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

Martín F. Ortiz-Genga, MD,[a,b] Sofía Cuenca, MD,[c] Matteo Dal Ferro, MD,[d] Esther Zorio, MD, PhD,[d] Ricardo Salgado-Aranda, MD,[f] Vicente Climent, MD,[g] Laura Padrón-Barthe, PhD,[h] Iria Duro-Aguado, MD,[i] Juan Jiménez-Jáimez, MD, PhD,[j] Víctor M. Hidalgo-Olivares, MD,[k] Enrique García-Campo, MD,[l] Chiara Lanzillo, MD, PhD,[m] M. Paz Suárez-Mier, MD, PhD,[n] Hagith Yonath, MD, PhD,[o] Sonia Marcos-Alonso, MD, PhD,[p] Juan P. Ochoa, MD,[b] José L. Santomé, BSc,[b] Diego García-Giustiniani, MD,[b] Jorge L. Rodríguez-Garrido, MD,[b] Fernando Domínguez, MD,[c] Marco Merlo, MD,[d] Julián Palomino, MD, PhD,[l] María L. Peña, MD,[q] Juan P. Trujillo, MD, PhD,[b] Alicia Martín-Vila, PharmD,[l] Davide Stolfo, MD,[d] Pilar Molina, MD, PhD,[r] Enrique Lara-Pezzi, PhD,[h,s] Francisco Calvo, MD, PhD,[l] Eyal Nof, MD,[o] Leonardo Calò, MD,[m] Roberto Barriales-Villa, MD, PhD,[a,p] Juan R. Gimeno-Blanes, MD, PhD,[t] Michael Arad, MD, PhD,[o] Pablo García-Pavia, MD, PhD,[c] Lorenzo Monserrat, MD, PhD[a,b]

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

Authors' Affiliations

[a]Instituto de Investigación Biomédica, A Coruña, Spain
[b]Health in Code SL, A Coruña, Spain
[c]Heart Failure and Inherited Cardiac Diseases Unit. Department of Cardiology, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain
[d]Cardiovascular Department, Azienda Ospedaliero - Universitaria Ospedali Riuniti, Trieste, Italy
[e]Hospital Universitario La Fe, Valencia, Spain
[f]Hospital Universitario de Burgos, Burgos, Spain
[g]Hospital General Universitario de Alicante, Alicante, Spain
[h]Myocardial Pathophysiology Area, Centro Nacional de Investigaciones Cardiovasculares, Melchor Fernández Almagro 3, Madrid 28029, Spain
[i]Hospital Clínico Universitario de Valladolid, Valladolid, Spain
[j]Hospital Universitario Virgen de las Nieves, Granada, Spain
[k]Complejo Hospitalario Universitario de Albacete, Albacete, Spain
[l]Complexo Hospitalario Universitario de Vigo, Vigo, Spain
[m]Policlinico Casilino, ASL Roma B, Rome, Italy
[n]Instituto Nacional de Toxicología y Ciencias Forenses, Madrid, Spain
[o]Sheba Medical Center, Tel-Aviv, Israel
[p]Complejo Hospitalario Universitario de A Coruña, A Coruña, Spain
[q]Hospital Universitario Virgen del Rocio, Sevilla, Spain
[r]Servicio de Patología, Instituto de Medicina Legal, Valencia, Spain
[s]National Heart and Lung Institute, Imperial College London, London, UK
[t]Hospital Universitario Virgen de la Arrixaca, Murcia, Spain

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Address for Correspondence
Dr. Martín Ortiz-Genga
Health in Code SL, As Xubias s/n
Edificio O Fortín, Hospital Marítimo de Oza
15006, A Coruna, Spain.
Telephone: +34 881 600 003
Fax: +34 981 167 093
E-mail: martin.ortiz@healthincode.com

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 we have included *FLNC* in the genetic screening of patients with inherited cardiomyopathies and sudden death.
OBJECTIVES 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 analyzed in patients with truncating *FLNC* mutations using immunohistochemistry.
RESULTS We identified 23 truncating mutations 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 of left ventricular dilation (68%), systolic dysfunction (46%), and myocardial fibrosis (67%); inferolateral negative T waves with low voltages on electrocardiogram (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 stainings 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 individuals harboring truncating mutations in *FLNC*.

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

ABBREVIATIONS
CMR = cardiac magnetic resonance
DCM = dilated cardiomyopathy
ECG = electrocardiogram
FLNC = Filamin C gene
HCM = hypertrophic cardiomyopathy
LOD = logarithm of the odds
LV = left ventricle/ventricular
RV = right ventricle/ventricular
VT = ventricular tachycardia

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 one 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 a nonspecified 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.

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 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 who agreed. All individuals 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, Inc., San Diego, California) with 2 x 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 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 (13).

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 (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 protein's sequence (nonsense or frameshift) or that could alter the splicing process according to the predictions of 5 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 (HF) death, or cardiac transplant 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 statistically significant. Statistical analyses were performed using SPSS Statistics Version 21.0 (IBM Corporation, Armonk, New York).

Two-point logarithm of the odds (LOD) score was 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

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 was performed using a specific antibody against the N-terminal extreme of filamin C. 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 min in the 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 4º C. Slides were TBS-T-washed and incubated with a horseradish peroxidase goat anti-rabbit secondary antibody for 30 min at room temperature. Vectastain ABC kit was used to amplify the signal, and 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 of them with predominant LV 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 (HCM) **(Online Table 1**). The prevalence of truncating *FLNC* mutations in this heterogeneous global cohort was low (28 out of 2,877 patients screened = 0.97%). However, if we referred to the specific phenotypes where 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 individuals with restrictive cardiomyopathy (2.2%).

Patients Studied

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 to 71). Eleven (92%) of 12 healthy mutation carriers were <40 years at last follow-up (mean 32 ± 16 years; range: 6 to 72). None of the 67 noncarriers were 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, 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 due to 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 of them presented LV involvement as well. Five (14%) of the 36 affected relatives with available information showed mild right ventricular (RV) abnormalities. Mild 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 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 to V3 was recorded in 18% of carriers evaluated. No individuals showed epsilon waves. A signal average electrocardiogram (ECG) was positive in 4 of 6 individuals tested.

Ventricular arrhythmias were extremely frequent among carriers (82%). Frequent ventricular extrasystoles (>500/24 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 (ages 22 and 25 years, respectively), and fibrosis was confirmed on cardiac histology (**Figures 2A and 2B**). Three of 13 probands without a CMR study showed myocardial fibrosis confined to the left ventricle on necropsy/explanted heart (1 died suddenly at age 17 years, and the other 2 were transplanted at ages 1 and 60 years, respectively) (**Figures 2C and 2D**). Endomyocardial biopsy of the RV 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 LV on CMR (n = 13) or in the necropsy (n = 2); 1 relative showed fibrosis on endomyocardial biopsy of the RV. 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 HF 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 (<2-fold of upper normal value) was found in only 3 of 40 evaluated carriers. Only 1 of them (proband from family 31277) presented muscle weakness in the lower limbs during follow-up. This patient had been diagnosed with restrictive cardiomyopathy at 29 years and had received a cardiac transplant at age 45. An electromyography study at 59 years 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 **Online Figure 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 to 68), being the first manifestation of the disease in 4 of them. All subjects with available data presented LV systolic dysfunction (n = 9; mean LVEF: 39.6 ± 12%; range: 21 to 4) 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 nonsustained VT.

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

Five carriers underwent heart transplantation due to 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).

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 to 80); 65% occurred in individuals ≤50 years old. **Figure 3** shows the survival curve for sudden death/appropriate defibrillator shock/HF 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 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 dilated cardiomyopathy and its clinical diagnostic criteria have not been formally established. Many authors have suggested that ventricular arrhythmias coming from a fibrotic LV wall in the absence of RV involvement 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 RV 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 (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 for 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 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 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.

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 *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. In fact, Begay et al. 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 (24). 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. Since most previous publications focused on skeletal myopathy and cardiac examinations were not routinely performed, subtle cardiac abnormalities only detectable through Holter ECG 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/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 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 showed 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 ECG or LV systolic dysfunction on echocardiogram. Unfortunately, the assessment of ventricular arrhythmias on Holter ECG 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 due to the low 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 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: Truncating mutations in FLNC, previously related to skeletal myopathy, can also be associated 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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Figure Legends

Central Illustration: Truncating *FLNC* Mutations and 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 dilation 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 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. CH1 = calponin homology domain 1; CH2 = calponin homology domain 2; (#) = mutations identified in 2 unrelated families.

Figure 2: LV Myocardial Fibrosis observed in two 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 age 22, was found to have **(A)** circumferential subepicardial fibrosis (arrows) on necropsy on the same localization **(B)** as late-gadolinium enhancement (arrows) detected on cardiac magnetic resonance performed prior to death Also seen are **(C)** circumferential LV intramyocardial fibrosis (arrows) on necropsy and **(D)** myocardial fibrosis (light blue) affecting the anterior wall of the left ventricle (Masson's trichrome, 2x) of the proband of family 26958, who died suddenly at age 17 after a soccer mat.

Figure 3: Major Cardiovascular Events. In families with truncating FLNC mutations, freedom from sudden death/appropriate defibrillator shock/heart failure death/heart transplant in all clinically or genetically affected individuals **(A)** and discriminated by sex **(B)** decreased as individuals aged.

Figure 4: Filamin C Localization in Cardiac Tissue. Immunohistochemical staining shows the presence of filamin C only in intercalated disks of 3 controls **(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.

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 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 **(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*	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 >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
LVEF <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 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 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									

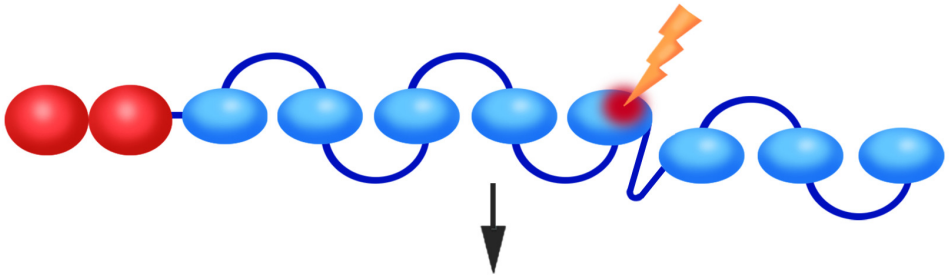
Clinical myopathy	28	1	4	48	0	0	76	1	1
Elevated CK plasma levels	21	2	10	19	1	5	40	3	8
OTHER									
Palmoplantar keratoderma	27	1	4	49	3	6	76	4	5
EVENTS									
Sudden death	28	5	18	54	7	13	82	12	15
Appropriate ICD shock	28	4	14	54	4	7	82	8	10
Heart failure death	28	0	0	54	0	0	82	0	0
Heart transplant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

Values are n unless otherwise indicated.

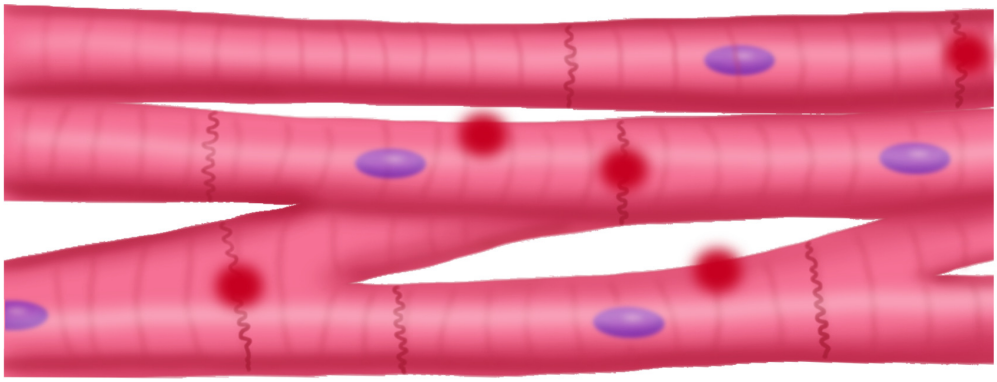
*Includes bundle branch block.

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

Truncating FLNC Mutation Produces an Abnormal Protein

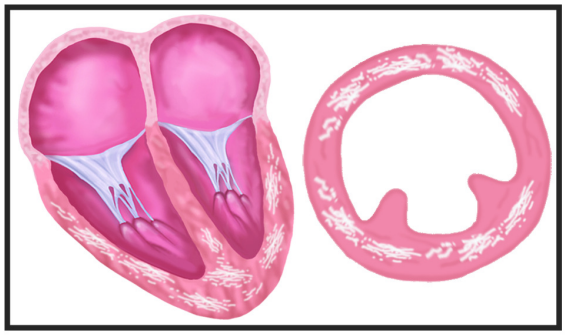


Alteration of Intercalated Disks and Costameres Weakens Myocytes' Adhesion

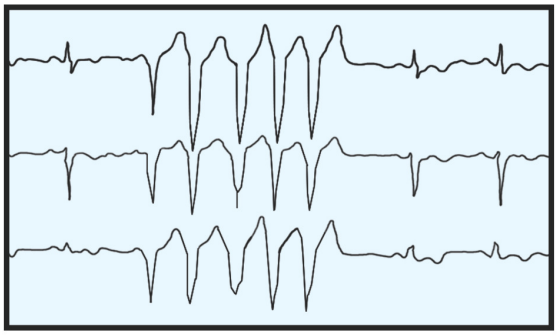


Dilated/Arrhythmogenic Cardiomyopathies

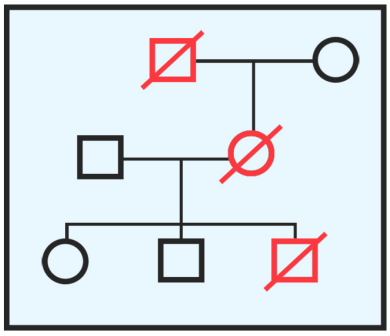
Left Ventricular Dilation and Systolic Dysfunction with Myocardial Fibrosis

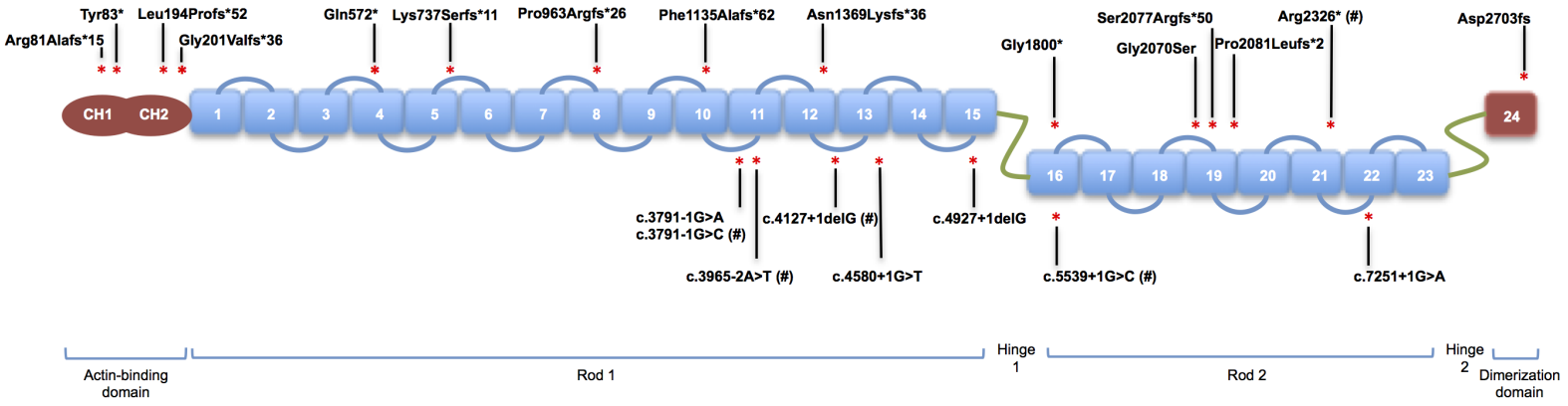


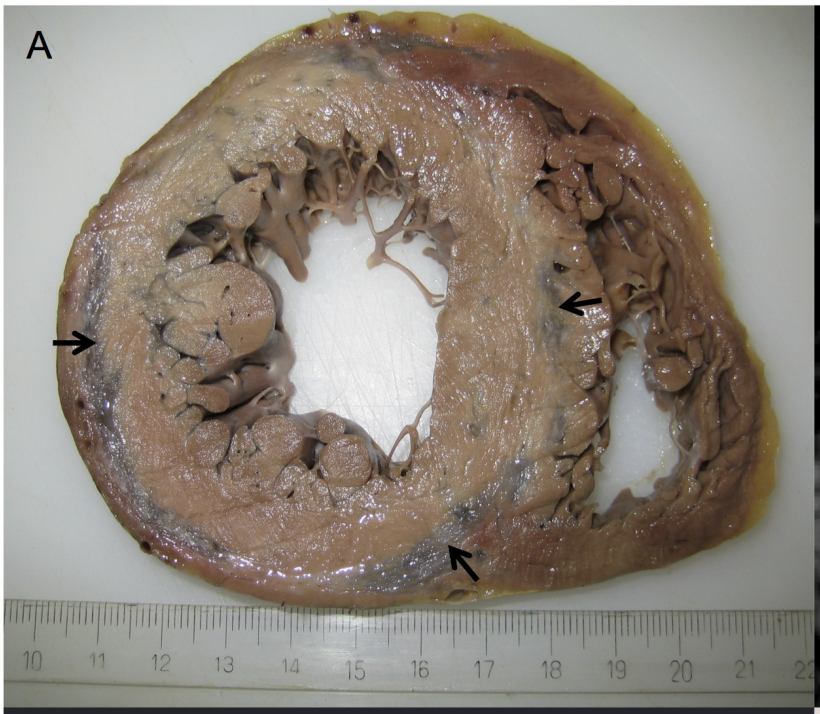
Ventricular Arrhythmias

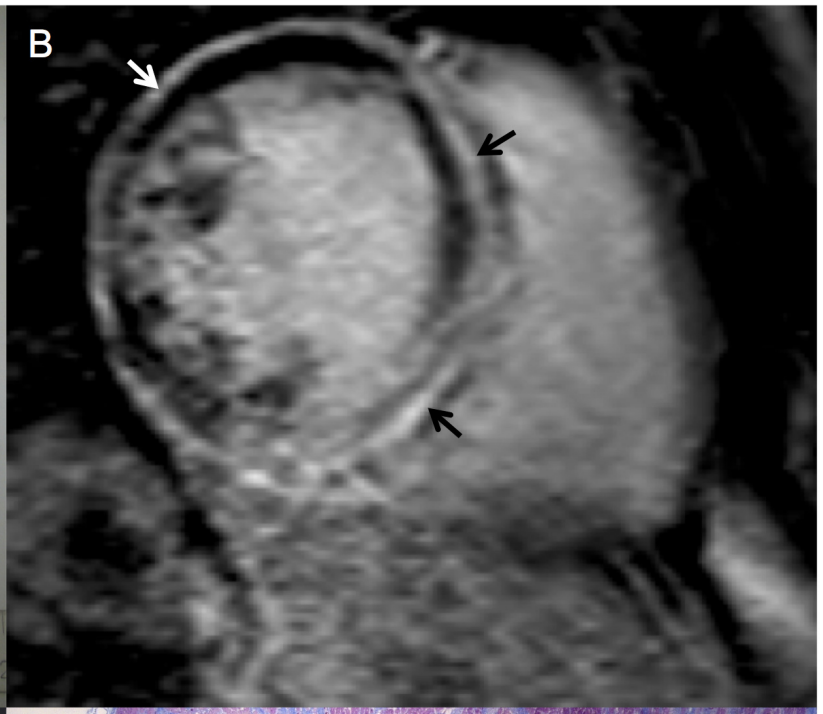


Familial Sudden Cardiac Death




Tyr83*
Arg81Alafs*15
Leu194Profs*52
Gly201Valfs*36
Gln572*
Lys737Serfs*11
Pro963Argfs*26
Phe1135Alafs*62
Asn1369Lysfs*36
Gly1800*
Ser2077Argfs*50
Gly2070Ser
Pro2081Leufs*2
Arg2326* (#)
Asp2703fs
CH1
CH2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
c.3791-1G>A
c.3791-1G>C (#)
c.3965-2A>T (#)
c.4127+1delG (#)
c.4580+1G>T
c.4927+1delG
c.5539+1G>C (#)
c.7251+1G>A
Actin-binding domain
Rod 1
Hinge 1
Rod 2
Hinge 2
Dimerization domain


A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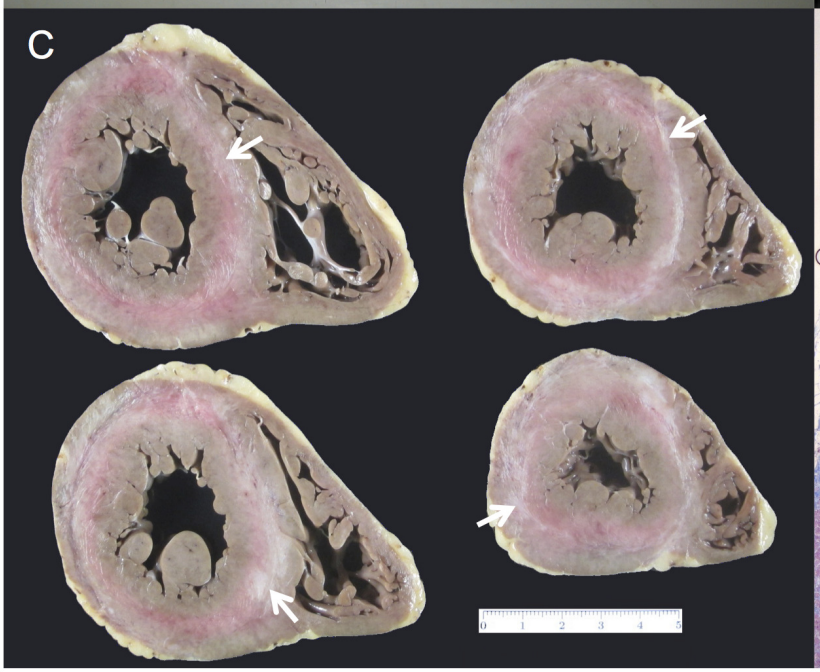
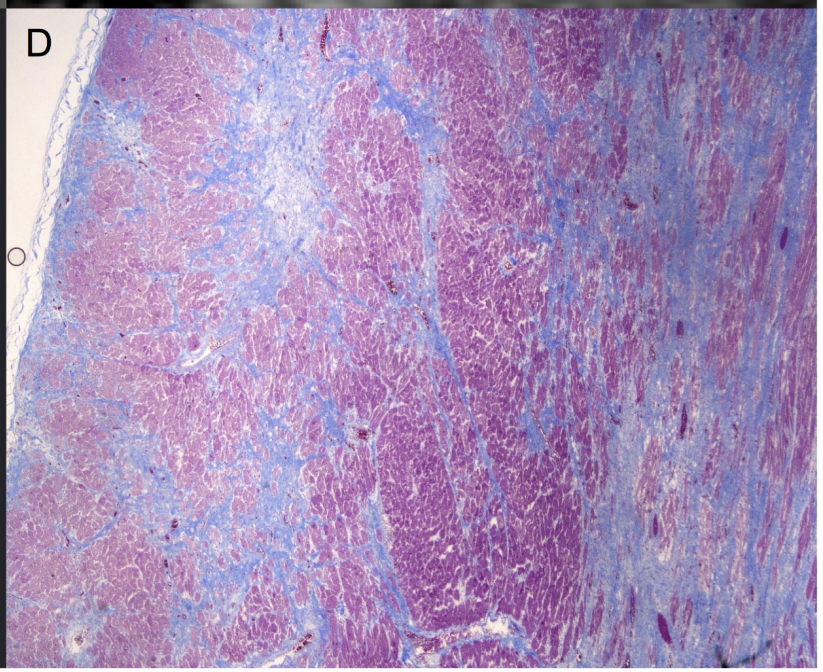

C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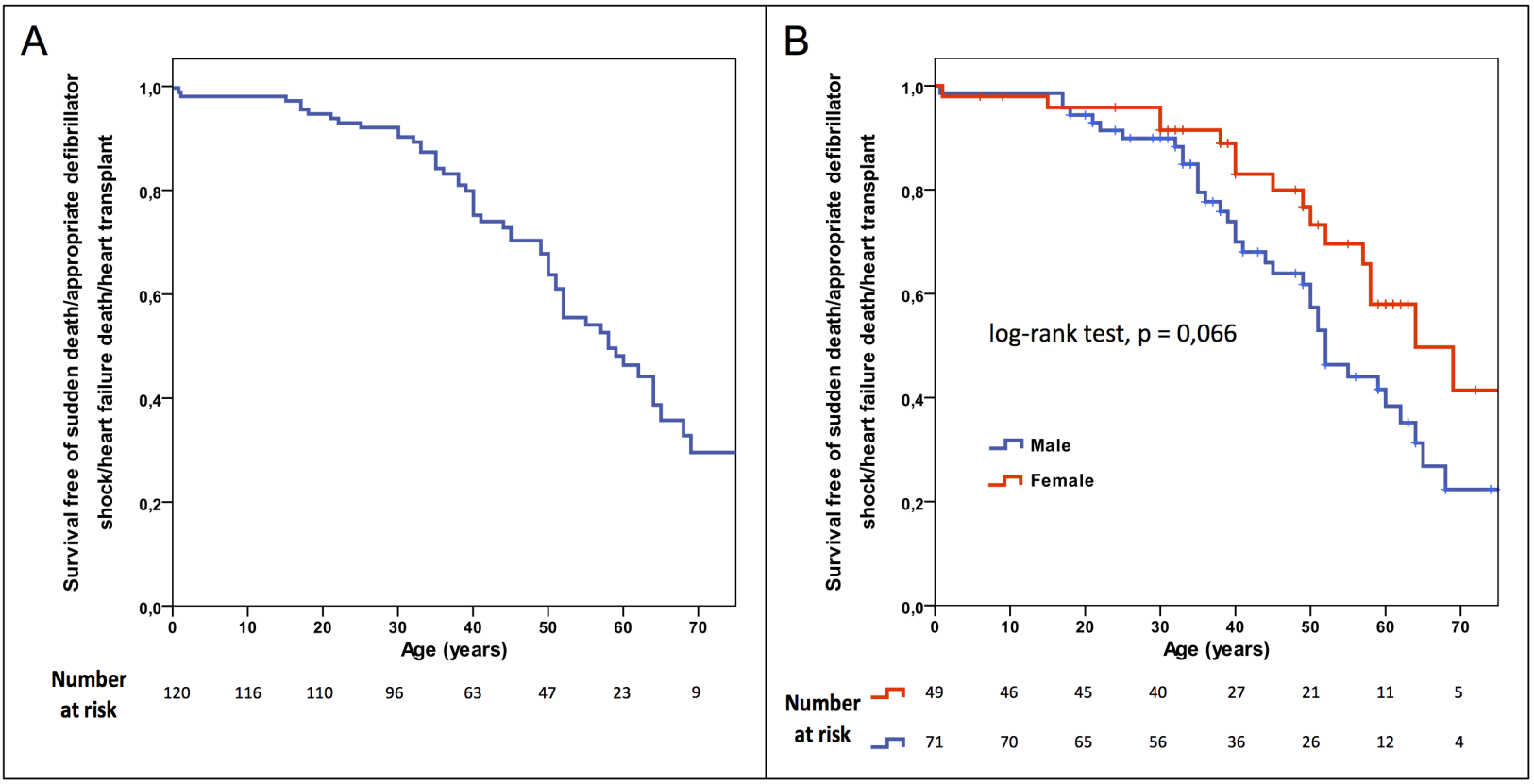
A
Survival free of sudden death/appropriate defibrillator shock/heart failure death/heart transplant
1,0
0,8
0,6
0,4
0,2
0,0
0
10
20
30
40
50
60
70
Age (years)
Number at risk
120
116
110
96
63
47
23
9
B
Survival free of sudden death/appropriate defibrillator shock/heart failure death/heart transplant
log-rank test, p = 0,066
Male
Female
Age (years)
Number at risk
49
46
45
40
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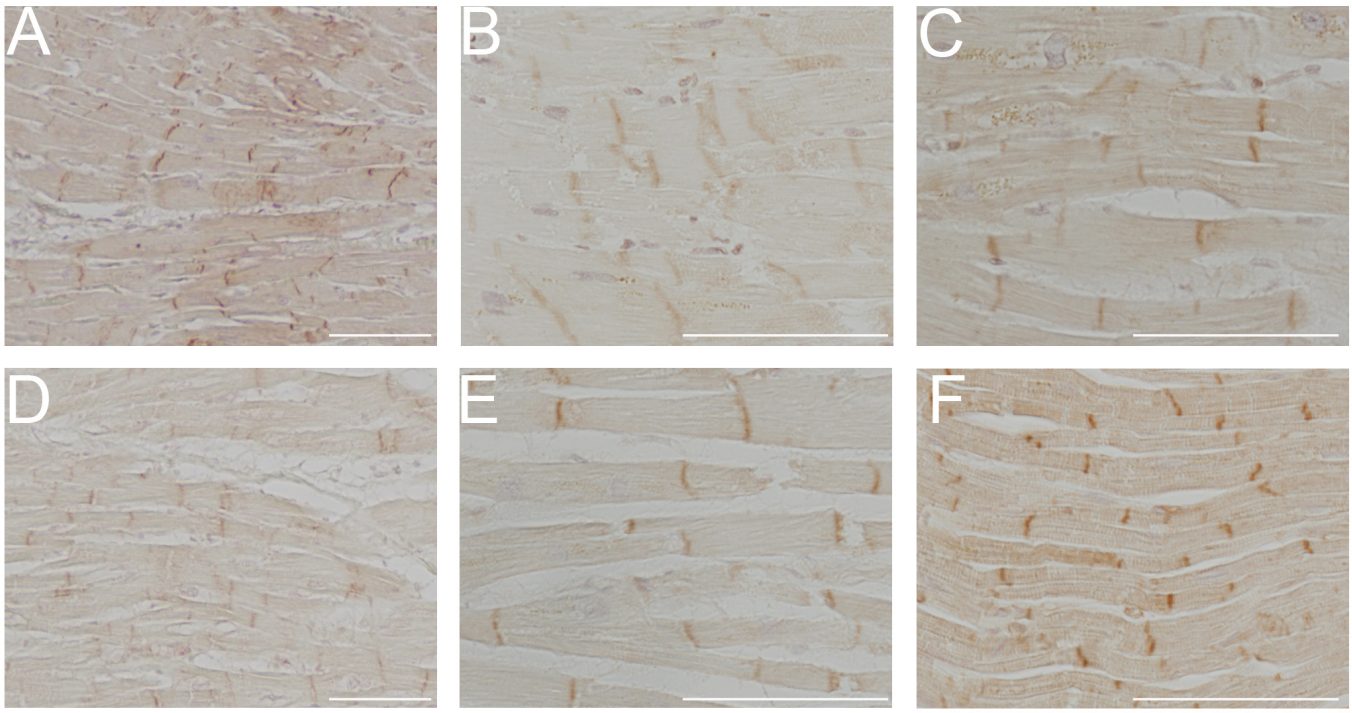
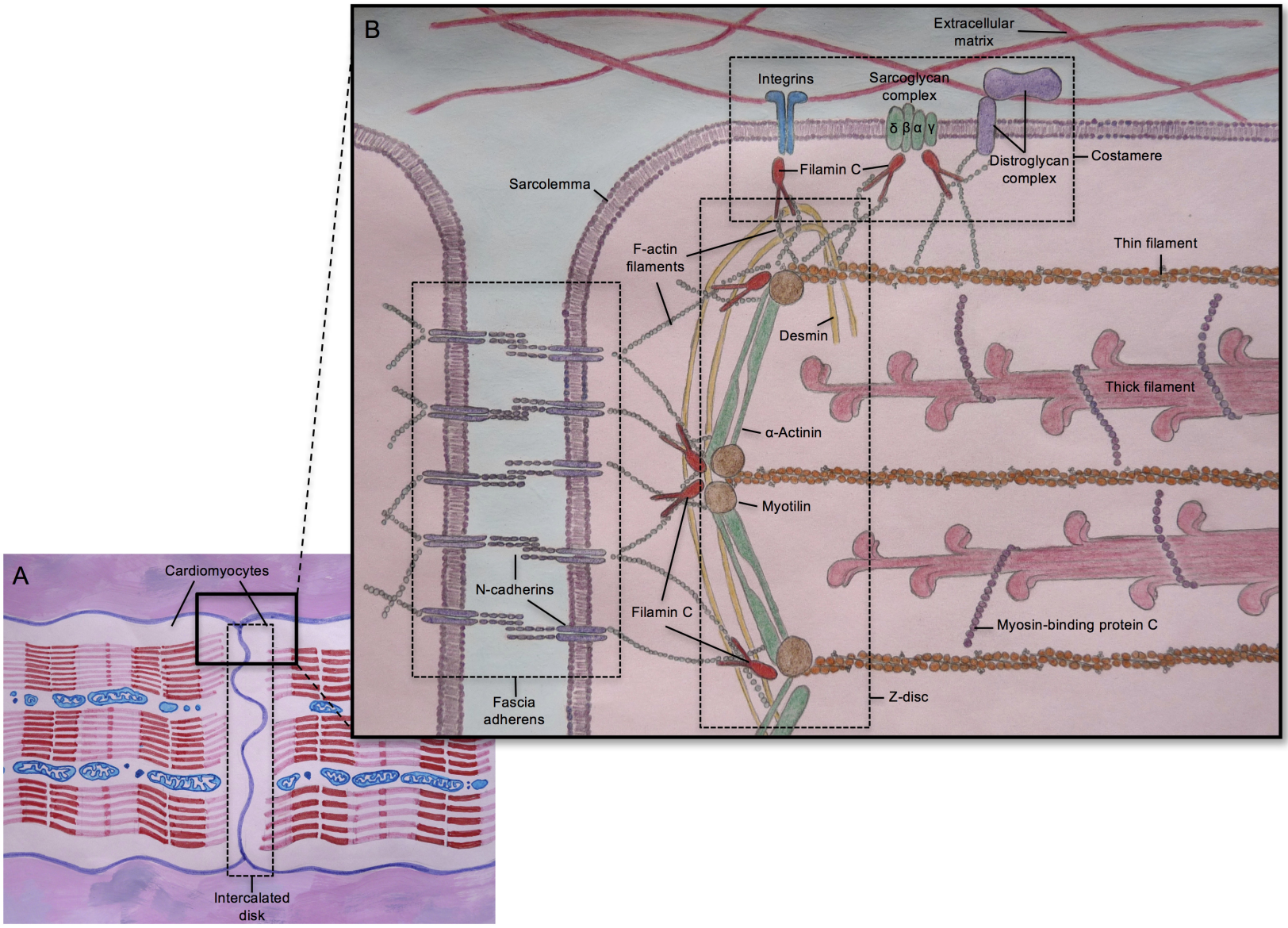
21
11
5
71
70
65
56
36
26
12
4


A
B
C
Controls
D
E
F
FLNC patients


A
Cardiomyocytes
Intercalated disk
B
Extracellular matrix
Integrins
Sarcoglycan complex
δ β α γ
Distroglycan complex
Costamere
Filamin C
Sarcolemma
F-actin filaments
Thin filament
Desmin
Thick filament
α-Actinin
Myotilin
N-cadherins
Filamin C
Myosin-binding protein C
Fascia adherens
Z-disc

Supplemental Material

TRUNCATING *FLNC* MUTATIONS ARE ASSOCIATED WITH HIGH-RISK DILATED AND ARRHYTHMOGENIC CARDIOMYOPATHIES

AUTHORS

Martín F. Ortiz-Genga, MD[1,2]; Sofía Cuenca, MD[3]; Matteo Dal Ferro, MD[4]; Esther Zorio, MD, PhD[5]; Ricardo Salgado-Aranda, MD[6]; Vicente Climent, MD[7]; Laura Padrón-Barthe PhD[8]; Iria Duro-Aguado, MD[9]; Juan Jiménez-Jáimez, MD, PhD[10]; Víctor M. Hidalgo-Olivares, MD[11]; Enrique García-Campo, MD[12]; Chiara Lanzillo, MD, PhD[13]; M. Paz Suárez-Mier, MD, PhD[14]; Hagith Yonath, MD, PhD[15]; Sonia Marcos-Alonso, MD, PhD[16]; Juan P. Ochoa, MD[2]; José L. Santomé, BSc[2]; Diego García-Giustiniani, MD[2]; Jorge L. Rodríguez-Garrido, MD[2]; Fernando Domínguez, MD[3]; Marco Merlo, MD[4]; Julián Palomino, MD, PhD[12]; María L. Peña, MD[17]; Juan P. Trujillo, MD, PhD[2]; Alicia Martín-Vila, PharmD[12]; Davide Stolfo MD[4]; Pilar Molina MD, PhD[18]; Enrique Lara-Pezzi PhD[8,19]; Francisco Calvo, MD, PhD[12]; Eyal Nof, MD[15]; Leonardo Calò, MD[13]; Roberto Barriales-Villa, MD, PhD[1,16]; Juan R. Gimeno-Blanes, MD, PhD[20]; Michael Arad, MD, PhD[15]; Pablo García-Pavia, MD, PhD[3]; Lorenzo Monserrat, MD, PhD[1,2].

AFFILIATIONS

1- Instituto de Investigación Biomédica, A Coruña, Spain
2- Health in Code SL, A Coruña, Spain
3- Heart Failure and Inherited Cardiac diseases Unit. Department of Cardiology, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain
4- Cardiovascular Department, Azienda Ospedaliero - Universitaria Ospedali Riuniti, Trieste, Italy
5- Hospital Universitario La Fe, Valencia, Spain
6- Hospital Universitario de Burgos, Burgos, Spain
7- Hospital General Universitario de Alicante, Alicante, Spain
8- Myocardial Pathophysiology Area, Centro Nacional de Investigaciones Cardiovasculares, Melchor Fernández Almagro 3, Madrid 28029, Spain
9- Hospital Clínico Universitario de Valladolid, Valladolid, Spain
10- Hospital Universitario Virgen de las Nieves, Granada, Spain
11- Complejo Hospitalario Universitario de Albacete, Albacete, Spain
12- Complexo Hospitalario Universitario de Vigo, Vigo, Spain
13- Policlinico Casilino, ASL Roma B, Rome, Italy
14- Instituto Nacional de Toxicología y Ciencias Forenses, Madrid, Spain
15- Sheba Medical Center, Tel-Aviv, Israel
16- Complejo Hospitalario Universitario de A Coruña, A Coruña, Spain
17- Hospital Universitario Virgen del Rocio, Sevilla, Spain
18- Servicio de Patología, Instituto de Medicina Legal, Valencia, Spain
19- National Heart and Lung Institute, Imperial College London, London, UK
20- Hospital Universitario Virgen de la Arrixaca, Murcia, Spain

Table of contents:

Table S1: List of different referring phenotypes related with inherited cardiovascular diseases evaluated through next generation sequencing.

Referring phenotype	Number of studies
Hypertrophic cardiomyopathy	1078
Dilated cardiomyopathy	508
Arrhythmogenic cardiomyopathy	219
Ascending aortic disease/Marfan Syndrome	214
Long QT syndrome	188
Left ventricular non-compaction-hypertrabeculation	159
Brugada syndrome	135
Sudden death of unknown cause	131
Idiopathic ventricular fibrillation	52
Catecholaminergic Polymorphic Ventricular Tachycardia	49
Restrictive Cardiomyopathy	45
Noonan Syndrome/Cardiofaciocutaneous Syndrome	30
Cardiac Conduction Disease	19
Ehlers-Danlos Syndrome	16
Hereditary hemorrhagic telangiectasia/Primary pulmonary hypertension	16
Short QT syndrome	11
Familial Atrial Fibrillation	7
TOTAL	2877

Table S2: List of 213 genes related to inherited cardiovascular diseases and sudden death included in our custom probe library.

Gene name	Codified protein
AARS2	Alanine--tRNA ligase, mitochondrial
ABCC9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
ACAD9	Acyl-CoA dehydrogenase family member 9, mitochondrial
ACADM	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial
ACADVL	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial
ACTA1	Actin, alfa 1, skeletal muscle
ACTA2	Actin, aortic smooth muscle
ACTC1	Actin, alpha cardiac muscle 1
ACTN2	Alpha-actinin-2
ACVRL1	Serine/threonine-protein kinase receptor R3
ADAMTSL4	ADAMTS-like protein 4
AGK	Acylglycerol kinase, mitochondrial
AGL	Glycogen debranching enzyme
AGPAT2	1-acyl-sn-glycerol-3-phosphate acyltransferase beta
AKAP9	A-kinase anchor protein 9
ALMS1	Alstrom syndrome protein 1
ANK2	Ankyrin 2
ANK3	Ankyrin-3
ANKRD1	Ankyrin repeat domain-containing protein 1
APOA5	Apolipoprotein A-V
APOB	Apolipoprotein B-100
APOC3	Apolipoprotein C-III
ATPAF2	ATP synthase mitochondrial F1 complex assembly factor 2
BAG3	BAG family molecular chaperone regulator 3
BMPR1B	Bone morphogenetic protein receptor type-1B
BMPR2	Bone morphogenetic protein receptor type II
BRAF	Serine/threonine-protein kinase B-raf
BSCL2	Seipin
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C
CACNA1D	Voltage-dependent L-type calcium channel subunit alpha-1D
CACNA2D1	Voltage-dependent calcium channel subunit alpha-2/delta-1
CACNB2	Voltage-dependent L-type calcium channel subunit beta-2
CALM1	Calmodulin
CALM2	Calmodulin
CALR3	Calreticulin 3
CAPN3	Calpain-3
CASQ2	Calsequestrin-2
CAV1	Caveolin-1
CAV3	Caveolin-3
CBL	E3 ubiquitin-protein ligase CBL
CBS	Cystathionine beta-synthase

CETP	Cholesteryl ester transfer protein
COL1A1	Collagen alpha-1(I) chain
COL1A2	Collagen alpha-2(I) chain
COL3A1	Collagen alpha-1(III) chain
COL5A1	Collagen alpha-1(V) chain
COL5A2	Collagen alpha-2(V) chain
COQ2	4-hydroxybenzoate polyprenyltransferase, mitochondrial
COX15	Cytochrome c oxidase assembly protein COX15 homolog
COX6B1	Cytochrome c oxidase subunit 6B1
CRELD1	Cysteine-rich with EGF-like domain protein 1
CRYAB	Alpha-crystallin B chain
CSRP3	Cysteine and glycine-rich protein 3
CTF1	Cardiotrophin 1
CTNNA3	Catenin alpha-3
DES	Desmin
DLD	Dihydrolipoyl dehydrogenase, mitochondrial
DMD	Dystrophin
DNAJC19	Mitochondrial import inner membrane translocase subunit TIM14
DOLK	Dolichol kinase
DSC2	Desmocollin 2
DSG2	Desmoglein 2
DSP	Desmoplakin
DTNA	Dystrobrevin alpha
ELN	Elastin
EMD	Emerin
ENG	Endoglin
EYA4	Eyes absent homolog 4
FAH	Fumarylacetoacetase
FBN1	Fibrillin 1
FBN2	Fibrillin 2
FHL1	Four and a half LIM domains protein 1
FHL2	Four and a half LIM domains 2
FHOD3	FH1/FH2 domain-containing protein 3
FKRP	Fukutin-related protein
FKTN	Fukutin
FLNA	Filamin-A
FLNC	Filamin-C
FOXD4	Forkhead box protein D4
GAA	Lysosomal alpha-glucosidase
GATA4	Transcription factor GATA-4
GATA6	Transcription factor GATA-6
GATAD1	GATA zinc finger domain-containing protein 1
GDF2	Growth/differentiation factor 2
GFM1	Elongation factor G, mitochondrial
GJA1	Gap junction alpha-1 protein

GJA5	Gap junction alpha-5 protein
GLA	Alpha-galactosidase A
GLB1	Beta-galactosidase
GNPTAB	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta
GPD1L	Glycerol-3-phospate dehydrogenase 1-like protein
GUSB	Beta-glucuronidase
HCN4	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4
HFE	Hereditary hemochromatosis protein
HRAS	GTPase HRas
JAG1	Jagged-1
JPH2	Junctophilin 2
JUP	Junction plakoglobin
KCNA5	Potassium voltage-gated channel subfamily A member 5
KCND3	Potassium voltage-gated channel subfamily D member 3
KCNE1	Potassium voltage-gated channel subfamily E member 1
KCNE1L	Potassium voltage-gated channel subfamily E member 1-like protein
KCNE2	Potassium voltage-gated channel subfamily E member 2
KCNE3	Potassium voltage-gated channel subfamily E member 3
KCNH2	Potassium voltage-gated channel subfamily H member 2
KCNJ2	Inward rectifier potassium channel 2
KCNJ5	G protein-activated inward rectifier potassium channel 4
KCNJ8	ATP-sensitive inward rectifier potassium channel 8
KCNK3	Potassium channel subfamily K member 3
KCNQ1	Potassium voltage-gated channel subfamily KQT member 1
KLF10	Krueppel-like factor 10
KRAS	GTPase KRas
LAMA2	Laminin subunit alpha-2
LAMA4	Laminin subunit alpha-4
LAMP2	Lysosome-associated membrane glycoprotein 2
LDB3	LIM domain-binding protein 3
LDLR	Low density lipoprotein receptor
LIAS	Lipoyl synthase, mitochondrial
LMNA	Prelamin-A/C
LRP6	Low-density lipoprotein receptor-related protein 6
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1
MAP2K2	Dual specificity mitogen-activated protein kinase kinase 2
MIB1	E3 ubiquitin-protein ligase MIB1
MLYCD	Malonyl-CoA decarboxylase, mitochondrial
MRPL3	39S ribosomal protein L3, mitochondrial
MRPS22	28S ribosomal protein S22, mitochondrial
MTO1	Protein MTO1 homolog, mitochondrial
MURC	Muscle-related coiled-coil protein
MYBPC3	Myosin-binding protein C, cardiac-type
MYH11	Myosin-11
MYH6	Myosin-6

MYH7	Myosin-7
MYL2	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform
MYL3	Myosin light chain 3
MYLK	Myosin light chain kinase, smooth muscle
MYLK2	Myosin light chain kinase 2, skeletal/cardiac muscle
MYOT	Myotilin
MYOZ2	Myozenin 2
MYPN	Myopalladin
NEBL	Nebulette
NEXN	Nexilin
NKX2-5	Homeobox protein Nkx-2.5
NOTCH1	Neurogenic locus notch homolog protein 1
NOTCH3	Neurogenic locus notch homolog protein 3
NPPA	Atrial natriuretic factor
NRAS	GTPase NRas
OBSL1	Obscurin-like protein 1
PCSK9	Proprotein convertase subtilisin/kexin type 9
PDHA1	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial
PDLIM3	PDZ and LIM domain protein 3
PHKA1	Phosphorylase b kinase regulatory subunit alpha, skeletal muscle isoform
PITX2	Pituitary homeobox 2
PKP2	Plakophilin 2
PLN	Cardiac phospholamban
PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1
PMM2	Phosphomannomutase 2
PRDM16	PR domain zinc finger protein 16
PRKAG2	5'-AMP-activated protein kinase subunit gamma-2
PRKG1	cGMP-dependent protein kinase 1
PSEN1	Presenilin-1
PSEN2	Presenilin 2
PTPN11	Tyrosine-protein phosphatase non-receptor type 11
RAF1	RAF proto-oncogene serine/threonine-protein kinase
RANGRF	Ran guanine nucleotide release factor
RBM20	Probable RNA-binding protein 20
RYR2	Ryanodine receptor 2
SCN10A	Sodium channel protein type 10 subunit alpha
SCN1B	Sodium channel subunit beta-1
SCN2B	Sodium channel subunit beta-2
SCN3B	Sodium channel subunit beta-3
SCN4B	Sodium channel subunit beta-4
SCN5A	Sodium channel protein type 5 subunit alpha
SGCA	Alpha-sarcoglycan
SGCB	Beta-sarcoglycan
SGCD	Delta-sarcoglycan
SHOC2	Leucine-rich repeat protein SHOC-2

SKI	Ski oncogene
SLC22A5	Solute carrier family 22 member 5
SLC25A4	ADP/ATP translocase 1
SLC2A10	Solute carrier family 2, facilitated glucose transporter member 10
SLMAP	Sarcolemmal membrane-associated protein
SMAD1	Mothers against decapentaplegic homolog 1
SMAD3	Mothers against decapentaplegic homolog 3
SMAD4	Mothers against decapentaplegic homolog 4
SMAD9	Mothers against decapentaplegic homolog 9
SNTA1	Alpha-1-syntrophin
SOS1	Son of sevenless homolog 1
SPRED1	Sprouty-related, EVH1 domain-containing protein 1
SURF1	Surfeit locus protein 1
TAZ	Tafazzin
TBX1	T-box transcription factor TBX1
TBX20	T-box transcription factor TBX20
TBX5	T-box transcription factor TBX5
TCAP	Telethonin
TGFB2	Transforming growth factor beta-2
TGFB3	Transforming growth factor, beta 3
TGFBR1	TGF-beta receptor type-1
TGFBR2	TGF-beta receptor type-2
TMEM43	Transmembrane protein 43
TMEM70	Transmembrane protein 70, mitochondrial
TMPO	Thymopoietin
TNNC1	Troponin C, slow skeletal and cardiac muscles
TNNI3	Troponin I, cardiac muscle
TNNT2	Troponin T, cardiac muscle
TPM1	Tropomyosin alpha-1 chain
TRDN	Triadin
TRIM63	E3 ubiquitin-protein ligase TRIM63
TRPM4	Transient receptor potential cation channel subfamily M member 4
TSFM	Elongation factor Ts, mitochondria
TTN	Titin
TTR	Transthyretin
TXNRD2	Thioredoxin reductase 2, mitochondrial
VCL	Vinculin

Table S3. List of mutations identified in the *FLNC* gene with their predicted functional effect on the protein and their frequency in different public databases.

Exon/ Intron	Chr DNA level name	c.DNA	Protein	Predicted functional effect	1KG	EVS	ExAC	Previously reported
Ex1	Chr7:128470932delC	c.241delC	p.Arg81Alafs*15	Introduces a premature stop codon at amino acid 95, after 14 novel residues. Truncation in the CH1 domain at the ABD.	-	-	-	-
Ex1	Chr7:128470940C>G	c.249C>G	p.Tyr83*	Truncation in the CH1 domain at the ABD.	-	-	-	-
Ex2	Chr7:128475608_128475626delTGGTGGACAACTGCGCCCC	c.581_599delTGGTGGACAACTGCGCCCC	p.Leu194Profs*52	Truncation in the CH2 domain at the ABD.	-	-	-	-
Ex3-6	Chr7:128476498_128478081delinsTGCCCCGGGAGGGGTGCCTCAGTCTCCCTGTCCCTCTG	c.602-716_1010delinsTGCCCCGGGAGGGGTGCCTCAGTCTCCCTGTCCCTCTG	p.Gly201Valfs*36	Complete deletion of exons 3, 4, 5, and part of 6. Truncation in the CH2 domain. Eliminates the splicing acceptor of exon 3. Possible skipping of exon 3 with a loss of amino acids 201-233 (CH2 domain at the ABD), frame shift at this point, addition of 3 new amino acids, and stop codon at position (c.711). Implies the loss of the last 2524 amino acids.	-	-	-	-
Ex11	Chr7:128480925C>T	c.1714C>T	p.Gln572*	Truncation in the Ig-repeat 4.	-	-	-	-
Ex14	Chr7:128482371delT	c.2208delT	p.Lys737Serfs*11	Truncation in the Ig-repeat 5.	-	-	-	-
Ex19	Chr7: 128483926delC	c.2888delC	p.Pro963Argfs*26	Truncation in the Ig-repeat 8.	-	-	1/120492	-
Ex21	Chr7:128484921_128484922dupGCGAGTACACCATCAACATCCTG	c.3402_3403dupGCGAGTACACCATCAACATCCTG	p.Phe1135Alafs*62	Truncation in the Ig-repeat 10.	-	-	-	-
Int21	Chr7:128486043G>A	c.3791-1G>A	-	Eliminates the splicing acceptor of exon 22. Possible skipping of exon 22 with a loss of amino acids 1265-1322 (Ig-repeat 11).	-	-	-	-
Int21	Chr7:128486043G>C	c.3791-1G>C (2)	-	Eliminates the splicing acceptor of exon 22. Possible skipping of exon 22 with a loss of amino acids 1265-1322 (Ig-repeat 11).	-	-	1/118498	Two cases with dilated cardiomyopathy (1,2)
Int22	Chr7:128486353A>T	c.3965-2A>T (2)	-	Eliminates the splicing acceptor of exon 23. Possible skipping of exon 23 with a loss of amino acids 1322-1376) (Ig-repeat 11-12), frame shift at this point, addition of 8 new amino acids, and stop codon at position 1330 (c.4154). Implies the loss of the last 1403 amino acids.	-	-	-	-
Ex23	Chr7:128486496_128486497dupA	c.4106_4107dupA	p.Asn1369Lysfs*36	Truncation in the Ig-repeat 12.	-	-	-	-
Int23	Chr7:128486518delG	c.4127+1delG (2)	-	Abolishes the splicing donor of exon 23. Two alternatives: 1) A possible loss of amino acid 1376 (Ig-repeat 12), frame shift at this point, addition of 6 new amino acids, and stop codon at position (c.4127+18). Implies the loss of the last 1350 amino acids. 2) Possible skipping of exon 23 (Ig-repeat 12), frame	-	-	-	-

				shift at this point, addition of 9 new amino acids, and stop codon at position 1386 (c.4154). Implies the loss of the last 1401 amino acids.				
Int26	Chr7:128488123G>T	c.4580+1G>T	-	Eliminates the splicing donor of exon 26. Possible skipping of exon 26 with a loss of amino acids 1486-1527 (Ig-repeat 13), frame shift at this point, addition of 80 new amino acids, and stop codon at position 1566 (c.4817). Implies the loss of the last 1239 amino acids.	-	-	-	-
Int28	Chr7:128489037delG	c.4927+1delG	-	Abolishes the splicing donor of exon 28. Two alternatives: 1) A possible loss of amino acid 1643 (Ig-repeat 15), frame shift at this point, addition of 53 new amino acids, and stop codon at position (c.4928-38). Implies the loss of the last 1083 amino acids. 2) Possible skipping of exon 28, (Ig-repeat 15), frame shift at this point, addition of 22 new amino acids, and stop codon at position 1665 (c.4994). Implies the loss of the last 1145 amino acids.	-	-	-	-
Ex32	Chr7:128490537G>T	c.5398G>T (2)	p.Gly1800*	Truncation in the Ig-repeat 16.	-	-	-	-
Int33	Chr7:128490998G>C	c.5539+1G>C	-	Abolishes the splicing donor of exon 33. Possible skipping of exon 33 with a loss of amino acids 1800-1846 (Ig-repeat 16).	-	-	-	-
Ex37	Chr7:128493085G>A	c.6208G>A	p.Gly2070Ser	The splicing is likely to be altered (as predicted by 3 software tools) but the consequences are not predictable.	-	-	-	-
Ex38	Chr7:128493545delT	c.6231delT	p.Ser2077Argfs*50	Truncation in the Ig-repeat 19.	-	-	-	-
Ex38	Chr7:128493554_128493573delCCCAAGCAAGGTGGACATCA	c.6240_6259delCCCAAGCAAGGTGGACATCA	p.Pro2081Leufs*2	Truncation in the Ig-repeat 19.				-
Ex41	Chr7:128494715C>T	c.6976C>T (2)	p.Arg2326*	Truncation in the Ig-repeat 21.	-	-	1/111082	-
Int43	Chr7:128495369G>A	c.7251+1G>A	-	Abolishes the splicing donor of exon 43. Possible skipping of exon 43 (Ig-repeat 21 and 22), frame shift at this point, addition of 23 new amino acids, and stop codon at position 2440 (c.7320). Implies the loss of the last 346 amino acids.	-	-	-	Two families with dilated cardiomyopathy (3)
Ex48	Chr7:128498506delG	c.8107delG	p.Asp2703Thrfs*69	Replacement of 23 amino acids at the C-terminal tail (dimerization domain) and extension of 45 amino acids.	-	-	-	-

ABD= actin binding domain. Ig-repeat= immunoglobulin-like repeat. CH1= calponin homology domain 1. CH2= calponin homology domain 2. (2)= mutations identified in two unrelated families. Reference sequence for names at chromosomal DNA level: NC_000007.13; cDNA level: NM_001458.4; protein level: NP_001449.3. 1KG= 1000 Genomes Project. EVS= Exome Variant Server. ExAC= Exome Aggregation Consortium. Frequency is expressed Nº of alternative alleles/Total Nº of alleles.

Table S4: Detailed clinical information on *FLNC* mutation carriers.

Family	Ind	Mutation	Age Dx	Age LFU	Sex	FH SD	Clinical press	SM	Pheno	LV DD	LV EF	MWT	RV affect	CK	Rhythm	CCD	Low Volt	Neg Tw	Ter QRS>55	Vent arrhyt	EPS	LGE on MRI	LV NC	Pathology	Events	Age at event	ICD	Comments
25767	III:1	Ser2077Argfs*50	48	52	M	+	D	-	DCM	83	27	normal	+	N	S	LBBB	-	n/a	n/a	n/a	n/a	n/a	-	No fibrosis; myocyte degeneration; RV linphocytic infiltration	HTx	52		
25767	III:2	Ser2077Argfs*50	59	59	M		P, AF	-	DCM	67	35	10	-	n/a	S	-	-	V4-V6	+	n/a	n/a	n/a	-	n/a	n			
25767	IV:1	Ser2077Argfs*50		33	F		n	-	Healthy	42	65	9	-	n/a	S	-	-	-	-	n/a	n/a	n/a	-	n/a	n			
25767	IV:3	Ser2077Argfs*50		30	F		n	-	Healthy	44	65	6	-	n/a	S	-	-	-	-	n/a	n/a	n/a	-	n/a	n			
26958	II:1	Tyr83*	17	17	M	+	SD	-	LDACM	n/a	n/a	normal	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	LV subepicardial fibrosis	SD	17		SD after a soccer match.
26958	I:1	Tyr83*	49	49	M		n	-	Affected?	53	60	12	+	n/a	S	-	-	-	-	-	n/a	n	-	n/a	n			Mild dyskinetic zone/saculation on anterior RV wall on cardiac-MRI.
27103	III:1	c.3791-1G>C	45	52	F	+	n	-	DCM	58	35	8	-	n/a	S	-	-	-	-	FVE, NSVT, SVT	n/a	n/a	-	n/a	aICDs	52	2º	SVT on effort on routine effort test. ICD discharge: VT on exercise. Positive delay potencials on SAECG.
27103	IV:1	c.3791-1G>C		26	M		n	-	Healthy	normal	normal	normal	-	n/a	S	-	-	-	-	-	n/a	n	-	n/a	n			Normal SAECG.

27348	II:3	Asn1369Lysfs*36	20	60	M	+	SVT	-	DCM	82	32	13	+	N	AF	-	+	V4-V6	+	SVT	n/a	n/a	-	LV endomyocardial fibroelastosis	aICDs; HTx	21; 60	2º	SVT requiring shocks (20,21,34 yo). Urgent HTx; severe RV systolic dysfunction.
27348	III:2	Asn1369Lysfs*36	34	38	F		FVE	-	Affected?	45	64	10	-	n/a	S	-	-	-	-	FVE	n/a	n/a	-	n/a	n			
29544	III:2	Pro963Argfs*26	0.25	1	F	-	D	-	DCM	40	22	normal	-	N	S	-	-	-	-	n/a	n/a	n/a	-	LV myocardial fibrosis and endomyocardial fibroelastosis	HTx	1		
29544	II:1	Pro963Argfs*26	40	40	F		n	-	Affected?	dilated	47	10	-	n/a	S	-	+	-	-	-	n/a	n	+	n/a	n			Mild LV dilatation and systolic dysfunction.
29544	III:1	Pro963Argfs*26	9	9	F		n	-	Affected?	46	69	6	-	n/a	S	-	-	-	-	n/a	n/a	n/a	+	n/a	n			Bicuspid aortic valve with mild insufficiency.
29876	III:3	c.4127+1delG	19	25	M	+	S, P	-	LDACM	61	54	11	-	N	S	-	-	-	-	FVE, SVT	n/a	LV (subepicardial)	+	LV fibrosis	aICDs; SD	25	2º	SVT playing football, ICD implant, death on electric storm. Palmoplantar keratosis.
29876	II:5	c.4127+1delG	43	57	M		S, P	-	DCM	60	50	12	-	N	S	-	-	-	-	SVT	SVT	n/a	-	n/a	aICDs	51	2º	Syncope playing football secondary to SVT. Several ICD interventions due to SVT. Palmoplantar keratosis.
29876	II:3	c.4127+1delG	60	60	F		n	-	LDACM	51	67	12	-	n/a	S	-	-	-	-	FVE	n/a	LV (intramyocardial)	-	n/a	n			

29876	II:4	c.4127+1delG	35	59	M		D, S	-	DCM	64	43	11	-	n/a	AF	LAFB	-	-	-	FVE, NSVT	n/a	n	-	n/a	n			Palmoplantar keratosis.
29876	II:7	c.4127+1delG	54	56	M		n	-	LDACM	normal	mild	normal	-	n/a	S	-	-	n/a	n/a	FVE, NSVT	n/a	LV (subepicardial)	-	n/a	n			Palmoplantar keratosis.
29876	III:5	c.4127+1delG	21	21	M		n	-	LDACM	51	52	10	-	n/a	S	-	-	-	-	-	n/a	LV (intramyocardial)	-	n/a	n			
31035	IV:1	c.7251+1G>A	26	33	F	+	NSVT	-	DCM	66	23	6	-	N	S	-	+	V3-V6	+	NSVT	-	n/a	-	n/a	n		1º	
31035	III:2	c.7251+1G>A	38	61	F		n	-	DCM	64	33	10	-	n/a	S	-	+	V1-V5	+	n/a	n/a	n/a	-	n/a	n		1º	
31035	IV:4	c.7251+1G>A		39	F		n	-	Healthy	52	68	6	-	n/a	S	-	-	-	-	-	n/a	n	-	n/a	n			
31035	III:5	c.7251+1G>A	58	63	F		n	-	DCM	66	35	8	-	n/a	S	-	-	V1-V6	+	FVE	n/a	n/a	-	n/a	n		1º	
31035	IV:6	c.7251+1G>A	39	39	F		n	-	Affected?	46	54	8	-	n/a	S	-	-	-	-	-	n/a	n/a	-	n/a	n			
31035	IV:7	c.7251+1G>A	40	40	F		SD	n/a	LDACM	n/a	n/a	normal	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	LV concentric subepicardial fibrosis	SD	40		
31277	II:2	c.4927+1 delG	29	55	F	-	D	+	RCM	32	66	14	-	Mild	AF	1ºAVB	-	V5-V6	-	n/a	n/a	n/a	-	RV fibrosis (endomyocardial biopsy)	HTx	45		
32406	III:2	Gln572*	20	22	M	+	P	-	LDACM	68	37	normal	+	n/a	S	-	+	V5-V6	-	FVE	n/a	LV (subepicardial and intramyocardial)	-	LV concentric subepicardial fibrosis	SD	22	1º (ind)	SD playing football (waiting ICD implant).
33319	II:1	c.4127+1delG	53	63	M	-	D	-	DCM	dilated	15	n/a	+	N	AF	-	n/a	n/a	n/a	n/a	n/a	LV (transmural)	n/a	n/a	n			
33319	III:2	c.4127+1delG	30	31	M		CP	-	LDACM	normal	57	normal	-	mild	S	-	-	-	-	n/a	n/a	LV (intramyocardial)	n/a	n/a	n			
33319	III:1	c.4127+1delG		32	F		n	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n			
33541	II:3	c.5539+1G>C	60	68	M	+	D, CP, FVE	-	DCM	60	40	11	-	N	AF	LAFB	-	Inf, V4-V6	-	FVE, NSVT	n/a	LV (subepicardial and intramyocardial)	-	n/a	n		1º	
33541	II:5	c.5539+1G>C	55	63	M		SD	-	DCM	dilated	45	11	-	N	S	-	+	-	-	SVT	n/a	LV (subepicardial and intramyocardial)	-	n/a	SD; aICDs	55	2º	Multiple appropriate ICD shocks due to SVT/VF.

33541	II:6	c.5539+1G>C	59	60	F		S, D, P	-	Affected?	50	55	9	-	N	S	-	-	-	-	FVE	n/a	LV (intramyocardial)	-	n/a	n			
33541	III:1	c.5539+1G>C		39	F		n	-	Healthy	38	60	8	-	N	S	-	-	-	-	-	n/a	n	-	n/a	n			
33675	II:1	Gly1800*	55	59	M	-	P	-	LDACM	59	45	9	+	X2	S	-	-	V3-V6	-	FVE, NSVT	n/a	LV (subepicardial)	+	n/a	n		1º	Positive delay potencials on SAECG.
33675	II:2	Gly1800*	60	63	M		n	-	Affected?	37	67	14	-	N	S	-	-	-	-	n/a	n/a	n	+	n/a	n			HTN.
36107	II:9	Arg2326*	54	59	F	+	D	-	DCM	dilated	20	normal	+	N	S	LBBB	+	n/a	n/a	FVE, NSVT	n/a	LV (subepicardial)	-	n/a	HTx	57		Emergent HTx.
36107	II:4	Arg2326* (o)	52	58	F		D	n/a	DCM	dilated	25	normal	-	n/a	S	-	+	V1-V4	-	NSVT	n/a	LV basal septum	-	n/a	SD	58		Listed for HTx prior to SD.
36107	II:6	Arg2326*	57	59	F		S, P	-	Affected?	54	56	8	-	N	S	-	+	Inf, V1-V6	+	FVE	-	n	-	n/a	n			
36107	III:7	Arg2326*		33	M		n	-	Healthy	54	60	8	-	N	S	-	-	-	-	n/a	n/a	n	-	n/a	n			
36107	III:2	Arg2326*	33	34	M		S, P	-	DCM	60	46	11	+	N	S	-	-	-	+	FVE	n/a	LV (subepicardial)	-	n/a	aICDs	33	1º	Multiple SVT treated by the ICD.
36107	III:3	Arg2326*	33	38	F		D	-	DCM	57	35	12	-	N	S	-	-	-	-	FVE, NSVT	n/a	LV (intramyocardic)	-	n/a	n		1º (ind)	Declined ICD.
36203	III:1	Gly1800*	38	43	M	+	P	-	LDACM	dilated	50	10	-	n/a	S	-	-	Inf, V6	-	FVE, NSVT	n/a	LV (subepicardial and intramyocardial)	-	n/a	n		1º	Abnormal Q wave DIII and aVF.
36203	III:3	Gly1800*	28	38	M		P	-	LDACM	54	51	11	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	LV fibrosis posterior wall	SD	38		
36203	II:1	Gly1800*	71	74	M		S, CP	-	LDACM	46	41	11	-	N	S	-	-	Inf	n/a	FVE	n/a	LV (subepicardial and intramyocardial)	-	n/a	n		1º	Abnormal Q wave in DIII.
36203	IV:2	Gly1800*		6	F		n	-	Healthy	28	70	6	-	n/a	S	-	-	V1-V3 (6 years)	-	n/a	n/a	n/a	-	n/a	n			
37286	IV:2	Asp2703Thrfs*69	24	24	F	+	Stroke	-	DCM	63	26	7	-	N	S	-	+	Inf, V1-V3	-	NSVT	n/a	n	+	n/a	Stroke	24		

37286	III:1	Asp2703Thrfs*69	43	59	M		S	-	DCM	70	40	7	n/a	N	AF	-	-	Inf	-	SVT	-	n/a	n/a	n/a	aICDs	51	1º	2 ICD shocks on VF.
37296	II:2	Arg2326*	34	43	F	+	D	-	DCM	70	35	11	-	N	PM	-	+	Inf, V3-V6	n/a	FVE, NSVT	VF	n/a	-	n/a	aICDs	38	1º	Heart failure during pregnancy. Diastolic restrictive filling pattern. VF induction on EPS. 3 appropriate ICD shocks due to SVT (38, 39, 40 yo) and 1 due to VF (38 yo).
37302	I:1	Gly2070Ser	50	68	M	+	D	-	DCM	78	21	normal	+	n/a	S	1ºAVB	-	n/a	n/a	NSVT	n/a	n/a	-	n/a	SD	68		Heart failure at presentation; sudden death in worsening heart failure
37302	II:1	Gly2070Ser	24	34	M		n/a	n/a	Affected?	n/a	normal	normal	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n			Perimyocardytis .
37302	II:2	Gly2070Ser	24	32	M		n/a	n/a	Affected?	n/a	normal	normal	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n			Perimyocardytis.
41721	II:2	c.3791-1G>A	42	61	F	+	P	-	DCM	56	43	9	-	N	S	LAFB+ RBBB	-	-	-	-	n/a	n	-	n/a	n			
41721	II:4	c.3791-1G>A	44	52	M		n	-	DCM	65	45	10	-	N	S	-	-	-	-	NSVT	-	n	-	n/a	n			NSVT during effort test.
41721	II:7	c.3791-1G>A (o)			F		n/a	n/a	DCM	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	SD	?		
41721	III:1	c.3791-1G>A	20	34	M		n	-	DCM	64	49	10	-	N	S	-	-	-	-	FVE, NSVT	n/a	n/a	-	n/a	n			Positive delay potencials on SAECG.
41721	III:5	c.3791-1G>A	27	33	M		n	-	DCM	57	34	11	-	N	S	-	+	V3-V6	-	FVE, NSVT	n/a	n	-	n/a	SD	33		
47263	III:2	Phe1135Alafs*62	53	55	F	+	n	-	DCM	56	20	12	-	N	S	-	-	-	-	FVE, NSVT	n/a	n/a	-	n/a	n		1º	CRT-D
47253	IV:3	Phe1135Alafs*62		24	M		n	-	Healthy	54	68	8	-	n/a	S	-	-	-	-	n/a	n/a	n/a	-	n/a	n			
47263	IV:1	Phe1135Alafs*62		30	F		n	-	Healthy	50	73	9	-	n/a	S	-	-	-	-	n/a	n/a	n/a	-	n/a	n			

47263	IV:2	Phe1135Alafs*62	28	29	M		n	-	Affected?	60	53	11	-	n/a	S	-	-	-	+	n/a	n/a	n	-	n/a	n			Abnormal Q wave DIII.
48028	III:1	Lys737Serfs*11	58	62	F	+	D, FVE, NSVT	-	DCM	49	35	9	-	N	S	-	-	-	+	FVE, NSVT	n/a	LV (intramyocardial)	-	n/a	n			
48028	III:2	Lys737Serfs*11	44	60	F		D, P	-	DCM	57	40	8	+	n/a	S	-	-	-	+	FVE, NSVT	n/a	n/a	-	RV fibrosis (endomyocardial biopsy)	n			
48028	IV:1	Lys737Serfs*11		31	F		n	-	Healthy	46	60	7	-	n/a	S	-	-	-	-	n/a	n/a	n/a	-	n/a	n			
48102	II:1	c.3791-1G>C	44	50	M	+	S	-	DCM?	52	50	11	-	n/a	S	-	+	Inf	-	NSVT	n/a	n/a	-	n/a	SD	44	2º	NSVT on effort test and posterior akinesis on echo before an abborted SD.
48102	II:2	c.3791-1G>C	48	48	F		FVE	-	Affected?	normal	57	normal	+	n/a	S	-	+	-	-	FVE	NSVT	n	-	n/a	n			Mild regional RV anterior wall hypokinesis with normal RV size.
48102	I:1	c.3791-1G>C		78	M		n	-	Unknown	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n			CABG. Known to have "arrhythmias" (reported as extrasystoles) after surgery, treated with sotalol.
48924	IV:4	p.Leu194Profs*52	21	21	M	+	P	-	DCM	60	38	8	-	N	S	-	-	Inf, V4-V6	-	FVE, NSVT	n/a	n	-	n/a	n		1º	
48924	III:2	p.Leu194Profs*52	60	60	M		SD	-	DCM	n/a	n/a	n/a	+	n/a	S	-	+	-	-	n/a	n/a	n/a	-	Bi-ventricular dilatation (fibrosis not evaluated)	SD	60		
48924	III:1	p.Leu194Profs*52	51	51	F		n	-	Affected?	51	49	10	-	N	S	-	-	-	-	-	n/a	n	-	n/a	n			
48924	IV:1	p.Leu194Profs*52		18	M		n	-	Healthy	49	60	10	-	n/a	S	-	-	-	-	-	n/a	n	-	n/a	n			Myocarditis aged 13.
49537	I:1	c.3965-2A>T	55	66	M	-	D, CP, SVT	-	DCM	81	15	8	+	N	S	LBBB	-	n/a	n/a	FVE, NSVT, SVT	n/a	n/a	-	n/a	aICDs	62	2º	

49537	II:1	c.3965-2A>T	36	41	M		P, SVT	-	DCM	52	42	11	-	n/a	S	-	-	-	-	SVT	n/a	n/a	-	n/a	n		2º	
49537	III:3	c.3965-2A>T	34	38	M		P	-	DCM	61	45	10	-	N	S	-	-	-	-	FVE	n/a	LV (intramyocardial)	+	n/a	n		1º	
48888	IV:1	Pro2081Leufs*2	33	34	M	+	n	-	DCM	66	40	11	-	n/a	S	-	-	-	-	FVE, NSVT	n/a	LV (subepicardial and intramyocardial)	-	n/a	n		1º	
48888	IV:2	Pro2081Leufs*2	36	36	M		n	-	DCM	58	56	9	-	n/a	S	-	-	-	-	FVE	n/a	LV (subepicardial and intramyocardial)	-	n/a	n			Positive delay potencials on SAECG.
48956		c.4580+1G>T	53	75	M	-	S, CP, SVT	-	LDACM	49	47	14	-	N	S	-	+	V4-V6	+	FVE, NSVT, SVT	n/a	LV (transmural)	-	n/a	n		2º	
48868		Gly201Valfs*36	47	48	M	+	D, CP, NSVT	-	DCM	71	36	11	+	N	S	LBBB	n/a	n/a	n/a	FVE, NSVT	n/a	n	-	n/a	Stroke	47		ECG not informative due to LBBB.
49818		c.3965-2A>T	62	64	M	+	D	-	DCM	54	18	13	+	N	S	-	+	Inf	+	FVE, NSVT, SVT	n/a	n/a	-	n/a	n			LVEF recovered to 40% after medical treatment.
51118	II:1	Arg81Alafs*15	48	49	F	-	S	-	LDACM	57	40	8	-	N	S	-	-	-	-	NSVT	NSVT	LV (subepicardial and intramyocardial)	-	n/a	n		1º	
51118	II:2	Arg81Alafs*15	48	49	F		CP	-	LDACM	52	45	10	-	N	S	-	+	Inf, V4-V6, DI, aVL	-	FVE, NSVT	n/a	LV (subepicardial and intramyocardial)	-	n/a	n			
51118	I:2	Arg81Alafs*15		72	F		n	-	Healthy	48	60	10	-	N	S	-	-	-	-	n/a	n/a	n/a	-	n/a	n			

Grey shaded files indicate probands. Fam= familiy number. Ind= individual identification according to position in the pedigree. (o)= obligate carrier of the mutation. Age Dx= age at diagnosis. Age LFU= age at last follow-up. M= male; F= female. FHSD= family history of sudden death. Clinical press= signs or symptoms at clinical presentation (D= dyspnea; S= syncope; P= palpitations; AF= atrial fibrillation; NSVT= non-sustained ventricular tachycardia SVT= sustained ventricular tachycardia; FVE= frequent ventricular ectopics; SD= sudden death; CP= chest pain). SM= presence of clinical signs of skeletal myopathy. Pheno= phenotype (DCM= dilated cardiomyopathy; LDAC= left-dominant arrhythmogenic cardiomyopathy; RCM= restrictive cardiomyopathy; affected?= individual possibly affected but not fulfilling diagnosis of a specific cardiomyopathy). LVDD= left ventricular diastole diameter. LVEF= left ventricular ejection fraction (mild= mild depression). MLVWT= maximal left ventricular wall

thickness. RV affect= right ventricular affection (akinesia, dyskinesia, aneurysm, dilatation, systolic dysfunction). CK= plasma levels of creatine-kinase (N= normal value; mild= mild elevation). Rhythm= cardiac rhythm (S= sinus rhythm; AF= atrial fibrillation; PM= pace-maker). CCD= cardiac conduction defects (1ºAVB= first-degree AV block; LBBB= left bundle branch block; RBBB= right bundle branch block; LAFB= left anterior fascicular block). Low volt= low QRS voltage amplitude on limb leads. Neg Tw= ECG leads with abnormal negative T waves (inf= DII, DIII, aVF). Vent arrhyt= evidence of ventricular arrhythmias (SVT= sustained ventricular tachycardia; NSVT= non-sustained ventricular tachycardia; FVE= frequent ventricular ectopics). EPS= result of electrical endocavitary stimulation. LGE on MRI= presence and localization of late gadolinium enhancement on cardiac magnetic resonance images. LVNC= signs of myocardial non-compaction of the left ventricular wall (not necessarily fulfilling diagnosis of left ventricular non-compaction cardiomyopathy). Pathology= findings in the pathological evaluation of cardiac necropsy/explanted heart/endomyocardial biopsy. Events= cardiovascular events (SD= sudden death; HTx= heart transplant; aICDs= appropriate ICD shock. ICD= implantable cardiac defibrillator indication (1º= primary prevention; 2º= secondary prevention; ind= indicated but not implanted. + = positive finding. - = negative finding. n/a= not available data. LV= left ventricle. RV= right ventricle. VF= ventricular fibrillation. SAECG= signal average ECG. CRT-D= cardiac resynchronization therapy pacemaker with defibrillation therapy. CABG= coronary artery by-pass graft.

Table S5: Logarithm (base ten) of odds (LOD) scores showing cosegregation of truncating mutations in FLNC with the cardiac phenotype in individual families and combined.

Family ID	Mutation	LOD-score (penetrance 99%)
51118	Arg81Alafs*15	0.1758
26958	Tyr83*	0
48924	p.Leu194Profs*52	0.0786
48868	Gly201Valfs*36	n/a
32406	Gln572*	0.095
48028	Lys737Serfs*11	0.0783
29544	Pro963Argfs*26	0.5977
47263	Phe1135Alafs*62	0.0787
41721	c.3791-1G>A	0.3268
27103	c.3791-1G>C	0
48102	c.3791-1G>C	0.2984
49537	c.3965-2A>T	0.3942
49818	c.3965-2A>T	n/a
27348	Asn1369Lysfs*36	0.6364
29876	c.4127+1delG	1.6253
33319	c.4127+1delG	0.9117
48956	c.4580+1G>T	n/a
31277	c.4927+1 delG	n/a
33675	Gly1800*	0.2981
36203	Gly1800*	0.1753
33541	c.5539+1G>C	0.8491
37302	Gly2070Ser	0
25767	Ser2077Argfs*50	0.0789
48888	Pro2081Leufs*2	0.0781
36107	Arg2326*	1.3955
37296	Arg2326*	n/a
31035	c.7251+1G>A	1.3671
37286	Asp2703Thrfs*69	0
	Total	**9.539**

LOD score for each family calculated by means of Superlink-Online SNP tool with the following settings: disease mutant gene frequency= 0.001, dominant mode of inheritance, penetrance= 99%, θ=0. Individuals ≤40 years without clear clinical affection were consider "unknown phenotype". n/a= not available family information.

Supplementary Appendix figure legends

Figure S1: Circles, females. Squares, males. Arrows indicate the proband. Clinical status defined by cardiac evaluations or clinical records: black, affected; N, unaffected; vertical bar, possibly affected; slash, deceased; ?, unknown. Genotypes are indicated: +, FLNC mutation present; 0, obligate carrier; -, FLNC mutation absent. Ages below some individuals indicate the age of sudden death (SD), diagnosis of the phenotype (DCM, dilated cardiomyopathy), or last clinical follow-up in healthy carriers. yo= years old. HTx= heart transplant. PP= palmo-plantar keratoderma. SVT= sustained ventricular tachycardia. HFD= heart failure death. PM= pacemaker.

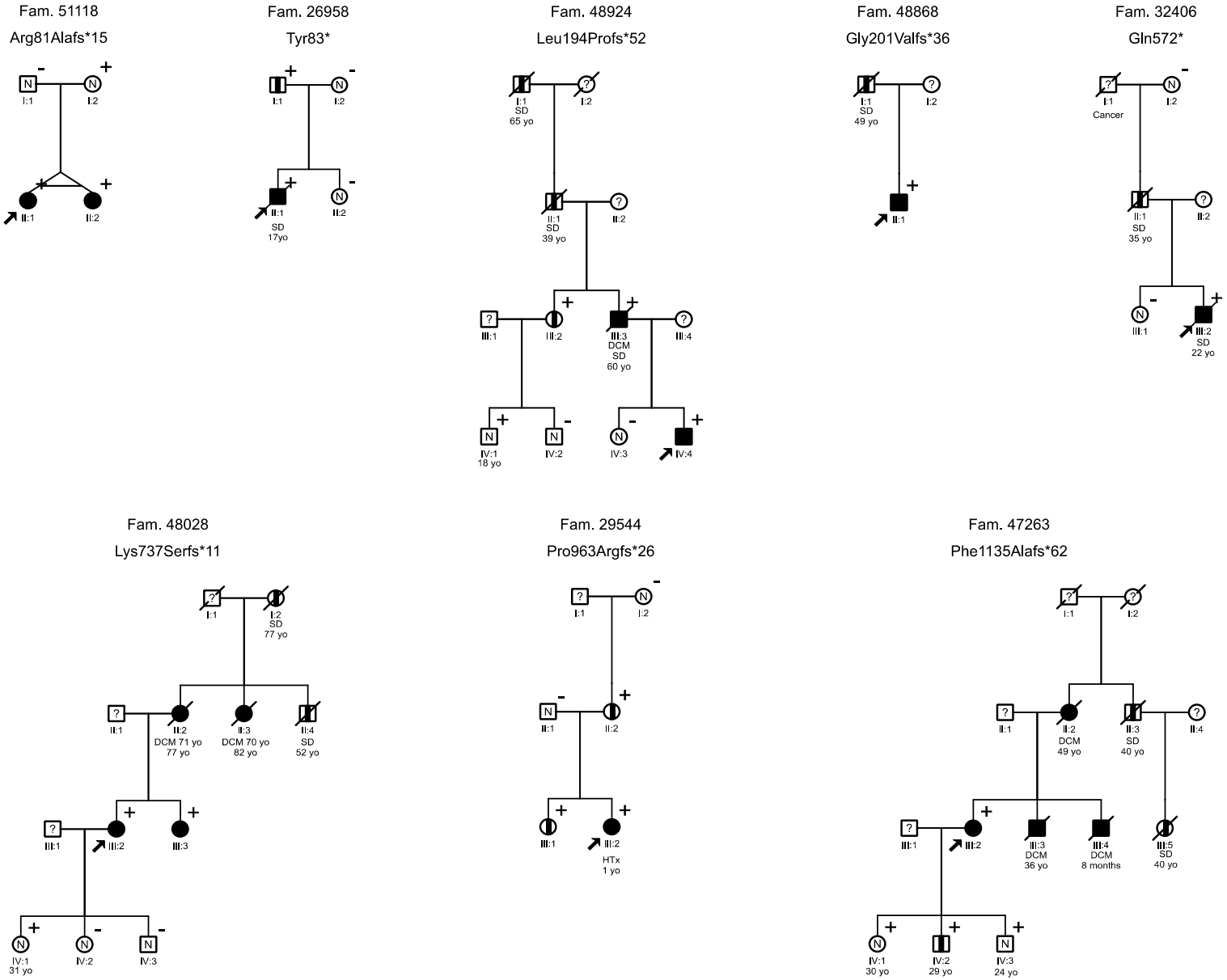
Figure S2: ECG from carrier III:2 of family 31035 (Panel A). ECG from carrier II:1 of family 37296 (proband) (Panel B). ECG from carrier III:1 (proband) of family 36203 (Panel C). ECG from carrier II:6 of family 36107 (Panel D).

Figure S4: Proband III:3 of family 29876. Presented with syncope due to sustained ventricular tachycardia while playing soccer. A cardiac defibrillator was implanted as secondary prevention. Died during a ventricular arrhythmic storm a few days later. Picture shows 10/12 failed appropriate defibrillator shock.

Supplementary Appendix references

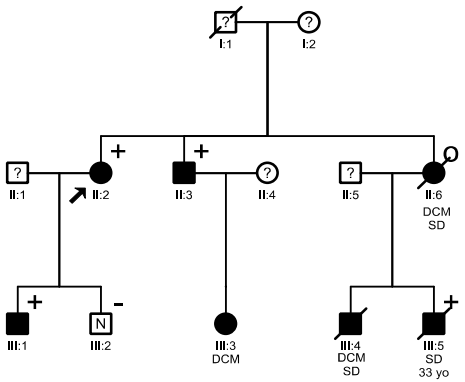
1. Golbus JR, Puckelwartz MJ, Dellefave-Castillo L, et al. Targeted analysis of whole genome sequence data to diagnose genetic cardiomyopathy. Circ Cardiovasc Genet 2014;7:751–9.
2. Deo RC, Musso G, Tasan M, et al. Prioritizing causal disease genes using unbiased genomic features. Genome Biol 2014;15:3274.
3. Begay RL, Tharp CA, Martin A, et al. FLNC Gene Splice Mutations Cause Dilated Cardiomyopathy. J Am Coll Cardiol Basic Trans Science 2016. DOI: 10.1016/j.jacbts.2016.05.004.

Figure S1: Pedigrees of families with truncating mutations in *FLNC*.



Fam. 41721

c.3791-1G>A



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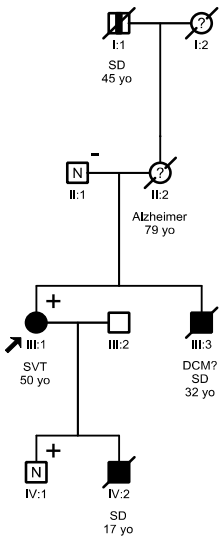
c.3791-1G>C



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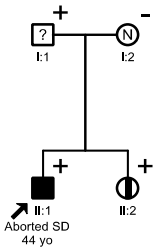
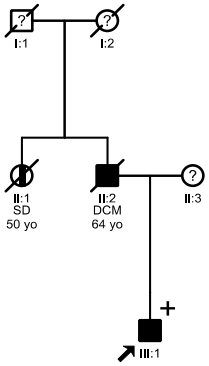
c.3791-1G>C



Fam. 49818

c.3965-2A>T



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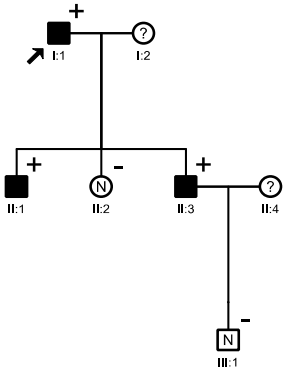
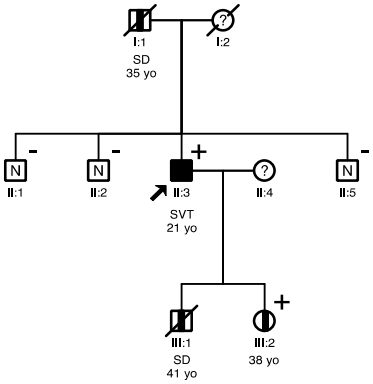
c.3965-2A>T



Fam. 27348

Asn1369Lysfs*36



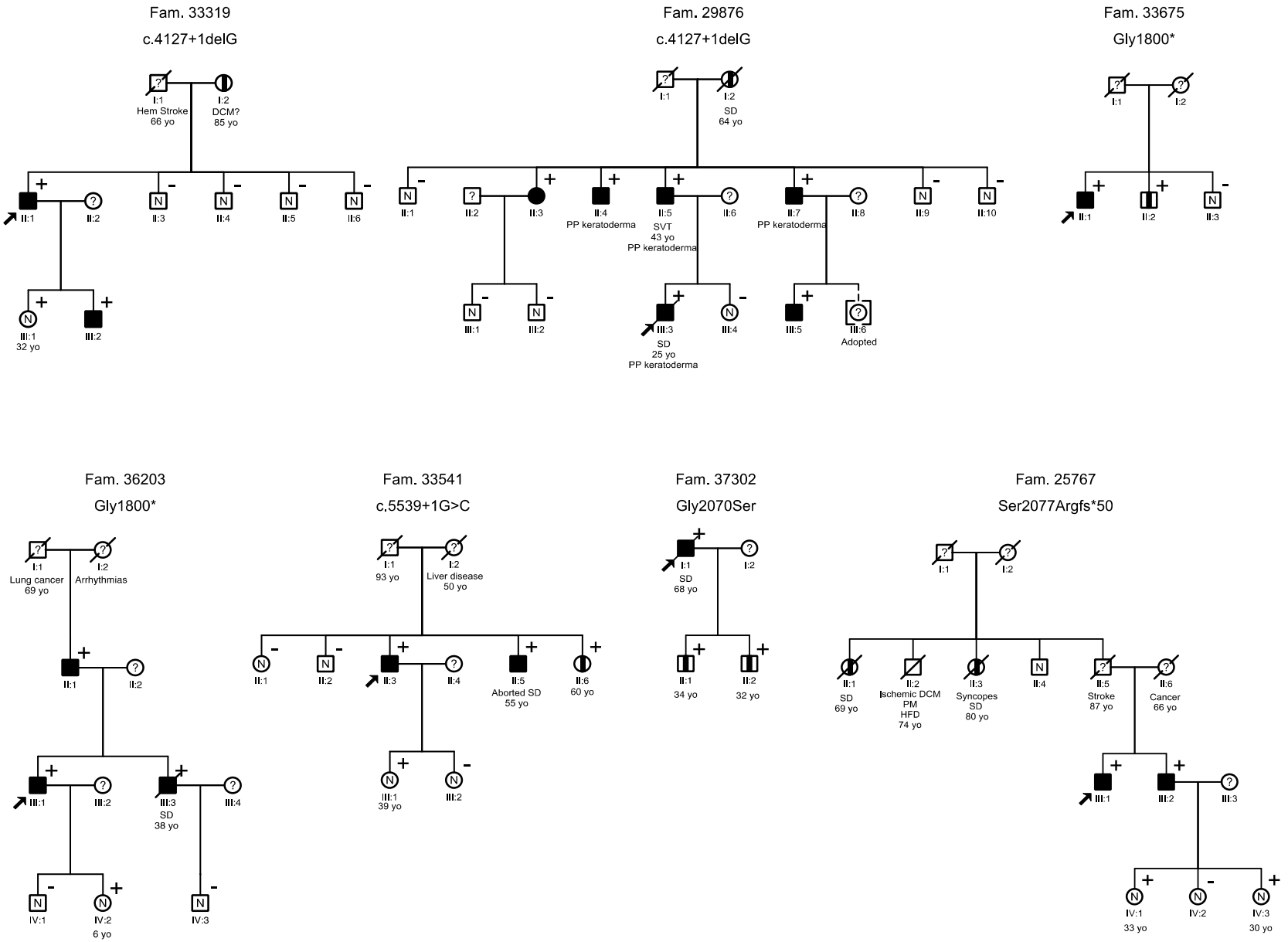

Fam. 33319
c.4127+1delG
I:1
Hem Stroke
66 yo
I:2
DCM?
85 yo
II:1
II:2
II:3
II:4
II:5
II:6
III:1
32 yo
III:2
Fam. 29876
c.4127+1delG
I:1
I:2
SD
64 yo
II:1
II:2
II:3
II:4
PP keratoderma
II:5
SVT
43 yo
PP keratoderma
II:6
II:7
PP keratoderma
II:8
II:9
II:10
III:1
III:2
III:3
SD
25 yo
PP keratoderma
III:4
III:5
III:6
Adopted
Fam. 33675
Gly1800*
I:1
I:2
II:1
II:2
II:3
Fam. 36203
Gly1800*
I:1
Lung cancer
69 yo
I:2
Arrhythmias
II:1
II:2
III:1
III:2
III:3
SD
38 yo
III:4
IV:1
IV:2
6 yo
IV:3
Fam. 33541
c.5539+1G>C
I:1
93 yo
I:2
Liver disease
50 yo
II:1
II:2
II:3
II:4
II:5
Aborted SD
55 yo
II:6
60 yo
III:1
39 yo
III:2
Fam. 37302
Gly2070Ser
I:1
SD
68 yo
I:2
II:1
34 yo
II:2
32 yo
Fam. 25767
Ser2077Argfs*50
I:1
I:2
II:1
SD
69 yo
II:2
Ischemic DCM
PM
HFD
74 yo
II:3
Syncopes
SD
80 yo
II:4
II:5
Stroke
87 yo
II:6
Cancer
66 yo
III:1
III:2
III:3
IV:1
33 yo
IV:2
IV:3
30 yo

Figure S1 (continued)

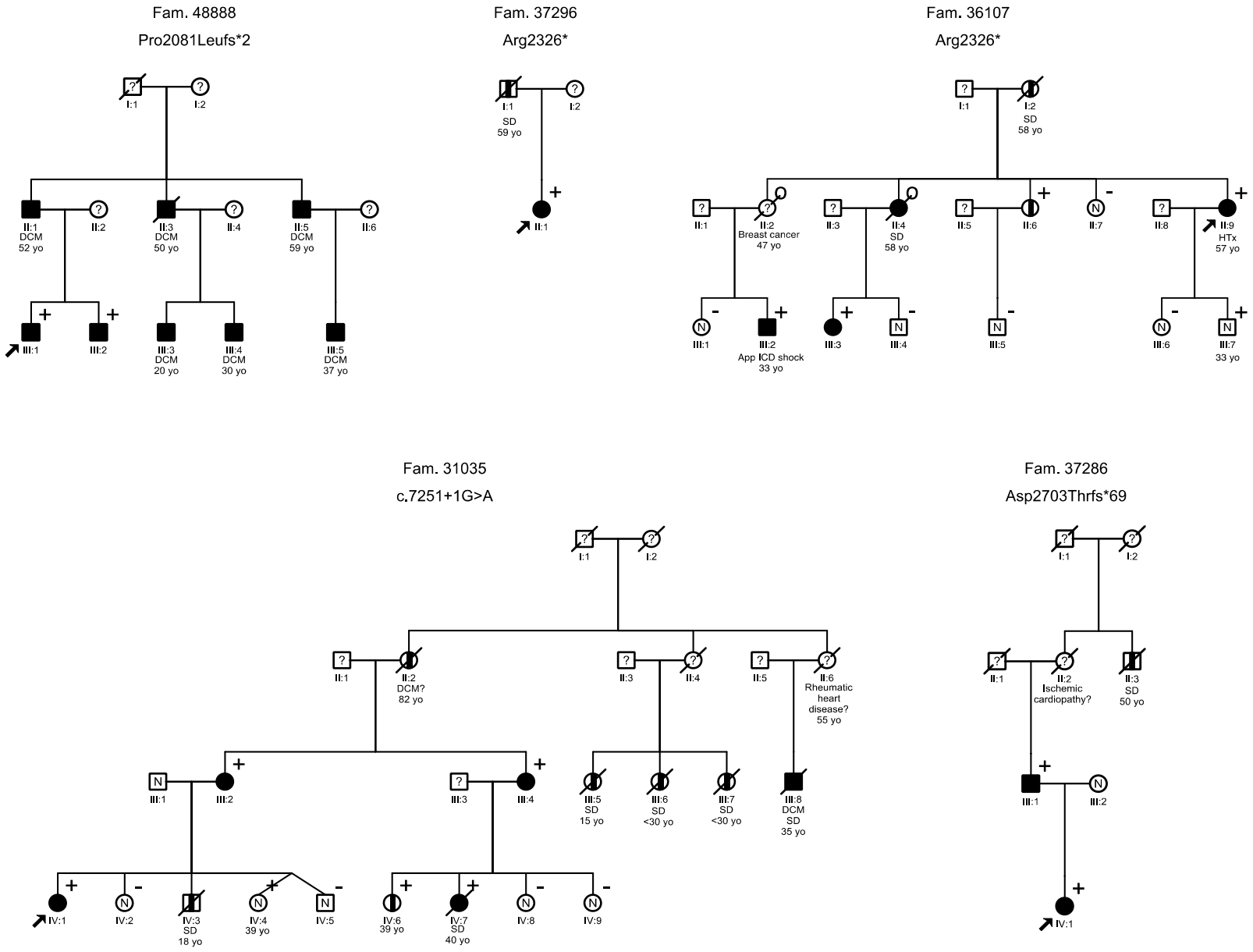


Figure S2: Typical ECG findings in affected carriers of FLNC truncating mutations.

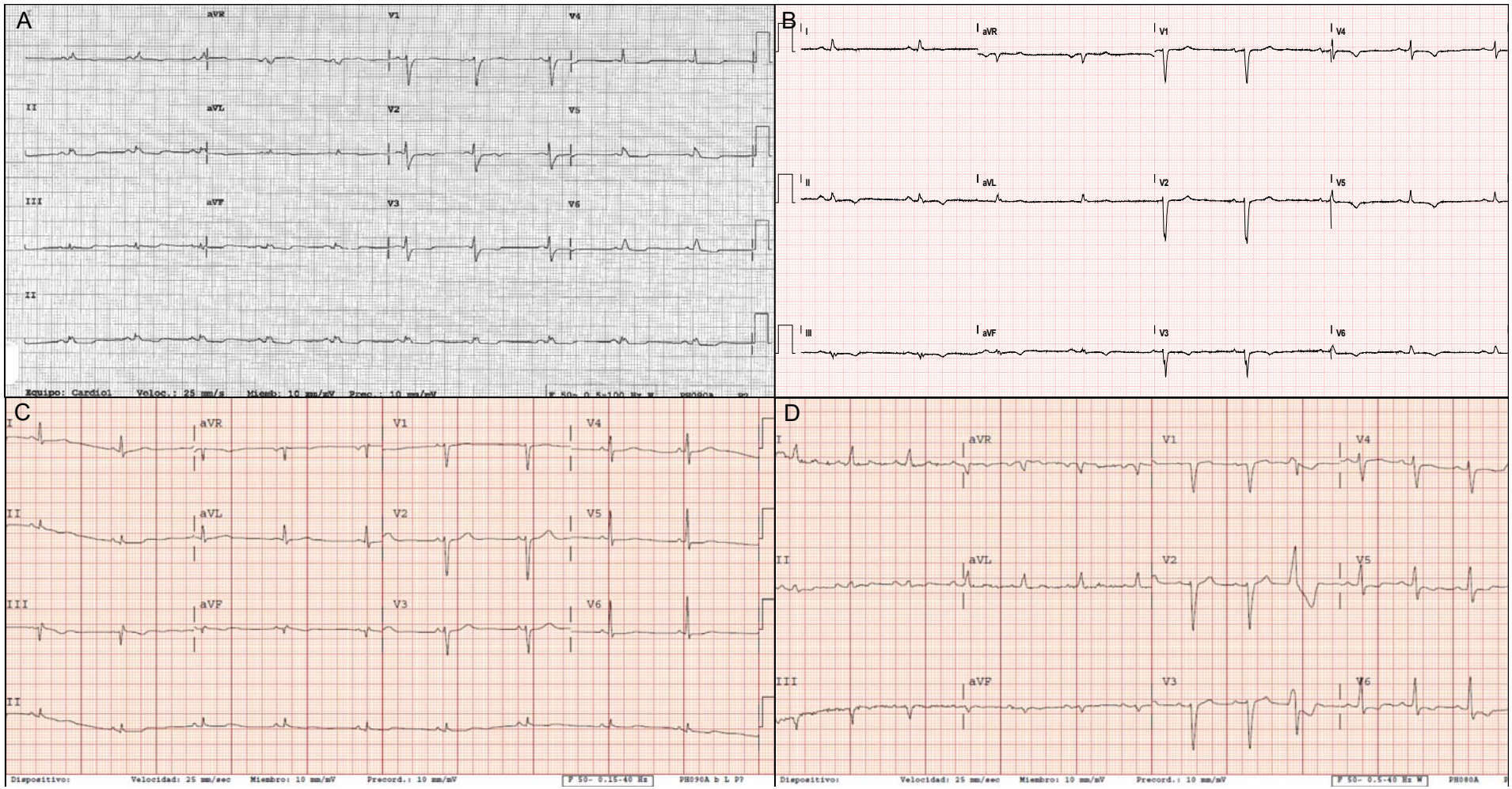


Figure S3: Palmo-plantar keratoderma in carrier II:6 from family 29876.

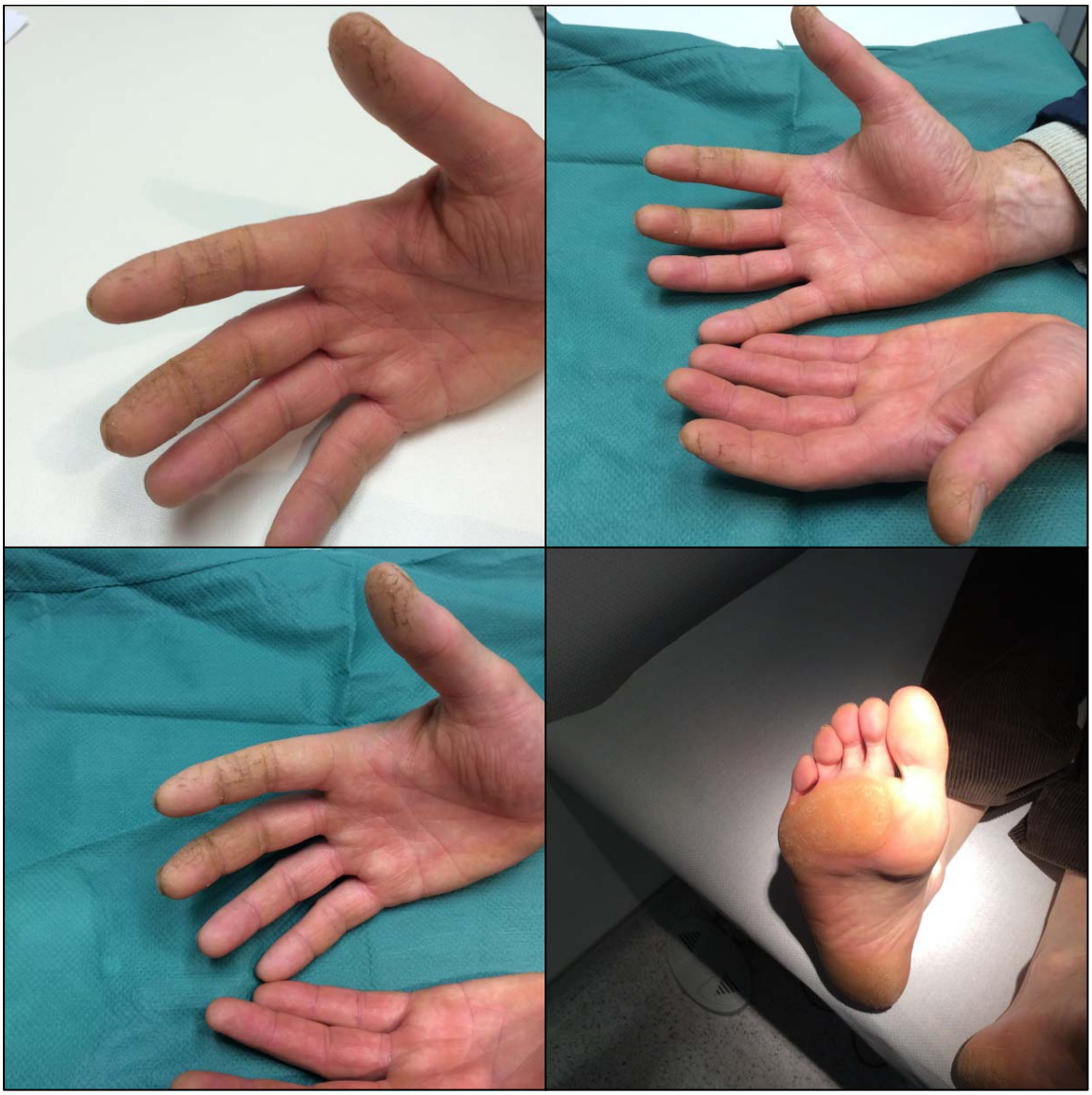


Figure S4: Appropriate defibrillator shock in a patient with a FLNC truncating mutation.