Truncating *FLNC* mutations are associated with high-risk dilated and arrhythmogenic cardiomyopathies

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Brief Title: *FLNC* mutations and high-risk cardiomyopathy

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Abstract
Background: Filamin C (encoded by the *FLNC* gene) is essential for sarcomere attachment to the plasmatic membrane. Mutations in *FLNC* have been associated with myofibrillar myopathies, and cardiac involvement has been reported in some carriers. Accordingly, since 2012 we have included *FLNC* in the genetic screening of patients with inherited cardiomyopathies and sudden death.
Objective(s): We aimed to demonstrate the association between truncating mutations in *FLNC* and the development of high-risk dilated and arrhythmogenic cardiomyopathies.
Methods: *FLNC* was studied by next-generation sequencing in 2,877 patients with inherited cardiovascular diseases. We identified a characteristic phenotype in probands with truncating mutations in *FLNC*. Clinical and genetic evaluation of 28 affected families was performed. Localization of filamin C in cardiac tissue was analysed in patients with truncating *FLNC* mutations using immunohistochemistry.
Results: Twenty-three truncating mutations were identified in 28 probands previously diagnosed with dilated, arrhythmogenic, or restrictive cardiomyopathies. Truncating *FLNC* mutations were absent in patients with other phenotypes, including 1,078 individuals with hypertrophic cardiomyopathy. Fifty-four mutation carriers were identified among 121 screened relatives. The phenotype consisted in left ventricular dilatation (68%) and systolic dysfunction (46%), left ventricular myocardial fibrosis (67%), inferolateral negative T waves and low voltages on ECG (33%), ventricular arrhythmias (82%), and frequent sudden cardiac death (40 cases in 21/28 families). Clinical skeletal myopathy was not observed. Penetrance was >97% in carriers older than 40 years. Truncating mutations in *FLNC* cosegregated with this phenotype with a dominant inheritance pattern (combined LOD score: 9.5). Immunohistochemical stainings of myocardial tissue showed no abnormal filamin C aggregates in patients with truncating *FLNC* mutations.
Conclusions: Truncating mutations in *FLNC* cause an overlapping phenotype of dilated and left dominant arrhythmogenic cardiomyopathies complicated by frequent premature sudden death. Prompt implantation of a cardiac defibrillator should be considered in affected individuals harboring truncating mutations in *FLNC*.

Key Words: filamin C, *FLNC*, mutation, dilated cardiomyopathy, arrhythmogenic cardiomyopathy, sudden death.

Abbreviations
FLNC: Filamin C gene.
LOD score: logarithm of the odds score.
ECG: electrocardiogram.

Introduction

Filamins cross-link actin filaments forming a widespread network in cardiac and skeletal muscles cells (1). Their principal function is the anchorage of membrane proteins to the cytoskeleton (2, 3). Gamma filamin or filamin C is one of the three filamin-related proteins, and it is encoded by the *FLNC* gene (4). Filamin C also binds to several proteins in the Z-disk of the sarcomere (5-7).

Mutations in *FLNC* were initially related to a particular form of skeletal myofibrillar myopathy associated in some cases with a non-specified form of "cardiomyopathy" (8-17). For that reason, since 2012 we have included *FLNC* in the genetic screening of patients with inherited cardiomyopathies and sudden death.

Here we describe a characteristic form of cardiomyopathy caused by truncating mutations in *FLNC* in the absence of clinical skeletal myopathy. The phenotype appears as an overlapping of dilated and arrhythmogenic cardiomyopathies, characterized by variable degrees of left ventricular dilatation and systolic dysfunction, prominent subepicardial and/or intramyocardial fibrosis of the left ventricle, frequent ventricular arrhythmias, and sudden cardiac death.

Methods

Patients

From February 2012 to August 2015, *FLNC* was evaluated by next-generation sequencing in 2,877 patients with different inherited cardiovascular diseases (Online Table 1). The phenotypes were those established by each center prior to the genetic study. We identified 28 unrelated probands with truncating mutations in *FLNC*. Clinical and genetic familial cascade screenings were performed in those cases that agreed. All individuals gave their written consent to participate in this study. The project was approved by the different local ethics committees

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Genetic Studies

Coding exons and intronic boundaries of 213 genes (Online Table 2) related to inherited cardiovascular diseases and sudden death were captured using a custom probe library (SureSelect Target Enrichment Kit for Illumina paired-end multiplexed sequencing method; Agilent Technologies). Sequencing was performed using the Illumina HiSeq 1500 platform (Illumina, USA) with 2x100 base read length following Illumina protocols. Bioinformatics analysis was performed by means of a custom pipeline including software such as NovoAlign (Novocraft Technologies Sdn Bhd), SAMtools, and BCFtools (Sanger Institute) for variant calling and genotyping, and Annovar for variant annotation. Mean coverage for all the evaluated genes ranged between 250x and 400x. Read depth of every nucleotide from genes related to the referring phenotype was >30x. Those exons that did not fulfil this standard were complementary sequenced by the Sanger technique. All exons of *FLNC* were completely covered (>30x). Information regarding frequency in different populations (1,000 Genomes Project, Exome Variant Server, Exome Aggregation Consortium) was considered. The allele frequency threshold to consider a mutation clinically relevant was ≤0.1%. Pathogenicity of variants was classified according to current recommendations (18).

Those variants considered clinically relevant according to the patient's phenotype were confirmed using Sanger sequencing. There is a pseudogene located 53.6 kilobases downstream from the functional *FLNC* gene, which is 98% homologous to exons 46, 47, and 48. All the variants identified in those exons were sequenced using specific primers designed to confirm that

they corresponded to the real *FLNC* sequence and not to the pseudogene (19). Cascade genetic screening in relatives was performed using Sanger sequencing.

We considered truncating mutations in *FLNC* those variants that introduce a premature stop codon in the protein's sequence (nonsense or frameshift) or that could alter the splicing process according to the predictions of five *in silico* tools: MaxEntScan, Splice-Site Finder (SSF), HSF, NNSPLICE, and GeneSplicer. All the genetic variants included in the present study were predicted to disrupt the protein function.

Statistical Analysis

The cumulative probability for the occurrence of sudden death, appropriate defibrillator shock, heart failure death, or cardiac transplant was estimated by using the Kaplan-Meier method, and factors were compared by using the log-rank (Mantel-Cox) method. Survival was calculated from birth. A two-sided p-value <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp).

Two-point LOD score was calculated in 23 families using the Superlink-Online SNP tool (http://cbl-hapw.cs.technion.ac.il/superlink-snp) with the following settings: disease mutant gene frequency=0.001, dominant mode of inheritance, penetrance=99%, $\theta=0$.

Immunohistochemistry

In order to analyze the presence of filamin C aggregates as a potential cause of myocardial injury, we compared 3 patients with truncating mutations in *FLNC* (26958-II:1, 36203-III:3, and 25767-III:1) with 3 control samples. Myocardial samples from patients were obtained from necropsy or explanted hearts. Immunohistochemistry analysis using a specific antibody against the N-terminal extreme of filamin C was performed. In brief, 5-micron-thick

sections were obtained from paraffin-embedded tissue blocks from patients with truncating *FLNC* mutations and controls. After rehydration, samples were heated for 15 minutes in the microwave oven to induce epitope retrieval in Tris-EDTA Buffer, pH 9.0 and were subsequently incubated at room temperature (RT) for 15 minutes to enhance penetration of the antibody. Slides were then washed twice in a 0.1% Tween-20 Tris-buffered saline (TBS-T) solution and blocked with Avidin/Biotin Blocking reagent according to the manufacturer's protocol (Vector Laboratories). After washing with TBS-T and blocking with a 15% goat serum TBS-T solution, slides were incubated with rabbit polyclonal anti-filamin C gamma (1:50; MyBiosource, MBS2026155) raised against the N-terminal peptide of the protein overnight at 4 ºC. Slides were TBS-T-washed and incubated with a HRP goat anti-rabbit secondary antibody for 30 minutes at RT. Vectastain ABC kit was used to amplify the signal, and DAB substrate kit was used for peroxidase detection (Vector Laboratories, Burlingame, California). Counterstaining with hematoxylin was carried out before dehydration and mounting the slides with DPX. Pictures were taken with a Nikon 90i microscope at different magnifications.

Results

Prevalence of truncating mutations in FLNC

Twenty-three different truncating mutations in *FLNC* were identified in 28 unrelated probands (**Figure 1**; Online Table 3 and Table 4). Previous diagnoses were dilated cardiomyopathy in 20 patients, arrhythmogenic cardiomyopathy in 7 (all of them with predominant left ventricular involvement), and restrictive cardiomyopathy in 1. No pathogenic mutations in other genes were identified in any of them. We did not find truncating mutations in *FLNC* among 2,105 individuals with other inherited cardiovascular diseases, including 1,078 patients with hypertrophic cardiomyopathy (Online Table 1). The prevalence of truncating *FLNC*

mutations in this heterogeneous global cohort is low (28 out of 2,877 patients screened= 0.97%). However, if we refer to the specific phenotypes where this type of mutations were found, the proportion is significantly higher: 20 out of 508 patients with dilated cardiomyopathy (3.9%), 7 out of 219 patients with arrhythmogenic cardiomyopathy (3.2%), and 1 out of 45 individuals with restrictive cardiomyopathy (2.2%).

Phenotype description of patients with truncating mutations in FLNC

In total, 149 individuals (28 probands and 121 relatives) were clinically and genetically evaluated. Fifty-four relatives (45%) carried the *FLNC* mutation identified in the proband. Cardiac alterations were evidenced in 74% of relatives with the mutation (n=40). Mean age at presentation in affected carriers was 41±15 years (range 0.3-71). Eleven (92%) of 12 healthy mutation carriers were younger than 40 years at last follow-up (mean 32±16; range 6-72). None of the 67 non-carriers was clinically affected. Complete cosegregation of truncating mutations in *FLNC* with a particular cardiac phenotype was observed in 23 families who agreed to be investigated (combined LOD score: 9.5) (Online Table 5 and Online Figure 1).

Table 1 shows the clinical characteristics of carriers of truncating mutations in *FLNC*. Exertional dyspnea and palpitations were the most frequent presentation symptoms. Three probands were asymptomatic at the moment of diagnosis and were studied due to family history of sudden death. Forty-three percent of the affected relatives with positive genotype were asymptomatic and were diagnosed through family screening.

Most of the probands showed left ventricular dilatation (end-diastolic diameter 61±13 mm) and systolic dysfunction (ejection fraction 34±13%), which were also frequent among relatives (end diastolic diameter 53±9 mm; ejection fraction 52±12%). Structural abnormalities in the right ventricle (dilatation, akinesia, dyskinesia, or systolic dysfunction) were observed in

10 probands (36%). All of them presented left ventricular involvement as well. Five (14%) of the 36 affected relatives with available information showed mild right ventricular abnormalities. Mild hypertrophy (maximal wall thickness ≤14 mm) not fulfilling diagnostic criteria for hypertrophic cardiomyopathy was described in 10 carriers (13%).

Most patients were in sinus rhythm, and cardiac conduction defects were mild and uncommon. Negative T waves were frequently seen in left precordial (12%), inferior (6%), left and inferior (9%), or left and right precordial leads (4%), while no patient presented isolated negative T waves in right precordial leads. Low QRS voltages in the limb leads were found in 25% of mutation carriers (Online Figure 2). Terminal QRS duration >55 ms in leads V1-V3 was recorded in 18% of carriers evaluated. No individuals showed epsilon waves. Signal average ECG was positive in four of six individuals tested.

Ventricular arrhythmias were extremely frequent among carriers (82%). Frequent ventricular extrasystoles (>500/24 hours) and non-sustained ventricular tachycardia were the most common. Sustained ventricular tachycardia was recorded in 10 out of 55 carriers with available information, in 3 of them during exercise.

Electrophysiological study was performed in 8 patients, and ventricular arrhythmias were induced in 4 (two non-sustained ventricular tachycardias, one ventricular tachycardia, and one ventricular fibrillation).

The presence of myocardial fibrosis was assessed by magnetic resonance in 15 probands; 11 of them presented areas with late-gadolinium enhancement exclusively affecting the left ventricular wall. Two of these subjects died suddenly (ages 22 and 25, respectively), and fibrosis was confirmed on cardiac histology (**Figure 2A-B**). Three out of 13 probands without magnetic resonance study showed myocardial fibrosis confined to the left ventricle on necropsy/explanted

heart (one died suddenly at age 17, and the other two were transplanted at ages 1 and 60, respectively) (**Figure 2C-D**). Endomyocardial biopsy of the right ventricle revealed large amounts of fibrosis in the proband diagnosed with restrictive cardiomyopathy. Globally, 75% of those investigated probands developed cardiac fibrosis predominantly affecting the left ventricular wall. Among relatives with the mutation, 16 of 31 evaluated relatives (52%) showed significant amounts of myocardial fibrosis mainly affecting the left ventricle on magnetic resonance (n=13) or in the necropsy (n=2); one relative showed fibrosis on endomyocardial biopsy of the right ventricle. Left ventricular myocardial fibrosis was mainly subepicardial. A concentric pattern and extension to intramyocardial or transmural involvement was observed in some cases. Two patients who were transplanted due to advanced heart failure showed endomyocardial fibrosis.

At initial evaluation no patient suffered from muscle weakness nor showed signs of skeletal myopathy. Mild elevation of plasmatic creatine-kinase levels (less than two-fold of upper normal value) was found in only 3 out of 40 evaluated carriers. Only one of them (proband from family 31277) presented muscle weakness in the lower limbs during follow-up. This patient had been diagnosed of restrictive cardiomyopathy aged 29 and had received a cardiac transplant aged 45. An electromyography study at age 59 revealed moderate myopathic changes. However, this woman was under therapy with simvastatin and corticosteroids that could explain these findings.

Palmoplantar keratoderma was observed and cosegregated with the cardiac phenotype and the *FLNC* mutation (c.4127+1delG) in 4 members from family 29876 (Online Figures 1 and 3). This finding was not observed in other families from this series.

Events

Twelve carriers suffered cardiac arrest (mean age at event 42±16 years; range 17-68), being the first manifestation of the disease in 4 of them. All subjects with available data presented left ventricular systolic dysfunction (n=9; mean left ventricular ejection fraction: 39.6±12%; range 21-54) and myocardial fibrosis confined to the left ventricle (n=7). Ventricular arrhythmias had been investigated prior to the event in 6 of these 12 individuals, and all of them showed frequent ventricular extrasystoles and/or non-sustained ventricular tachycardia.

Twenty-six affected mutation carriers received or were recommended to have a cardiac defibrillator implanted. The indication was primary prevention in 17 (1 declined the indication and 1 died suddenly waiting for the implant) and secondary prevention in 9, after suffering a symptomatic sustained ventricular tachycardia (n=7) or after an aborted cardiac arrest (n=2); all of them exhibited left ventricular systolic dysfunction. Appropriate shocks were recorded in 3/15 (20%) primary prevention patients and in 5/9 (56%) secondary prevention cases (mean time to shock 53±39 months; range 0.1-96) (Online Figure 4).

Five carriers underwent heart transplantation due to markedly reduced ejection fraction (n=4) or restrictive filling with severe pulmonary hypertension (n=1). Mean age at transplantation was 43±24 years (range 1-60).

Considering both carriers (n=12) and affected relatives without genetic study (n=28), there have been 40 sudden deaths in 21 of the 28 evaluated families. Mean age at event was 44±17 years (range: 15-80); 65% occurred in individuals ≤50 years old. **Figure 3** shows the survival curve for sudden death/appropriate defibrillator shock/heart failure death/heart transplant in clinically or genetically affected individuals.

Immunohistochemistry

Immunohistochemical stainings of myocardial tissue from patients carrying truncating mutations in *FLNC* showed no abnormal filamin C aggregates in the cytoplasm. In contrast, we observed that antibodies against filamin C stained the intercalated disk region in both patients and controls (**Figure 4**).

Discussion

We describe the association of truncating mutations in *FLNC* with a particular overlapping phenotype of dilated and left dominant arrhythmogenic cardiomyopathies complicated by frequent premature sudden death. Cosegregation of truncating *FLNC* mutations with this phenotype with a dominant mode of transmission was clearly demonstrated in this international series of 28 families. Mutation penetrance was >97% in carriers older than 40 years.

Carriers developed ventricular dilatation with reduced ejection fraction, especially affecting the left ventricle. The majority of the affected carriers had been diagnosed with dilated cardiomyopathy. However, a significant number of patients had been diagnosed with left-dominant arrhythmogenic cardiomyopathy. Diagnosis of arrhythmogenic cardiomyopathy is challenging and based on multicategorical criteria (20). Left-dominant arrhythmogenic cardiomyopathy mimics idiopathic dilated cardiomyopathy, and its clinical diagnostic criteria have not been formally established. Many authors have suggested that ventricular arrhythmias coming from a fibrotic left ventricular wall in the absence of right ventricular involvement could be an expression of left-dominant arrhythmogenic cardiomyopathy (21-23). This phenotype frequently presents with inferolateral negative T waves, mild-to-moderate left ventricular systolic dysfunction, and regional dyskinesia, all of them identified in several of our patients.

These patients with truncating mutations in *FLNC* share clinical characteristics both of desmosomal mutations and of laminopathies and desminopathies. Ventricular arrhythmias, likely

related to the presence of left ventricular myocardial fibrosis, and a high incidence of sudden death may appear in all of them (24, 25). Nevertheless, isolated or predominant right ventricular involvement, common in desmosomal mutations, was not observed in our patients. On the other hand, cardiac conduction abnormalities were mild and infrequent, while they are common and severe in patients with pathogenic lamin A/C, emerin, or desmin mutations (24-26). These differences likely reflect the involvement of different pathogenic mechanisms.

All mutations identified in our work are novel except for c.3791-1G>C. This genetic variant has been reported in two patients with dilated cardiomyopathy (27, 28). Similarly to our findings, significant ventricular arrhythmia was reported in one of them, and clinical signs of skeletal myopathy were absent in both. Segregation studies in the described families were quite limited.

Filamin C protein is widely expressed in cardiac myocytes and participates in mechanical, sensory, and signal transduction between sarcomeres and plasmatic membranes (2-4). Its participation in the attachment of the sarcomere´s Z-disk to the sarcolemma (costameres) and to the intercalated disks allows for cell-to-cell mechanical force transduction (7). Filamin C directly interacts with two protein complexes that link the subsarcolemmal actin cytoskeleton to the extracellular matrix: the dystrophin-associated glycoprotein and the integrin complexes (6). At intercalated disks, filamin C is located in the fascia adherens where myofiber ends reach the sarcolemma, adjacent to the position of desmosomal junctions (**Figure 5**) (29).

Several mutations in *FLNC* have been previously associated with a particular form of myofibrillar myopathy (8-17). This phenotype is mainly characterized by late-onset (usually starting in the fourth decade of life) skeletal myopathy, which usually initially involves proximal and later distal limb muscles. Cardiac involvement has been described in some patients, with

approximately 30% of carriers showing a non-specific and poorly characterized cardiomyopathy (9). History of early sudden death has been described in these families, but previous publications did not provide details about these findings (8-17). Mutations in *FLNC* previously identified in myofibrillar myopathy are mostly missense and in-frame indels. Only two truncating mutations were reported in those patients: a nonsense variant close to the C-terminal end of the protein and a frameshift variant producing a stop codon in exon 30 (8, 13). Abnormal cytoplasmic filamin C aggregates were demonstrated to play a pathogenic role in most of these cases.

Immunohistochemical analysis showed normal filamin C staining in intercalated disks. The absence of abnormal filamin C aggregates in the cardiomyocytes' cytoplasm of our patients with truncating *FLNC* mutations suggests that the mechanism involved in this type of mutations is different from that previously associated with myofibrillar myopathy. One potential explanation is that truncating mutations in *FLNC* would decrease the level of normal filamin C by means of haploinsufficiency. This alteration could affect mechanical force transduction at intercalated disks and costameres by weakening the binding of the Z-disk to the plasmatic membrane. Tissues exposed to high mechanical force generation, such as the left ventricular myocardium, could be particularly affected. Myocardial fibrosis, together with dilatation and systolic dysfunction of the left ventricle, could be the consequences of this functional alteration. In a previous study, a medaka fish harboring a homozygous nonsense mutation in *FLNC* showed early rupture of the myocardial ventricular wall and progressive skeletal muscle degeneration in late embryonic stages. The mutant embryo fish showed fewer sarcomere bundles attached to the intercalated disks and detachment of myofibrils from sarcolemma and intercalated disks, with focal Z-disk destruction (30).

Clinical signs of skeletal myopathy were specifically and systematically investigated among carriers. It is noteworthy that only one of the carriers in our series showed clinical signs of skeletal myopathy. Although skeletal biopsies were not performed, creatine-kinase levels were within the normal limits in almost all carriers who were investigated. Previous studies suggested that skeletal myopathy would be the main phenotype associated with pathogenic *FLNC* mutations. Our data show that cardiac disease would be the main consequence of truncating mutations in this gene. Since most previous publications focused on skeletal myopathy and cardiac examinations were not routinely performed, subtle cardiac abnormalities only detectable through Holter ECG and cardiac magnetic resonance could have been missed.

It has been suggested that mutations in *FLNC* could explain nearly 10% of cases of hypertrophic cardiomyopathy in patients without mutations in the main sarcomeric genes (31). Seven out of eight novel mutations identified in this work were missense variants. In line with our results, none of the carriers showed symptoms of skeletal myopathy. Moreover, muscle biopsies performed in two patients showed normal histology and histochemistry. It is noteworthy that in this report, patients with *FLNC* mutations showed lower left ventricular wall thickness than patients without mutations in *FLNC*. In fact, 65% of carriers who developed hypertrophy showed a maximal wall thickness ≤15 mm. Whether missense mutations lead to hypertrophic cardiomyopathy and truncating mutations to dilated/left dominant arrhythmogenic cardiomyopathies would need to be confirmed in future studies, but so far we have not identified any truncating *FLNC* mutation in more than 1,000 patients with hypertrophic cardiomyopathy. Our data clearly suggest that truncating *FLNC* mutations are not related to the development of hypertrophic cardiomyopathy.

Two novel missense mutations in *FLNC* have recently showed cosegregation with restrictive cardiomyopathy in two Caucasian families (32). We postulate that the molecular mechanism associated with these missense mutations could be different to truncating mutations. However, some clinical characteristics of these two families resemble our findings. Several carriers showed different amounts of myocardial fibrosis on cardiac histology. Moreover, some cases presented T wave abnormalities on ECG or left ventricular systolic dysfunction on echocardiogram. Unfortunately, the assessment of ventricular arrhythmias on Holter ECG and the evaluation of areas with late-gadolinium enhancement with cardiac magnetic resonance were not reported.

Conclusions

Truncating mutations in *FLNC* are associated with a characteristic cardiac phenotype that includes left ventricular dilatation with systolic dysfunction and myocardial fibrosis. Ventricular arrhythmias are extremely frequent, and families with these mutations show a high incidence of sudden cardiac death. We did not find evidence of skeletal myopathy in our series, suggesting a new and exclusive cardiac phenotype associated with this type of mutations.

FLNC should be systematically included in the genetic studies of patients diagnosed with dilated, arrhythmogenic, or restrictive cardiomyopathies. The identification of pathogenic truncating mutations should prompt a thorough clinical evaluation that should include magnetic resonance imaging and Holter ECG monitoring. Implantable defibrillators should probably be considered even in cases with only moderate systolic dysfunction in the presence of myocardial fibrosis and ventricular arrhythmias.

Perspectives

Competency in Medical Knowledge 1: Truncating mutations in *FLNC* cause a particular overlapping phenotype of dilated and arrhythmogenic cardiomyopathies.

Competency in Medical Knowledge 2: The presence of a truncating *FLNC* mutation should be suspected when a cardiomyopathy is characterized by left ventricular systolic dysfunction and/or dilatation (could be mild), myocardial fibrosis preferentially affecting the left ventricle, high burden of ventricular arrhythmia, and family history of sudden death.

Competency in Medical Knowledge 3: Mutations in *FLNC* have been originally and mainly related with skeletal myopathy. We demonstrate that truncating mutations in *FLNC* could lead to the development of cardiomyopathy in the absence of clinical skeletal affection.

Competency in Patient Care 1: Screening for *FLNC* mutations should be considered in genetic testing of patients with dilated, arrhythmogenic, and restrictive cardiomyopathies.

Competency in Patient Care 2: Clinical assessment of truncating *FLNC* mutation carriers should include cardiac MRI (to rule out myocardial fibrosis), Holter ECG, and stress test (to analyze the presence of ventricular arrhythmias).

Competency in Patient Care 3: Implantation of a cardiac defibrillator could be considered in those carriers with myocardial fibrosis and ventricular arrhythmias even if left ventricular systolic function is not severely affected.

Translational Outlook: Carriers of truncating mutations in *FLNC* showed no abnormal filamin C aggregates in myocardial tissue. Further studies are needed to assess the specific functional mechanism associated to truncating *FLNC* mutations.

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Figure Legends

Central Illustration. Truncating *FLNC* mutations causing dilated and arrhythmogenic cardiomyopathies. Truncating *FLNC* mutations could alter intercalated disks and costameres, weakening the attachment of myocytes. The consequence is a particular form of cardiomyopathy mainly characterized by left ventricular dilatation and systolic dysfunction, myocardial fibrosis predominantly affecting the left ventricle, and a high burden of ventricular arrhythmias that leads to sudden cardiac death.

Figure 1. Spatial distribution of truncating mutations in filamin C protein. Mutations affecting coding exonic regions are showed above the diagram; mutations affecting intronic canonical splicing sites are showed below the diagram. CH1= calponin homology domain 1. CH2= calponin homology domain 2. Boxes with numbers represent the 24 immunoglobulin-like repeats of filamin C. (#)= mutations identified in two unrelated families.

Figure 2. Left ventricular myocardial fibrosis observed in two carriers of truncating *FLNC* mutations. Top images belong to the proband of family 32406, who died suddenly playing soccer at age 22: necropsy showing circumferential subepicardial fibrosis (arrows) of the left ventricle (Panel A) on the same localization as late-gadolinium enhancement (arrows) detected on cardiac magnetic resonance performed prior to death (Panel B). Images on the bottom are from the proband of family 26958, who died suddenly at age 17 after a soccer match: circumferential left ventricular intramyocardial fibrosis (arrows) on necropsy (Panel A); myocardial fibrosis (light blue) affecting the anterior wall of the left ventricle (Masson's trichrome, 2x) (Panel B).

Figure 3. Kaplan–Meier estimates of major cardiovascular events in families with truncating *FLNC* mutations. Survival curves free of sudden death/appropriate defibrillator shock/heart failure death/heart transplant in all clinically or genetically affected individuals (Panel A) and discriminated by sex (Panel B).

Figure 4. Filamin C localization in cardiac tissue of patients. Immunohistochemical staining shows the presence of filamin C only in intercalated disks of controls (Panels A to C) and patients with truncating *FLNC* mutations (Panels D to E). Hematoxylin counterstain was used to detect cell nuclei. Scale bar 100μm.

Figure 5. Cellular interactions of filamin C. Cardiomyocytes bind to each other at their longitudinal extremes by means of intercalated disks (Panel A). Filamin C directly interacts with components of the fascia adherens, allowing for the attachment of Z-disk components to the intercalated disks. Filamin C is also localized at costameres that couple the sarcomere to the lateral sarcolemma and to the extracellular matrix (Panel B).

Table 1: Clinical characteristics of carriers of truncating mutations in *FLNC*.

	Probands (n=28; 17 males)			Relatives with the mutation (n=54; 28 males)			All carriers (n=82; 45 males)		
	Evaluated	Positive finding	%	Evaluated	Positive finding	%	Evaluated	Positive finding	%
PRESENTING SYMPTOMS									
Asymptomatic	28	3	11	51	30	59	79	33	42
Dyspnea	28	12	43	51	5	10	79	17	22
Chest pain	28	4	14	51	3	6	79	7	9
Muscle weakness	28	0	0	48	0	0	76	0	0
Syncope	28	4	14	51	7	14	79	11	14
Palpitations	28	6	21	51	9	18	79	15	19
Sudden death	28	1	4	51	3	6	79	4	5
Minor stroke	28	1	4	51	0	0	79	1	1
ECG									
Sinus rhythm	27	22	81	47	45	96	74	67	91
Atrial fibrillation	27	4	15	47	2	4	74	6	8
Pacemaker (atrial)	27	1	4	47	0	0	74	1	1
Cardiac conduction defects (incl. BBB)	27	8	30	47	1	2	74	9	12

Low voltages	25	9	36	47	9	19	72	18	25
Negative Tw all locations	21	13	62	46	9	20	67	22	33
Left precordial negative Tw	21	6	29	46	2	4	67	8	12
Right precordial negative Tw	21	0	0	46	0	0	67	0	0
Left + right precordial negative Tw	21	0	0	46	3	7	67	3	4
Inferior negative Tw	21	2	10	46	2	4	67	4	6
Inferior + left precordial negative Tw	21	4	19	46	2	4	67	6	9
Inferior + right precordial negative Tw	21	1	5	46	0	0	67	1	1
Epsilon wave	21	0	0	46	0	0	67	0	0
Terminal QRS>55ms	20	5	25	45	7	16	65	12	18
SAECG positive	3	2	67	3	2	67	6	4	67
CARDIAC STRUCTURAL									

AFFECTION									
LV dilatation	27	19	70	47	15	32	74	34	46
LVEF <55%	27	26	96	49	25	51	76	51	67
MLVWT ≥ 12mm	27	5	19	50	5	10	77	10	13
MLVWT ≥ 15 mm	27	0	0	50	0	0	77	0	0
LV hypertrabeculation	27	2	7	47	4	9	74	6	8
RV dilat/akin/dyskin/syst dysf	28	10	36	48	5	10	76	15	20
Myocardium fibrosis	20	15	75	31	16	52	51	31	61
LV fibrosis	19	14	74	30	15	50	49	29	59
RV fibrosis	20	1	5	31	1	3	51	2	4
ARRHYTHMIAS									
FVE (>500/24 hs)	23	16	70	32	17	53	55	33	60
NSVT	23	19	83	32	9	28	55	28	51
SVT	23	6	26	32	4	13	55	10	18
Ventricular arrhythmia (any)	23	22	96	32	23	72	55	45	82
EPS positive	3	2	67	5	2	40	8	4	50
SKELETAL MYOPATHY									

Clinical myopathy	28	1	4	48	0	0	76	1	1
Elevated CK plasma levels	21	2	10	19	1	5	40	3	8
OTHER									
Palmoplantar keratoderma	27	1	4	49	3	6	76	4	5
EVENTS									
Sudden death	28	5	18	54	7	13	82	12	15
Appropriate ICD shock	28	4	14	54	4	7	82	8	10
Heart failure death	28	0	0	54	0	0	82	0	0
Heart transplant	28	5	18	54	0	0	82	5	6
Stroke	28	2	7	54	0	0	82	2	2

BBB= bundle branch block. CK= creatine-kinase. EPS= electro-physiological study. FVE= frequent ventricular extrasystoles. ICD= implantable cardioverter defibrillator. LV= left ventricle. LVEF= left ventricular ejection fraction. MLVWT= maximal left ventricular wall thickness. NSVT= non-sustained ventricular tachycardia. RV= right ventricle. RV dilat/akin/dyskin/syst dysf= right ventricular dilatation, akinesia, dyskinesia, or systolic dysfunction. SAECG: signal-average ECG. SVT= sustained ventricular tachycardia. Tw= T wave. Terminal QRS>55ms= S wave upstroke of the QRS during >55 miliseconds.