not a common polymorphism. *TTN*:c.14563C>T affects a conserved position in an Ig repeat in the N2Ba-specific region of titin (Figure 1c).

Among the nine subjects carrying only *LMNA*:c.656A>C, six presented with typical manifestations of DCM, including CHF, dyspnea at rest with reduced maximal oxygen consumption (VO_2), and conduction defects including ventricular arrhythmias. Five of these persons underwent HTX at a mean age of 58 years (Supplementary Table S1). None of the single-heterozygote LMNA: c.656A>C mutation carriers showed signs of muscular dystrophy or atrioventricular conduction block.

In contrast, four of the five doubly heterozygote mutation carriers presented with severe DCM with disease onset at the age of 24 (V.13), 25 (V.15), 28 (V.17), and 35 (V.18) years characterized by CHF, NYHA IV, dyspnea at rest, ventricular arrhythmias, and episodes of cardiac arrest. Three of these patients (V.13, V.15, and V.17) underwent HTX at the age of 29, 33, and 35 years, respectively, whereas the fourth (V.18) is currently on the waiting list at age 36. In addition, an 18-year-old male double-heterozygote proband (V.8) was asymptomatic, except for sporadic tachycardia (Supplementary Table S2). All 14 probands were diagnosed with DCM by endomyocardial biopsy. None of the family members who were negative for *LMNA*:c.656A>C and *TTN*:c.14563C>T presented with signs of cardiac disease, except for a 55-year-old male diagnosed with ischemic cardiomyopathy and a child with patent ductus arteriosus (Figure 1a; Supplementary Figure S2 and Supplementary Table S3). A Kaplan–Meier plot of age to HTX showed a substantial difference between *LMNA*:c.656A>C single heterozygotes and *LMNA*:c.656A>C+*TTN*:c.14563C>T double heterozygotes (Figure 2a; Supplementary Table S4). There was also a clearly different distribution of VO_2 and ejection fraction (Figures 2b and c). Myonuclear size was significantly increased in *LMNA*:c.656A>C samples compared with healthy controls, with a further significant increase in *LMNA*:c.656A>C+*TTN*:c.14563C>T samples. Nuclear clustering (distance between nuclei to one another along the myofiber less than the length of a single nucleus) was also observed both in *LMNA*:c.656A>C samples and with an even higher incidence in *LMNA*:c.656A>C+*TTN*:c.14563C>T samples (Figure 3). Histochemical analysis showed moderate interstitial fibrosis in *LMNA* patient myocardium compared with controls. A pronounced increase of fibrosis was observed in *LMNA*:c.656A>C+*TTN*:c.14563C>T patient myocardium.

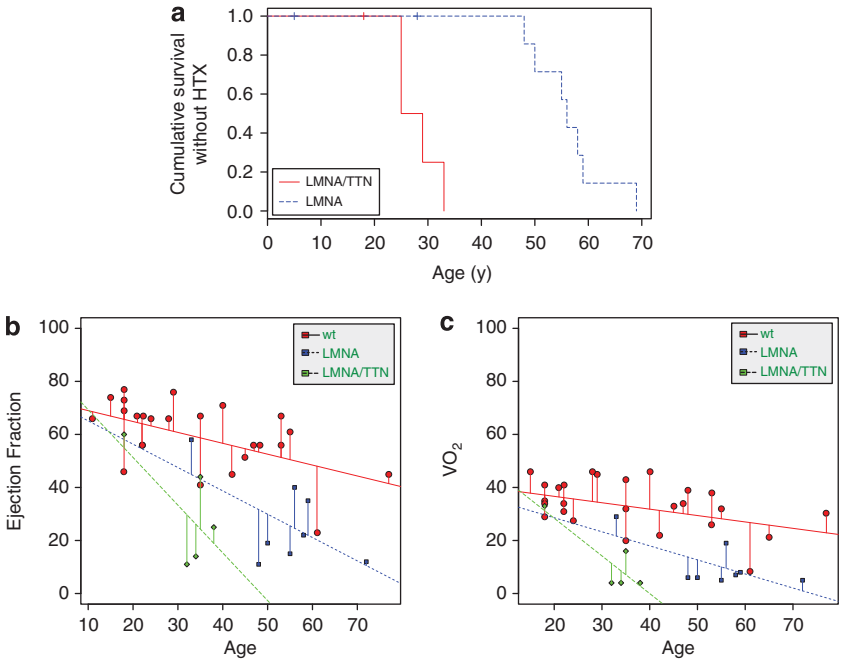

Figure 2 (**a**) Actuarial probability of survival without HTX in five patients with LMNA and TTN mutations compared with nine patients with only a LMNA mutation. (**b**) Comparison of ejection fraction *vs* age for the three groups (WT LMNA/TTN status, single-heterozygote status for LMNA:c.656A>C, and double-heteroyzgote status for LMNA:c.656A>C and TTN:c.14563C>T). A linear regression line is used to visualize the distribution of each group, and actual data points are plotted together with lines representing the residuals. (**c**) Comparison of maximum VO_2 *vs* age. A linear regression line is used to visualize the distribution of each group as before.

Importantly, in *LMNA*:c.656A>C + *TTN*:c.14563C>T, myocardium areas with marked sarcomere disorganization were observed (Figure 4).

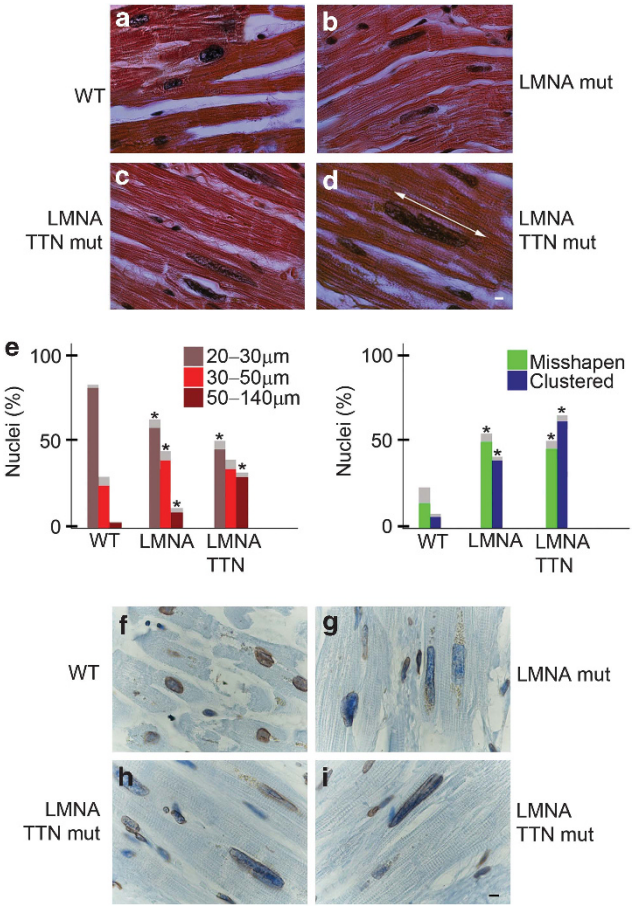

Figure 3 (**a–d**) Control (**a**) or laminopathic nuclei (**b–d**) were counted and scored for nuclear major axis length (20–30, 30–50, and 50–140 μm ranges). Nuclei from double-mutant myocardium (**c**, **d**) showed increased length and clustered (double arrow in **d** corresponds to 140 μm). (**e**) Percentage of nuclei in the 20–30, 30–50, and 50–140 μm ranges, 200 nuclei/sample, was scored from two different WT, *LMNA*-, or *LMNA/TTN*-mutated individuals. Statistically significant differences ($P<0.005$) relative to controls, calculated by the Mann–Whitney test, are indicated by asterisks. (**f–i**) Control (**f**) and laminopathic myocardium (**g–i**) were labeled using anti-lamin A/C antibody. Focal loss of lamin A/C and misshapen nuclei are clearly visible in specimens from patients with *LMNA* and *LMNA/TTN* mutations. Bar, 10 μm.

We additionally investigated the variant c.2327C>T in the *NUP133* gene and c.2540G>C in the *DIAPH3* gene. To date, no mutations in human disease have been identified in *NUP133*. A mutation in the 5′ UTR of *DIAPH3*, and associated with *DIAPH3* overexpression, was found in a family with auditory neuropathy.[34] Both variants are predicted to be disease-causing by MutationTaster.[35] However, only three of the four severely affected persons carried the *NUP133* variant, and only two of the four carried the *DIAPH3* variant (data not shown). Nonetheless, a modifying role for these variants cannot be completely ruled out. In addition, three of the severely affected persons (V.15, IV.17, and V.13) were shown to carry a mutation in the gene for thyroid-stimulating hormone receptor (*TSHR*). Mutations in this gene are a known cause of autosomal-dominantly inherited hyperthyroidism (MIM 609152). Each of the three persons has been under treatment (since the age of 20, 19, and 24 years) with methimazole for hyperthyroidism. Pretreatment values for TSH were not available.

DISCUSSION

Because the first use of NGS technologies for disease-gene identification and discovery,[36,37] the first descriptions of applications of WES to make clinical decisions in the care of patients have begun to appear,[38] and NGS methods for diagnostics are enabling genomic studies that were infeasible just a few years ago.[11,39–41] Previously, isolated cases of digenic inheritance underlying diseases such as HCM have been identified by targeted Sanger sequencing of candidate genes,[6–8,42] but clearly, WES approaches now allow a comprehensive and systematic approach to questions of genetic modifiers and digenic inheritance. We therefore hypothesized that all family members affected by DCM would segregate an identical mutation, but that the severely affected persons could have an additional variant in the same or a second gene. To test this hypothesis, we sequenced three of the severely affected persons and one unaffected relative and examined the data for variants that were present only in the affected persons. After initial analysis revealed candidates in two known DCM genes, *LMNA* and *TTN*, a comprehensive screen was conducted in the family for these variants. All affected family members were shown to have the variant LMNA:c.656A>C, which has been previously demonstrated in an unrelated 43-year-old man with DCM.[17] In addition, the four most severely affected family members were found to have a previously not described variant in the gene for titin, TTN:c.14563C>T (p L4855F).

Doubly heterozygous family members showed a substantially more severe clinical course, with respect to age of terminal CHF and heart transplantation, and a number of cardiological and histological

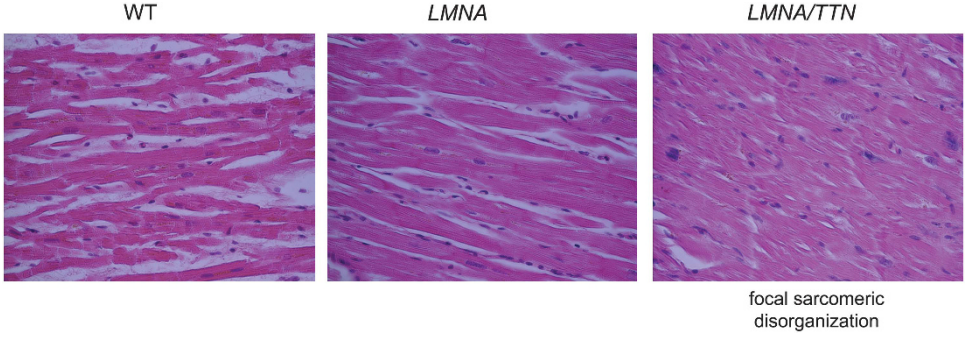

Figure 4 Hematoxilin and eosin staining of myocardium cryosections shows sarcomere disorganization in *LMNA/TTN*-mutant heart, but not in WT or *LMNA*-mutant myocardium.

parameters (Figures 2 and 3). We interpret these results as being consistent with a role for TTN:c.14563C>T as a modifier of DCM clinical severity in the *LMNA*-mutation carriers. A limitation of our study is the lack of functional data on the TTN mutation. However, we noted that c.14563C>T changes a leucine, a large aliphatic amino acid, to a phenylalanine, an aromatic amino acid. The pathogenicity prediction tool MutationTaster[35] predicts c.14563C>T to be a pathogenic substitution. Both amino acids have hydrophobic side chains often found in the interior of folded proteins. Based on literature data, a substitution of leucine to phenylalanine may result in a mild functional deficit of the affected protein, as was reported for the mutation L272F in the KvLQT1 gene[43] and for the mutation (F193L) in the KCNQ1 gene[44] observed in two different forms of long QT syndrome. We speculate that the TTN:c.14563C>T (p L4855F) identified in this study may lead to a mild defect in titin function that became manifest in combination with the *LMNA* mutation. Given the fact that truncating and missense mutations are relatively common in the general population,[1] it is tempting to speculate that variation in *TTN* may be a common genetic modifier in DCM. Further work will be required to determine whether the proposed modifier role of the *TTN* mutation results from defects in known titin functions, such as force transmission or mechanochemical signal transduction.[45,46] A recent study showed that mice heterozygous for a 2-bp *TTN* insertion were viable and demonstrated normal cardiac morphology. On the other hand, when the heterozygous mice were chronically exposed to angiotensin II or isoproterenol, the mice developed marked left ventricular dilatation.[47] Thus, a functional defect in titin may become only apparent under stress. Interestingly, lamin A binds the C-terminus of nuclear titin in a way that might contribute to mechanochemical transduction,[48,49] and thus one can speculate that a combination of *LMNA* and *TTN* mutations might have a synergistically deleterious effect on this function.

A limitation of our study is the small number of individuals doubly heterozygous for *LMNA* and *TTN* mutations. Although the Kaplan–Meier analysis showed a substantial difference between the *LMNA* and the *LMNA*/*TTN* groups, it cannot be ruled out that other genetic variants or environmental factors were actually responsible for the observed differences. However, no other genes associated with hereditary cardiomyopathy were observed to have rare variants in the sequenced individuals, and indeed, none of the genes with rare variants were associated with any kind of heart phenotype upon analysis with the Human Phenotype Ontology.[16]

Recently, an important role for truncating mutations of *TTN* has been demonstrated for DCM, but rare missense mutations were common in groups of DCM patients as well as in controls.[1] Our results suggest that *TTN* missense mutations may be modifiers of clinical course in familial DCM in the presence of a mutation in another gene associated with DCM. However, further comprehensive genotype/phenotype studies will be required to determine how commonly rare variants in multiple genes associated with DCM are associated with a particularly severe clinical course.

CONCLUSION

Our results show how WES can be used to address questions about clinical variability in human genetics. The *LMNA* and *TTN* mutational status may be useful in this family for risk stratification in persons at risk for familial DCM. Given the complexity of familial DCM, with nearly 40 loci and at least 33 currently known DCM genes that only explain a minority of cases,[50] substantially more data will be required to develop WES into a reliable tool for routine clinical diagnostics for familial DCM. A database with comprehensive genotype information and deep phenotyping data would go a long way toward providing clinicians and researchers with the tools needed to identify the remaining DCM genes, as well as the genetic correlates of variability in familial DCM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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