Truncating *FLNC* Mutations Are Associated With High-Risk Dilated and Arrhythmogenic Cardiomyopathies

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ABSTRACT

BACKGROUND Filamin C (encoded by the *FLNC* gene) is essential for sarcomere attachment to the plasmatic membrane. *FLNC* mutations have been associated with myofibrillar myopathies, and cardiac involvement has been reported in some carriers. Accordingly, since 2012, the authors have included *FLNC* in the genetic screening of patients with inherited cardiomyopathies and sudden death.

OBJECTIVES The aim of this study was to demonstrate the association between truncating mutations in *FLNC* and the development of high-risk dilated and arrhythmogenic cardiomyopathies.

METHODS *FLNC* was studied using next-generation sequencing in 2,877 patients with inherited cardiovascular diseases. A characteristic phenotype was identified 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 analyzed 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 patients with hypertrophic cardiomyopathy. Fifty-four mutation carriers were identified among 121 screened relatives. The phenotype consisted of left ventricular dilation (68%), systolic dysfunction (46%), and myocardial fibrosis (67%); inferolateral negative T waves and low QRS voltages on electrocardiography (33%); ventricular arrhythmias (82%); and frequent sudden cardiac death (40 cases in 21 of 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 logarithm of the odds score: 9.5). Immunohistochemical staining of myocardial tissue showed no abnormal filamin C aggregates in patients with truncating *FLNC* mutations.

CONCLUSIONS Truncating mutations in *FLNC* caused 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 patients harboring truncating mutations in *FLNC*.

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Filamins cross-link actin filaments, forming a widespread network in cardiac and skeletal muscles cells (1). Their principal function is to anchor membrane proteins to the cytoskeleton (2,3). Gamma filamin or filamin C is 1 of 3 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 an unspecified form of "cardiomyopathy" (8-12). For that reason, since 2012, we have included *FLNC* in the genetic screening of patients with inherited cardiomyopathies and sudden death.

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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 (LV) dilation and systolic dysfunction, prominent subepicardial and/or intramyocardial fibrosis of the left ventricle, frequent ventricular arrhythmias, and sudden cardiac death.

METHODS

From February 2012 to August 2015, *FLNC* was evaluated using 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 patients who agreed. All patients gave their written consent to participate in this study. The project was approved by the different local ethics committees.

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, Santa Clara, California). Sequencing was performed using the HiSeq 1500 platform (Illumina, San Diego, California) with 2 × 100 base read length following Illumina protocols. Bioinformatics analysis was performed by means of a custom pipeline including software for variant calling, genotyping, and annotation. Mean coverage for all the evaluated genes ranged between 250× and 400×. The read depth of every nucleotide from genes related to the referring phenotype was >30×. Those exons that did not fulfill this standard were complementary sequenced using the Sanger technique. All exons of *FLNC* were completely covered (>30×). 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 (13).

Those variants considered clinically relevant according to the patient's phenotype were confirmed using Sanger sequencing. There is a pseudogene located 53.6 kb 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, not to the pseudogene (14). Cascade genetic screening in relatives was performed using Sanger sequencing.

We defined truncating mutations in *FLNC* as those that introduce a premature stop codon in the

ABBREVIATIONS AND ACRONYMS

CMR = cardiac magnetic resonance

DCM = dilated cardiomyopathy

EF = ejection fraction

HCM = hypertrophic cardiomyopathy

LV = left ventricular

TBS-T = Tween-20 Tris-buffered saline

VT = ventricular tachycardia

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Manuscript accepted September 6, 2016.

protein's sequence (nonsense or frameshift) or that could alter the splicing process according to the predictions of 5 software tools: MaxEntScan, Splice-Site Finder, 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 of the occurrence of sudden death, appropriate defibrillator shock, heart failure death, or cardiac transplantation was estimated by using the Kaplan-Meier method, and factors were compared using the log-rank (Mantel-Cox) method. Survival was calculated from birth. A 2-sided p value < 0.05 was considered to indicate statistical significance. Statistical analyses were performed using SPSS Statistics version 21.0 (IBM, Armonk, New York).

Two-point logarithm of the odds scores were calculated in 23 families using the Superlink-Online SNP tool with the following settings: disease mutant gene frequency = 0.001, dominant mode of inheritance, penetrance = 99%, and $\theta = 0$.

IMMUNOHISTOCHEMISTRY. 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 subjects. Myocardial samples from patients were obtained from necropsy or explanted hearts. Immunohistochemistry analysis was performed using a specific antibody against the N-terminal extreme of filamin C. In brief, 5-μm-thick sections were obtained from paraffin-embedded tissue blocks from patients with truncating *FLNC* mutations and control subjects. After rehydration, samples were heated for 15 min in a microwave oven to induce epitope retrieval in Tris-EDTA buffer (pH 9.0) and were subsequently incubated at room temperature for 15 min 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, Burlingame, California). 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) (MBS2026155, MyBioSource, San Diego, California) raised against the N-terminal peptide of the protein overnight at 4°C. Slides were TBS-T-washed and incubated with a horseradish peroxidase goat antirabbit secondary antibody for 30 min at room temperature. The Vectastain ABC kit was used to amplify the signal, and the DAB substrate kit was used for peroxidase detection (Vector Laboratories). 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

Twenty-three different truncating mutations in *FLNC* were identified in 28 unrelated probands (**Figure 1**, Online Tables 3 and 4). Previous diagnoses were dilated cardiomyopathy (DCM) in 20 patients, arrhythmogenic cardiomyopathy in 7 (all with predominant LV involvement), and restrictive cardiomyopathy in 1. No pathogenic mutations in other genes were identified in any patients. We did not find truncating mutations in *FLNC* among 2,105 patients with other inherited cardiovascular diseases, including 1,078 patients with hypertrophic cardiomyopathy (HCM) (Online Table 1). The prevalence of truncating *FLNC* mutations in this heterogeneous global cohort was low (28 of 2,877 patients screened [0.97%]). However, if we referred to the specific phenotypes in which this type of mutation was found, the proportion was significantly higher: 20 of 508 patients with DCM (3.9%), 7 of 219 patients with arrhythmogenic cardiomyopathy (3.2%), and 1 of 45 patients with restrictive cardiomyopathy (2.2%).

PATIENTS STUDIED. In total, 149 subjects (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 found in 74% of relatives with the mutation (n = 40). Mean age at presentation in affected carriers was 41 ± 15 years (range: 0.3 to 71 years). Eleven of 12 healthy mutation carriers (92%) were <40 years of age at last follow-up (mean 32 ± 16 years; range: 6 to 72 years). None of the 67 noncarriers were clinically affected. Complete cosegregation of truncating mutations in *FLNC* with particular cardiac phenotypes was observed in 23 families that agreed to be investigated (combined logarithm of the odds score: 9.5) (Online Table 5, Online Figure 1).

In terms of clinical characteristics of carriers of truncating mutations in *FLNC* (**Table 1**), exertional dyspnea and palpitations were the most frequent presentation symptoms. Three probands were asymptomatic at the moment of diagnosis and were studied because of family history of sudden death. Of the affected relatives with positive genotype, 43% were asymptomatic and diagnosed through family screening.

Most of the probands showed LV dilation (end-diastolic diameter 61 ± 13 mm) and systolic dysfunction (ejection fraction [EF] 34 ± 13%), which were also

frequent among relatives (end-diastolic diameter 53 ± 9 mm; EF 52 ± 12%). Structural abnormalities in the right ventricle (dilation, akinesia, dyskinesia, or systolic dysfunction) were observed in 10 probands (36%). All had LV involvement as well. Five of the 36 affected relatives (14%) with available information showed mild right ventricular abnormalities. Mild LV hypertrophy (maximal wall thickness ≤14 mm) not fulfilling diagnostic criteria for HCM 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 (4%) leads, 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 V_1 to V_3 was recorded in 18% of carriers evaluated. No subjects showed epsilon waves. A signal-average electrocardiogram was positive in 4 of 6 subjects tested.

Ventricular arrhythmias were extremely frequent among carriers (82%). Frequent ventricular extrasystoles (>500 in 24 h) and nonsustained ventricular tachycardia (VT) were the most common. Sustained VT was recorded in 10 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 (2 nonsustained VTs, 1 VT, and 1 ventricular fibrillation).

The presence of myocardial fibrosis was assessed by cardiac magnetic resonance (CMR) in 15 probands; 11 of them presented areas with late gadolinium enhancement exclusively affecting the LV wall. Two of these subjects died suddenly (at ages 22 and 25 years, respectively), and fibrosis was confirmed on cardiac histology (**Figures 2A and 2B**). Three of 13 probands without CMR studies showed myocardial fibrosis confined to the left ventricle on necropsy or explanted heart (1 died suddenly at 17 years of age, and the other 2 were transplanted at 1 and 60 years of age, respectively) (**Figures 2C and 2D**). 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 LV wall. Among relatives with the mutation, 16 of 31 evaluated relatives (52%) showed significant amounts of myocardial fibrosis mainly affecting the left ventricle on CMR (n = 13) or on necropsy (n = 2); 1 relative showed fibrosis on

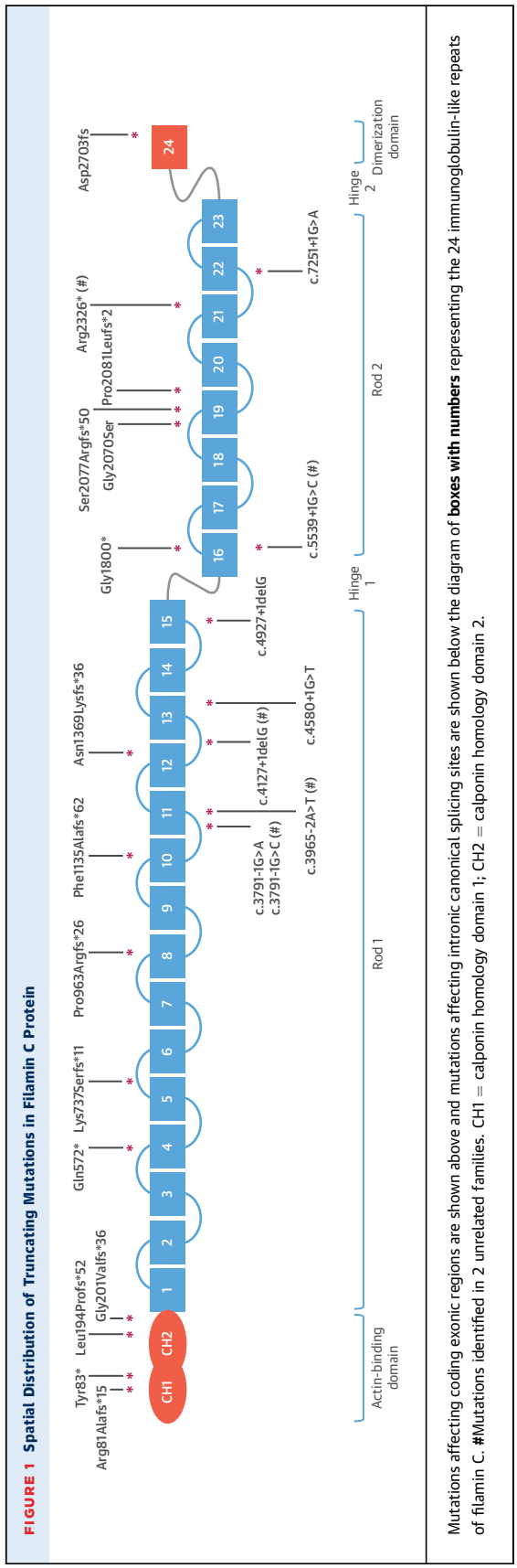


FIGURE 1 Spatial Distribution of Truncating Mutations in Filamin C Protein

Mutations affecting coding exonic regions are shown above and mutations affecting intronic canonical splicing sites are shown below the diagram of **boxes with numbers** representing the 24 immunoglobulin-like repeats of filamin C. **#**Mutations identified in 2 unrelated families. CH1 = calponin homology domain 1; CH2 = calponin homology domain 2.

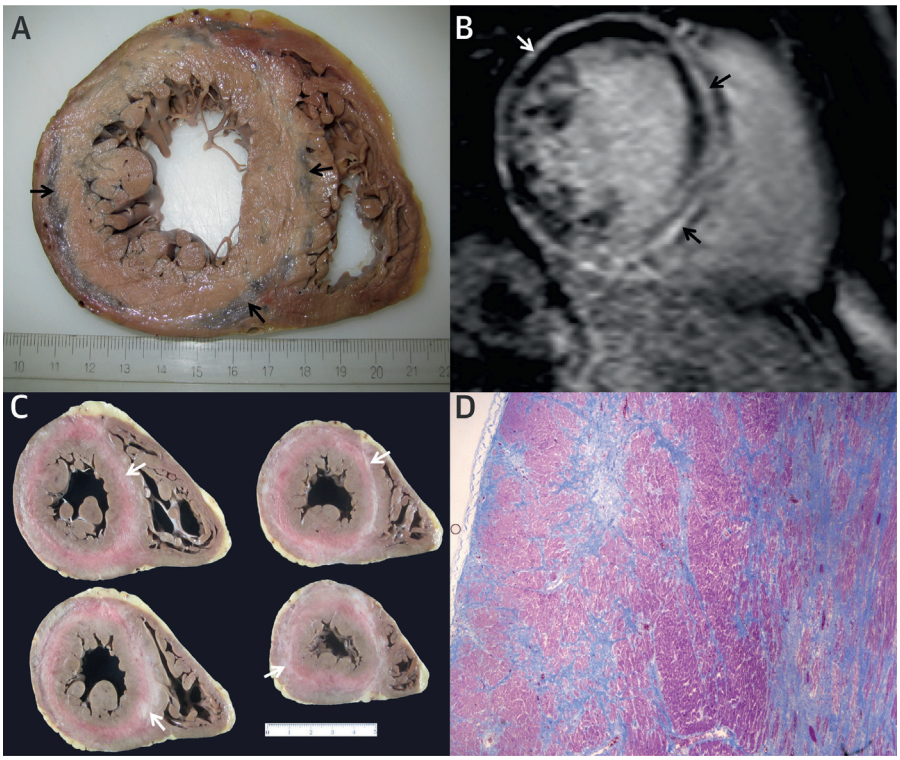
TABLE 1 Clinical Characteristics of Carriers of Truncating Mutations in *FLNC*

	Probands (n = 28; 17 Male)			Relatives With the Mutation (n = 54; 28 Male)			All Carriers (n = 82; 45 Male)		
	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*	27	8	30	47	1	2	74	9	12
Low voltages	25	9	36	47	9	19	72	18	25
Negative T-wave all locations	21	13	62	46	9	20	67	22	33
Left precordial negative T-wave	21	6	29	46	2	4	67	8	12
Right precordial negative T-wave	21	0	0	46	0	0	67	0	0
Left + right precordial negative T-wave	21	0	0	46	3	7	67	3	4
Inferior negative T-wave	21	2	10	46	2	4	67	4	6
Inferior + left precordial negative T-wave	21	4	19	46	2	4	67	6	9
Inferior + right precordial negative T-wave	21	1	5	46	0	0	67	1	1
Epsilon wave	21	0	0	46	0	0	67	0	0
Terminal QRS >55 ms	20	5	25	45	7	16	65	12	18
SAECG positive	3	2	67	3	2	67	6	4	67
Cardiac structural affection									
LV dilation	27	19	70	47	15	32	74	34	46
EF <55%	27	26	96	49	25	51	76	51	67
MLVWT ≥12 mm	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 dilation/akinesia/dyskinesia/systolic dysfunction	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 h)	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 transplantation	28	5	18	54	0	0	82	5	6
Stroke	28	2	7	54	0	0	82	2	2

*Includes bundle branch block.

CK = creatine kinase; ECG = electrocardiography; EF = ejection fraction; EPS = electrophysiological study; FVE = frequent ventricular extrasystoles; ICD = implantable cardioverter-defibrillator; LV = left ventricular; MLVWT = maximal left ventricular wall thickness; NSVT = nonsustained ventricular tachycardia; RV = right ventricular; SAECG = signal-average electrocardiogram; SVT = sustained ventricular tachycardia; terminal QRS >55 ms = S-wave upstroke of the QRS complex during >55 ms.

FIGURE 2 Left Ventricular Myocardial Fibrosis Observed in 2 Carriers of Truncating *FLNC* Mutations



Left ventricular (LV) fibrosis is seen in 2 carriers of truncating *FLNC* mutations. The proband of family 32406, who died suddenly playing soccer at 22 yrs of age, was found to have **(A)** circumferential subepicardial fibrosis **(black arrows)** on necropsy on the same localization **(B)** as late gadolinium enhancement **(black and white arrows)** detected on cardiac magnetic resonance performed prior to death. Also seen are **(C)** circumferential LV intramyocardial fibrosis **(white arrows)** on necropsy and **(D)** myocardial fibrosis **(light blue)** affecting the anterior wall of the left ventricle (Masson's trichrome, 2×) of the proband of family 26958, who died suddenly at 17 yrs of age after a soccer game.

endomyocardial biopsy of the right ventricle. LV myocardial fibrosis was mainly subepicardial. A concentric pattern and extension to intramyocardial or transmural involvement were observed in some cases. Two patients who underwent transplantation because of advanced heart failure showed endomyocardial fibrosis.

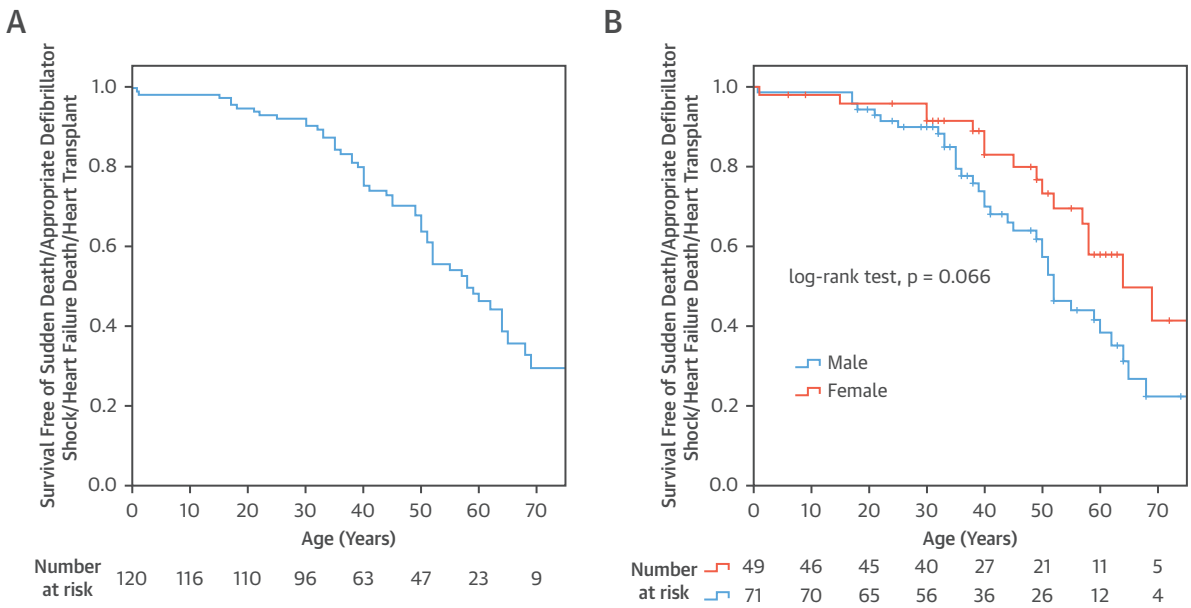
At initial evaluation, no patient had muscle weakness or showed signs of skeletal myopathy. Mild elevation of plasmatic creatine kinase levels (<2 times the upper normal value) was found in only 3 of 40 evaluated carriers. Only 1 of them (the proband from family 31277) had muscle weakness in the lower limbs during follow-up. This patient had been diagnosed with restrictive cardiomyopathy at 29 years of age and had undergone heart transplantation at age 45. An electromyographic study at 59 years of age revealed moderate myopathic changes. However, this woman was receiving therapy with simvastatin and corticosteroids, which 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 experienced cardiac arrest (mean age at event 42 ± 16 years; range: 17 to 68 years), which was the first manifestation of the disease in 4 of them. All subjects with available data presented LV systolic dysfunction (n = 9; mean EF: 39.6 ± 12%; range: 21% to 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 patients, and all of them showed frequent ventricular extrasystoles and/or nonsustained VT.

Twenty-six affected mutation carriers received or were recommended to receive cardiac defibrillators. The indication was primary prevention in 17 (1 declined the indication and 1 died suddenly while

FIGURE 3 Major Cardiovascular Events



In families with truncating *FLNC* mutations, freedom from sudden death, appropriate defibrillator shock, heart failure death, or heart transplantation in all clinically or genetically affected subjects **(A)** and discriminated by sex **(B)** decreased as subjects aged.

waiting for implantation) and secondary prevention in 9, after experiencing symptomatic sustained VT (n = 7), or after aborted cardiac arrest (n = 2); all of them exhibited LV systolic dysfunction. Appropriate shocks were recorded in 3 of 15 primary prevention patients (20%) and in 5 of 9 secondary prevention patients (56%) (mean time to shock 53 ± 39 months; range: 0.1 to 96 months) (Online Figure 4).

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

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

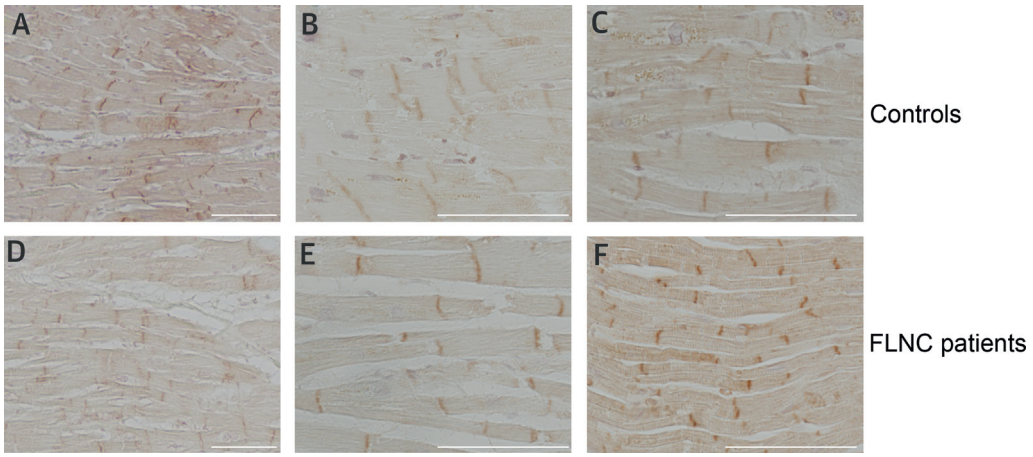
IMMUNOHISTOCHEMISTRY. Immunohistochemical staining of myocardial tissue from patients carrying truncating mutations in *FLNC* showed no abnormal filamin C aggregates in the cytoplasm. In contrast, we observed that antibodies against filamin C stained the intercalated disk region in both patients and control subjects (**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 (**Central Illustration**). 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 of age.

Carriers developed ventricular dilation with reduced EF, especially affecting the left ventricle. The majority of the affected carriers had been diagnosed with DCM. 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 (15). Left-dominant arrhythmogenic cardiomyopathy mimics idiopathic DCM and its clinical diagnostic criteria have not been formally established. Many investigators have suggested that ventricular arrhythmias coming from a fibrotic LV wall in the absence of right ventricular involvement

FIGURE 4 Filamin C Localization in Cardiac Tissue



Immunohistochemical staining shows the presence of filamin C only in intercalated disks of 3 control subjects **(A to C)** and 3 patients with truncating *FLNC* mutations **(D to F)**. Hematoxylin counterstain was used to detect cell nuclei. Scale bar = 100 μm.

could be an expression of left-dominant arrhythmogenic cardiomyopathy (16-18). This phenotype frequently presents with inferolateral negative T waves, mild to moderate LV systolic dysfunction, and regional dyskinesia, all of which were identified in several of our patients.

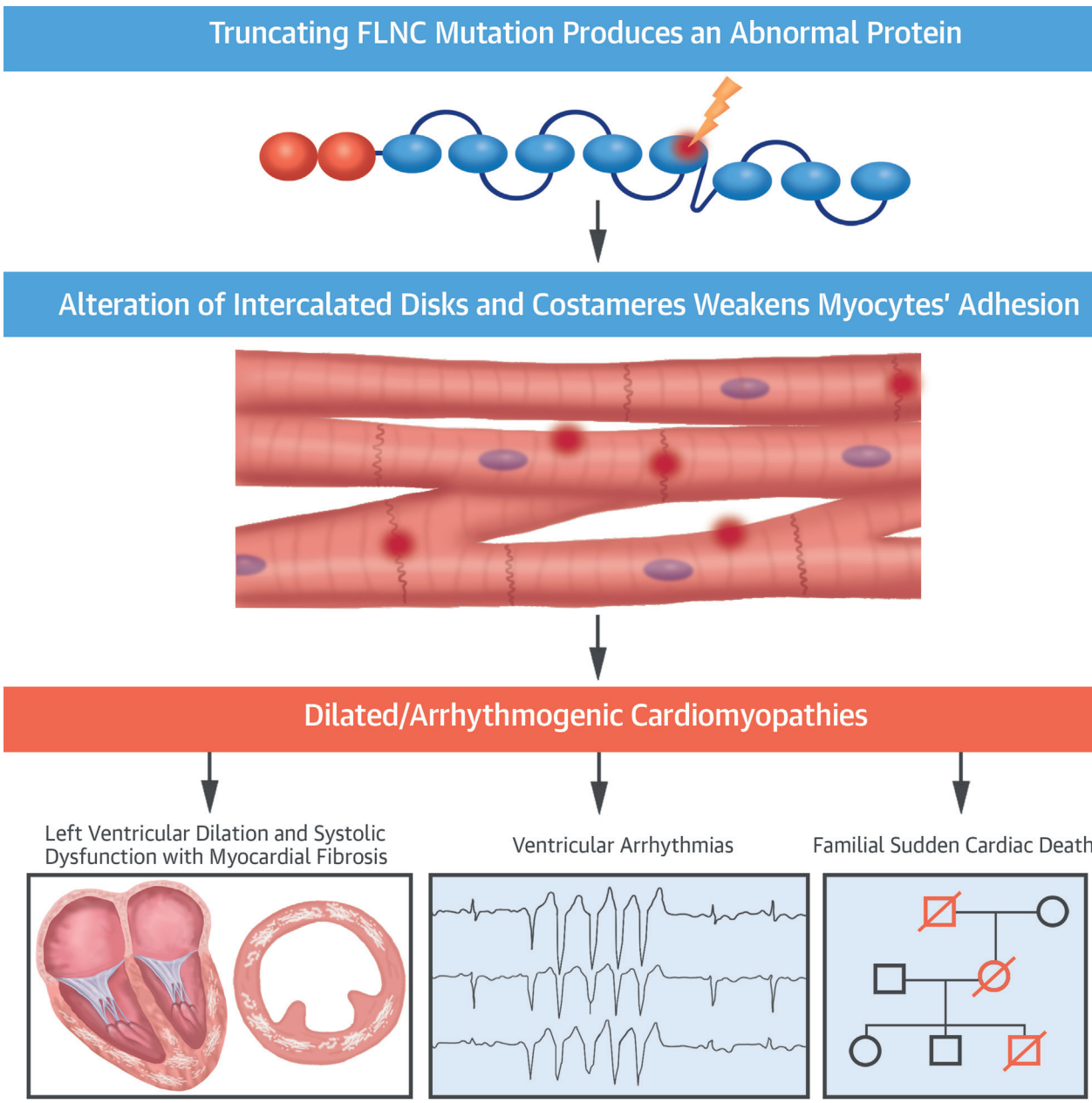
These patients with truncating mutations in *FLNC* share clinical characteristics both of desmosomal mutations and of laminopathies and desminopathies. Ventricular arrhythmias, likely related to the presence of LV myocardial fibrosis, and a high incidence of sudden death may appear in all of them (19,20). Nevertheless, isolated or predominant right ventricular involvement, common in desmosomal mutations, was not observed in our patients. In contrast, cardiac conduction abnormalities were mild and infrequent, while they are common and severe in patients with pathogenic lamin A/C, emerin, or desmin mutations (19-21). These differences likely reflect the involvement of different pathogenic mechanisms.

All mutations identified in our work were novel except for c.3791-1G>C and c.7251+1G>A, although both variants have been reported in patients with DCM (22-24). The phenotype of these cases resembles our findings: high prevalence of ventricular arrhythmias and sudden cardiac death (even in the absence of severe LV dilation and dysfunction) with no skeletal muscle involvement.

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 cell-to-cell mechanical force transduction (7). Filamin C directly interacts with 2 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**) (25).

Several mutations in *FLNC* have been previously associated with a particular form of myofibrillar myopathy (8-12). This phenotype is characterized mainly 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 nonspecific 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 (8-12). Mutations in *FLNC* previously identified in myofibrillar myopathy are mostly missense and in-frame indels. Only 2 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 (8,12). Abnormal cytoplasmic filamin C aggregates were demonstrated to play a pathogenic role in most of these cases.

CENTRAL ILLUSTRATION Truncating *FLNC* Mutations and Dilated and Arrhythmogenic Cardiomyopathies

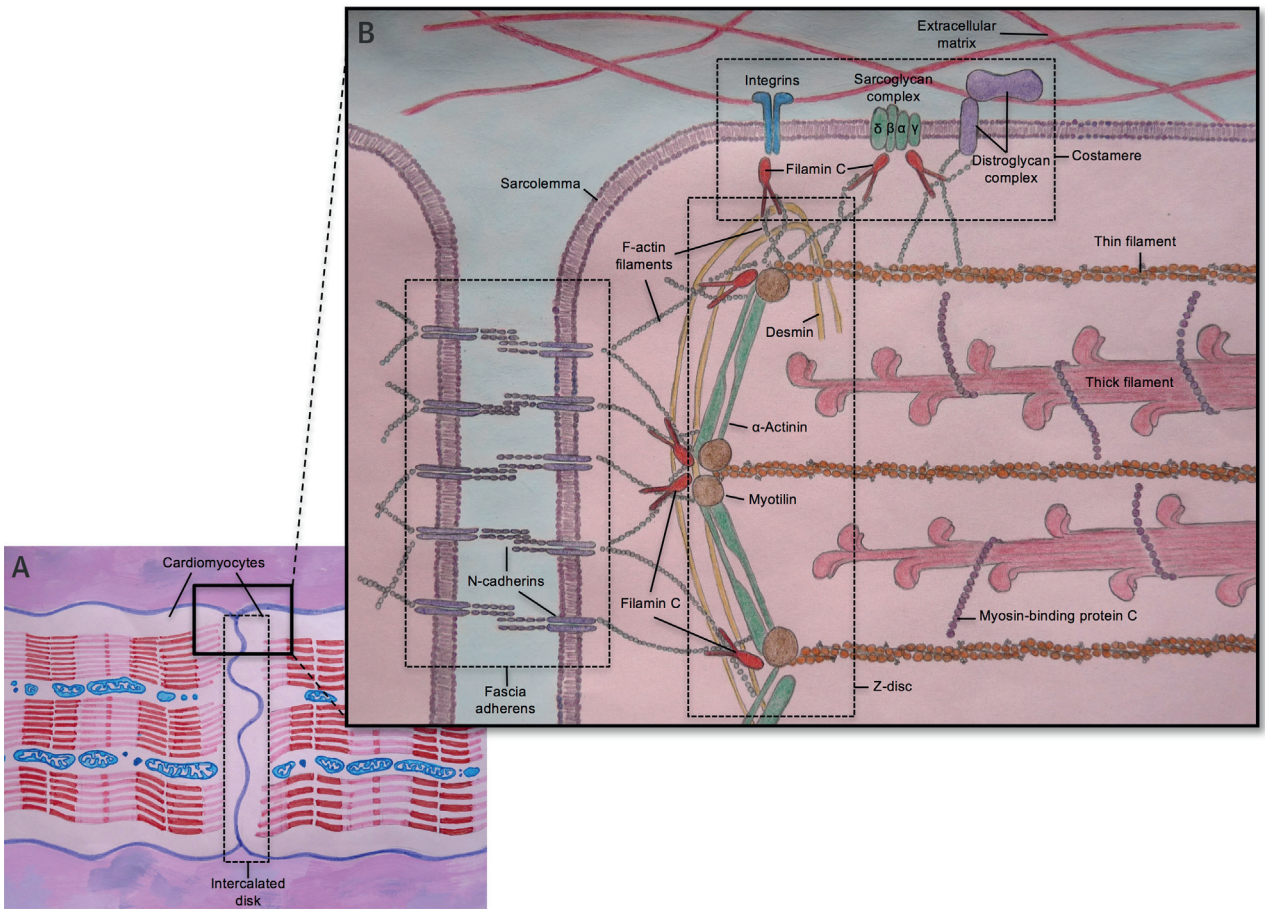


Ortiz-Genga, M.F. et al. J Am Coll Cardiol. 2016;68(22):2440–51.

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

Immunohistochemical analysis showed normal filamin C staining in intercalated disks. The absence of abnormal filamin C aggregates in the cytoplasm of cardiomyocytes 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

FIGURE 5 Cellular Interactions of Filamin C



Cardiomyocytes bind to each other at their longitudinal extremes by means of intercalated disks **(A)**. Filamin C directly interacts with components of the fascia adherens, allowing 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 **(B)**.

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 LV myocardium, could be particularly affected. Myocardial fibrosis, together with dilation and systolic dysfunction of the left ventricle, could be the consequences of this functional alteration (**Central Illustration**). In fact, Begay et al. (24) recently demonstrated in a zebrafish knockdown model that 2 splicing variants in *FLNC* produced a reduction in cardiac filamin C protein levels with Z-disk and sarcomere disorganization. These findings additionally supported haploinsufficiency as the underlying functional mechanism of truncating mutations in *FLNC*. 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 (26).

Clinical signs of skeletal myopathy were specifically and systematically investigated among carriers. It is noteworthy that only 1 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 showed that cardiac disease would be the main consequence of truncating mutations in this gene. Because most previous publications focused on skeletal myopathy and cardiac examinations were not routinely performed, subtle cardiac abnormalities detectable only through Holter electrocardiography and CMR could have been missed.

It has been suggested that mutations in *FLNC* could explain nearly 10% of cases of HCM in patients without mutations in the main sarcomeric genes (27). Seven of 8 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 2 patients showed normal histology and histochemistry. It is noteworthy that in this report, patients with *FLNC* mutations showed lower LV 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 HCM and truncating mutations to dilated or left-dominant arrhythmogenic cardiomyopathies would need to be confirmed in future studies, but so far we have not identified any truncating *FLNC* mutations in more than 1,000 patients with HCM. Our data clearly suggest that truncating *FLNC* mutations are not related to the development of HCM.

Two novel missense mutations in *FLNC* have recently shown cosegregation with restrictive cardiomyopathy in 2 Caucasian families (28). We postulate that the molecular mechanism associated with these missense mutations could be different to truncating mutations. However, some clinical characteristics of these 2 families resembled our findings. Several carriers showed different amounts of myocardial fibrosis on cardiac histology. Moreover, some cases presented T-wave abnormalities on electrocardiography or LV systolic dysfunction on echocardiography. Unfortunately, the assessment of ventricular arrhythmias on Holter electrocardiography and the evaluation of areas with late gadolinium enhancement with CMR were not reported.

STUDY LIMITATIONS. Patients were genetically screened for genes previously associated with inherited cardiac conditions. The presence of additional mutations in other genes contributing to the phenotype cannot be ruled out. Cosegregation studies were limited in some families because of the small number of relatives available for screening. Clinical assessment was incomplete in some carriers. The presence of skeletal myopathy among carriers was not assessed using more specific diagnostic tools such as magnetic resonance imaging or skeletal muscle biopsies.

The available data used for survival curves could be insufficient to accurately estimate the prognosis associated with truncating mutations in *FLNC*. Additional studies are needed to specifically determine the functional mechanisms behind the development of cardiomyopathy among carriers of truncating mutations in *FLNC*.

CONCLUSIONS

Truncating mutations in *FLNC* are associated with a characteristic cardiac phenotype that includes LV dilation 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 includes CMR imaging and Holter electrocardiographic monitoring. Implantable defibrillators should probably be considered even in cases with only moderate systolic dysfunction in the presence of myocardial fibrosis and ventricular arrhythmias.

ACKNOWLEDGMENTS The authors acknowledge Fondazione CR Trieste, Centre for Translational Cardiology Project; Silvia Moimas, PhD, of ICGEB Trieste, for participation in the genetic research studies; and Alicia Pallas Lozano and Sylvia Kinney for their help with English editing.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Truncating mutations in *FLNC*, previously related to skeletal myopathy, can also be associated with cardiomyopathy in the absence of clinical skeletal manifestations. These mutations cause an overlapping phenotype of dilated and arrhythmogenic cardiomyopathies, and should be suspected when a cardiomyopathy is characterized by LV systolic dysfunction and/or dilation, fibrosis, ventricular arrhythmias, and a family history of sudden death.

COMPETENCY IN PATIENT CARE: Patients with suspected *FLNC* mutations can be evaluated by genetic testing, CMR (to exclude myocardial fibrosis), cardiac arrhythmia monitoring, and stress testing (to evaluate ventricular arrhythmias).

TRANSLATIONAL OUTLOOK: Further studies are needed to clarify the mechanisms linking truncating *FLNC* mutations to the clinical manifestations of these cardiomyopathies and to determine whether implantation of a cardiac defibrillator improves survival in carriers with myocardial fibrosis and ventricular arrhythmias when LV systolic function is relatively well preserved.

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KEY WORDS filamin C, filaminopathy, genotype, prognosis, sudden death, ventricular arrhythmia