Filamin C Truncation Mutations Are Associated With Arrhythmogenic Dilated Cardiomyopathy and Changes in the Cell–Cell Adhesion Structures

Rene L. Begay, MS,a Sharon L. Graw, PhD,a Gianfranco Sinagra, MD,b Angeliki Asimaki, PhD,c Teisha J. Rowland, PhD,a Dobromir B. Slavov, PhD,a Katherine Gowan, BS,a Kenneth L. Jones, PhD,d Francesca Brun, MD,a Marco Merlo, MD,b Daniela Miani, MD,c Mary Sweet, BA,a Kalpana Devaraj, MD,c Eric P. Wartchow, BS,a Marta Gigli, MD,b Ilaria Puggia, MD,b Ernesto E. Salcedo, MD,b Deborah M. Garrity, PhD,a Amrut V. Ambardekar, MD,b Peter Buttrick, MD,b T. Brett Reece, MD,b Michael R. Bristow, MD,PhD,b Jeffrey E. Safitz, MD,PhD,b Luisa Mestroni, MD,a Matthew R.G. Taylor, MD,PhD,a

ABSTRACT

OBJECTIVES The purpose of this study was to assess the phenotype of Filamin C (FLNC) truncating variants in dilated cardiomyopathy (DCM) and understand the mechanism leading to an arrhythmogenic phenotype.

BACKGROUND Mutations in FLNC are known to lead to skeletal myopathies, which may have an associated cardiac component. Recently, the clinical spectrum of FLNC mutations has been recognized to include a cardiac-restricted presentation in the absence of skeletal muscle involvement.

METHODS A population of 319 U.S. and European DCM cardiomyopathy families was evaluated using whole-exome and targeted next-generation sequencing. FLNC truncation probands were identified and evaluated by clinical examination, histology, transmission electron microscopy, and immunohistochemistry.

RESULTS A total of 13 individuals in 7 families (2.2%) were found to harbor 6 different FLNC truncation variants (2 stopgain, 1 frameshift, and 3 splicing). Of the 13 FLNC truncation carriers, 11 (85%) had either ventricular arrhythmias or sudden cardiac death, and 5 (38%) presented with evidence of right ventricular dilation. Pathology analysis of 2 explanted hearts from affected FLNC truncation carriers showed interstitial fibrosis in the right ventricle and epicardial fibrofatty infiltration in the left ventricle. Ultrastructural findings included occasional disarray of Z-discs within the sarcomere. Immunohistochemistry showed normal plakoglobin signal at cell-cell junctions, but decreased signals for desmoplakin and synapse-associated protein 97 in the myocardium and buccal mucosa.

CONCLUSIONS We found FLNC truncating variants, present in 2.2% of DCM families, to be associated with a cardiac-restricted arrhythmogenic DCM phenotype characterized by a high risk of life-threatening ventricular arrhythmias and a pathological cellular phenotype partially overlapping with arrhythmogenic right ventricular cardiomyopathy. (J Am Coll Cardiol EP 2018;4:504–14) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Dilated cardiomyopathy (DCM) is a major cause of heart failure and disproportionately leads to cardiac transplantation (1–3). The condition is familial in ~50% of cases, and genetic variants residing in over 40 genes have been found to cause DCM through a variety of pathological mechanisms associated with perturbations of the cytoskeleton, intercalated disc region, nuclear envelope, and muscle sarcomere (1,3). Filamin C (FLNC) is an actin cross-linking protein (4) that provides structure for the sarcomere and is one of the largest Z-disc proteins (2,725 amino acids) in cardiac and skeletal muscle. FLNC also localizes to the sarcolemma, where it connects the muscle cell to the extracellular matrix and is involved in related signaling pathways (5).

Originally, FLNC gene mutations were associated with distal myofibrillar skeletal myopathies (MFM) (6), characterized by loss of myofibrils and filamentous intracellular aggregates of myocyte proteins, including desmin, dystrophin, and sarcoglycans. Further investigations have revealed that FLNC missense mutations may lead to hypertrophic cardiomyopathy (HCM) (7) and restrictive cardiomyopathy (RCM) (8). Recently, using whole exome sequencing, we identified an FLNC splicing variant as causing DCM in the absence of skeletal muscle involvement in 2 families (9), a finding further supported by the report of FLNC truncation variants in 4% of DCM and 3% of arrhythmogenic DCM patients (10–12).

In the current study, we report the characterization of the clinical features of FLNC truncating variant carriers, which include a prominent arrhythmogenic DCM phenotype (13), sarcomere structural changes by transmission electron microscopy (TEM), and changes in the distribution of cell-cell junction proteins in the myocardium and buccal mucosa. These structural and cellular changes overlap with arrhythmogenic right ventricular cardiomyopathy (ARVC), and represent a critical link between DCM and ARVC, leading to a more comprehensive understanding of complex familial arrhythmia syndromes as well as an appreciation of the need for mutation-directed clinical monitoring and treatment of this population.

**METHODS**

**STUDY POPULATION AND CARDIOMYOPATHY EVALUATION.** We analyzed 319 U.S. and European DCM families from the Familial Cardiomyopathy Registry. Study subjects underwent extensive clinical evaluations (details in the Online Appendix) (14). Medical records from deceased subjects were reviewed when available (9). Informed consent was obtained from living subjects, and local institutional review boards approved the study protocols.

**NEXT-GENERATION SEQUENCING AND BIOINFORMATIC ANALYSIS.** Twenty larger families were evaluated by whole-exome sequencing (9). In 299 smaller families, probands were evaluated using the Illumina TruSight One-Sequence panel (Illumina, Redwood City, California), which queries 4,813 genes associated with known clinical phenotypes (15). Briefly, subject deoxyribonucleic acid (DNA) was captured with the panel, sequenced on an HISEQ 2500 (Illumina) with v4 chemistry, and mapped with Genomic Short-read Nucleotide Alignment Program (version 2012-07-20) (16). Variants were called with the Genome Analysis Toolkit (version 2.1–8-g5efb575, Broad Institute, Cambridge, Massachusetts) and classified with Annovar (version 2012-07-28) (17). Functional predictions were made with the database for Nonsynonymous SNPs and Their Functional Predictions (version 2.0) (18). All variants were confirmed by Sanger sequencing (19). Variants

---

University Hospital S. Maria della Misericordia, Udine, Italy; 2Department of Pathology, University of Colorado, Colorado Hospital, Aurora, Colorado; 3Department of Pathology, Children’s Hospital Colorado, Aurora, Colorado; 4Center for Cardiovascular Research and Department of Biologics, Colorado State University, Fort Collins, Colorado; and the 5Department of Surgery, University of Colorado Denver, Aurora, Colorado. This study was supported by National Institutes of Health grants R01 HL69071 and HL116906 (to Dr. Mestroni), R01 HL6906 (to Dr. Saffitz), and 1R23HL06795 and R01HL109209 (to Dr. Taylor); and by the CRTrieste Foundation and GENERALI Foundation (to Dr. Sinagra). This work is also supported in part by a Trans-Atlantic Network of Excellence grant from the Leduq Foundation (14-CVD 03) and by National Center for Advancing Translation Sciences at the National Institutes of Health Colorado CTSA Grant Number UL1 TR000102. Dr. Mestroni has served as a consultant for Array BioPharma. Dr. Taylor has served on the Speakers Bureau of GeneDx. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

All authors attest that they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the JACC: Clinical Electrophysiology author instructions page.

Manuscript received October 5, 2017; revised manuscript received November 20, 2017, accepted December 7, 2017.
predicted to be damaging in at least 1 of the prediction algorithms were retained, whereas missense and truncation variants present in >1% in the 1000 Genomes Project were discarded (9,20).

Variant frequency information was obtained from the 6,500 National Human, Lung, and Blood Institute Exome Sequencing Project (21) and the Exome Aggregation Consortium (ExAC, Cambridge, Massachusetts) (22) on February 27, 2017, and was cross-referenced to the ClinVar database (23). Gene variant locations are provided in reference to FLNC transcript NM_001458. Cosegregation analysis was performed when DNA from biological relatives was available. Variants in other cardiomyopathy-related genes were also identified using the Illumina TruSight One Sequencing Panel, as described in the previous text, and confirmed using Sanger sequencing.
HISTOLOGY AND TRANSMISSION ELECTRON MICROSCOPY. Cardiac muscle tissue was obtained from 2 affected siblings of family DNFDC057 (Figure 1), including left ventricular (LV) tissue at the time of LV assist device placement in subject II:1 and LV and right ventricular (RV) tissue from the explanted heart of subject II:2. Fresh heart tissue samples were processed according to standard histology protocols for hematoxylin and eosin, Masson’s trichrome, and immunohistochemistry staining and fixed for TEM. Details can be found in the Online Appendix.

IMMUNOSTAINING. Cotton-tipped swabs (Medi-Choice, Mechanicsville, Virginia) were used to collect buccal mucosa cells from 2 siblings with truncating variants in \( FLNC \) and from normal control subjects. Each cheek was rubbed with a slight rolling and scraping motion, and the resulting material was smeared on standard microscope slides. Immunostaining of buccal mucosa and myocardial tissue was performed as previously described (24), and is detailed in the Online Appendix. For immunofluorescence microscopy, antibodies included: mouse monoclonal anti-plakoglobin (P8087, Sigma-Aldrich, St. Louis, Missouri), mouse monoclonal anti-connexin 43 (Cx43) (Millipore, Burlington, Massachusetts), mouse monoclonal anti-N-cadherin (Sigma-Aldrich), mouse monoclonal anti-desmoplakin (Fitzgerald, Acton, Massachusetts), mouse monoclonal anti-synapse-associated protein 97SAP97 (Santa Cruz, Dallas, Texas), and rabbit polyclonal antiglycogen synthase kinase 3 \( \beta \) (GSK3\( \beta \)) (Cell Signaling Technology, Danvers, Massachusetts). Immunostained preparations were analyzed by confocal microscopy (LSM-510, Zeiss, Oberkochen, Germany).

RIBONUCLEIC ACID SEQUENCING OF EXPLANTED HEART TISSUE. Ribonucleic acid (RNA) was extracted from frozen LV tissue using the mirVana miRNA isolation kit (Thermo Fisher Scientific, Waltham, Massachusetts) enriched for total RNA according to manufacturer’s instructions with the exception of replacing the lysis/binding buffer with mechanical homogenization in TRIzol (Thermo Fisher Scientific). The library was sequenced \( 1/2 \times 50 \) (Illumina HiSeq 2500). Reads were filtered for quality and aligned to the GRCh37hg19 reference human genome using the Genomic Short-read Nucleotide Alignment Program. Transcripts aligning to \( FLNC \) were visualized using the Integrative Genomics Viewer (Broad Institute).

RESULTS

IDENTIFICATION OF \( FLNC \) TRUNCATIONS. Pathogenic/likely pathogenic variants have been identified in approximately 40% of dilated cardiomyopathy samples from the Familial Cardiomyopathy Registry (data not shown), and include \( TTN \) (11%), sarcomeric genes (10%), structural cytoskeleton genes (5%), \( LMNA \) (4%), ion channel genes (2%), and other rare genes (5%). A total of 6 \( FLNC \) truncation variants (Figure 1), including 2 \( FLNC \) stopgain (families DNFDC079 and TSSDC130), 1 frameshift (DNFDC057), and 3 splicing variants (families DNFDC195, TSFDC029, TSFDC031, and TSFDC043), were identified in 7 of the 319 DCM families, for an overall frequency of 2.2% (7 of 319) (Figure 2, Table 1), 100-fold more frequent than the 0.02% \( FLNC \) loss of function variants reported in ExAC (22). Five of the \( FLNC \) truncation variants were absent from the 1000 Genomes Project, 6,500 Exome Sequencing Project, ExAC, and ClinVar databases; c.805C>T had a minor allele frequency of \( 8.3 \times 10^{-6} \) in ExAC (Online Table 1). \( FLNC \) variants p.Y2381Gfs21X and p.G1891Vfs61X have been previously reported (9). The 6 \( FLNC \) truncation variants reported here (Figures 1 and 2) occur in the immunoglobulin domains of the
### TABLE 1 Clinical Phenotype Features of FLNC Truncation Carriers and DCM-Affected Individuals

<table>
<thead>
<tr>
<th>Family</th>
<th>DNFDC057</th>
<th>DNFDC079</th>
<th>DNFDC195</th>
<th>TSFDC029</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>II:1</td>
<td>II:2</td>
<td>II:1</td>
<td>II:2</td>
</tr>
<tr>
<td>II:1</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Age at diagnosis, yrs</td>
<td>59</td>
<td>54</td>
<td>33</td>
<td>39</td>
</tr>
<tr>
<td>Variation</td>
<td>Frameshift</td>
<td>Stopgain</td>
<td>Splicing</td>
<td>Splicing</td>
</tr>
<tr>
<td>Nucleotide change</td>
<td>c.5669-1delG</td>
<td>c.2119C&gt;T</td>
<td>c.2930-1G&gt;T</td>
<td>c.7251+1A&gt;G</td>
</tr>
<tr>
<td>Secondary mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NYHA functional class**

<table>
<thead>
<tr>
<th></th>
<th>III</th>
<th>II</th>
<th>III</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>DOE, fatigue</td>
<td>Palpitations, SOB</td>
<td>Pre-syncope, SOB</td>
<td>Syncope</td>
</tr>
<tr>
<td>Arrhythmias</td>
<td>PACs, PVCs, NSVT</td>
<td>PVCs, AF, sustained VT (1997)</td>
<td>No arrhythmias</td>
<td>Multiform PVCs</td>
</tr>
<tr>
<td>ECG</td>
<td>AVB1, incomplete LBBB</td>
<td>AVB1, LVH</td>
<td>Non-specific ST changes</td>
<td>PM, AICD, non-specific ST changes</td>
</tr>
<tr>
<td>LVEDD, cm</td>
<td>6.4</td>
<td>5.6</td>
<td>5.4</td>
<td>7.2</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>45</td>
<td>10</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>CK, U/l†</td>
<td>118</td>
<td>106</td>
<td>66</td>
<td>52</td>
</tr>
<tr>
<td>RV</td>
<td>Normal</td>
<td>Mild dilatation</td>
<td>Dilatation</td>
<td>Mild dilatation and dysfunction</td>
</tr>
<tr>
<td>Follow-up, yrs</td>
<td>11</td>
<td>11</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>

Families DNFDC057, TSFDC029, and TFDC031 are previously reported variants (9). *Individual III:6 died before enrollment; DNA was not available for genetic testing. †Normal CK level is <223 U/l.

---

### TABLE 1 Continued

<table>
<thead>
<tr>
<th>Family</th>
<th>TSFDC029</th>
<th>TSFDC031</th>
<th>TSFDC043</th>
<th>TSFDC130</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>III:6†</td>
<td>III:7</td>
<td>IV:1</td>
<td>I:2</td>
</tr>
<tr>
<td>III:6†</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Age at diagnosis, yrs</td>
<td>35</td>
<td>34</td>
<td>27</td>
<td>44</td>
</tr>
<tr>
<td>Variation</td>
<td>Splicing</td>
<td>Splicing</td>
<td>Splicing</td>
<td>Stopgain</td>
</tr>
<tr>
<td>Nucleotide change</td>
<td>c.7251+1A&gt;G</td>
<td>c.7251+1A&gt;G</td>
<td>c.3791-1G&gt;A</td>
<td>c.805C&gt;T</td>
</tr>
<tr>
<td>Secondary mutation</td>
<td>SCNSA</td>
<td>c.5270delT; p.F1757fs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>I</td>
<td>I</td>
<td>II</td>
<td>I</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Asymptomatic, SCD</td>
<td>Palpitations</td>
<td>Fatigue, palpitations</td>
<td>SOB, Pre-syncope</td>
</tr>
<tr>
<td>Arrhythmias</td>
<td>NA</td>
<td>No arrhythmias</td>
<td>PACs, PVCs (93/24h)</td>
<td>NSVT, couplet (154/24h)</td>
</tr>
<tr>
<td>ECG</td>
<td>NA</td>
<td>Normal</td>
<td>RBBB</td>
<td>Normal</td>
</tr>
<tr>
<td>LVEDD, cm</td>
<td>NA</td>
<td>4.9</td>
<td>5.3</td>
<td>5.7</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>NA</td>
<td>63</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>CK, U/l†</td>
<td>NA</td>
<td>50</td>
<td>80</td>
<td>103</td>
</tr>
<tr>
<td>RV</td>
<td>NA</td>
<td>Normal</td>
<td>Normal</td>
<td>NA</td>
</tr>
<tr>
<td>Outcome</td>
<td>Cardiomegaly at autopsy</td>
<td>NYHA functional class I, LVEF 52%</td>
<td>NYHA functional class II, LVAD (2016)</td>
<td>NYHA functional class II, LVEF 51%</td>
</tr>
<tr>
<td>Follow-up, yrs</td>
<td>15</td>
<td>15</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>

SAECG = signal-averaged electrocardiography; SB = sinus bradycardia; SCD = sudden cardiac death; SOB = shortness of breath; ST = sinus tachycardia; VT = ventricular tachycardia.
FLNC protein with no apparent geographical clustering or relationships to HCM, RCM, and MFM FLNC variants described to date. The presence of a secondary "likely pathogenic" unique variant was found in a known cardiomyopathy-related gene in the TSFDC043 proband (SCN5A c.5270delT; p.F1757fs, not reported in ExAC and ClinVar databases) who did not show signs of Brugada or long QT syndromes. Finally, FLNC missense variants detected in the overall population of 319 families are charted to the FLNC protein in Online Figure 1 and details are reported in Online Table 2.

RNA-Seq was performed on the explanted heart of DNFDC057 patient II:2. The majority of FLNC transcripts (141 of 162 total reads; 87.0%) were wildtype, and the remaining reads predominantly contained the c.5669-1delG, resulting in a frameshift and subsequent premature stop codon (as nucleotide 5670 is also a G, splicing occurs normally but introduces a frameshift into exon 34). These data support a mechanism of nonsense-mediated decay leading to haploinsufficiency, consistent with our zebrafish studies in which morpholino knockdown of the flnc transcript led to a cardiac phenotype.

**CLINICAL PHENOTYPES OF FLNC TRUNCATION AFFECTED INDIVIDUALS.** Clinical features of FLNC truncation carriers are reported in Table 1, and a detailed clinical description is reported in the Online Appendix. None of the patients fulfilled Task Force criteria of ARVC (25). Comprehensive physical examination of the skeletal muscle of all probands revealed no skeletal muscle abnormalities; serum creatine kinase levels were normal in all tested probands.

Supraventricular and ventricular arrhythmias as well as conduction disease were prominent clinical features (Figure 1, Table 1). Ventricular arrhythmias appear to originate from the LV or appeared polymorphic. Sudden cardiac death (SCD) occurred before the age of 55 years in 5 of 22 (23%) confirmed (n = 13) or suspected by family history (n = 9) truncation carriers. RV involvement with dilatation and/or dysfunction was present in 5 of 13 (38%) cases. The overall penetrance of FLNC truncation variants in our 7 families was 92% (1 unaffected of 13 total confirmed truncation carriers).

**HISTOLOGICAL AND ULTRASTRUCTURAL CARDIAC ANALYSIS OF FLNC G1891Vfs61X VARIANT.** Cardiac muscle tissue was studied from 2 affected siblings of family DNFDC057. In the proband II:2, light microscopic analysis of the LV from the explanted heart showed myocyte hypertrophy, diffuse interstitial fibrosis, and focal replacement fibrosis (Figure 3A). Epicardial fat tissue infiltrated focally into the LV myocardium, especially in areas of replacement fibrosis containing clusters of degenerating myocytes with myofibrillar loss (Figure 3A). The RV showed increased interstitial fibrosis and mild fatty infiltration (Figure 3B). In the affected sibling II:1, cardiac tissue taken upon LV assist device placement showed similar cardiac tissue pathology in the LV (Online Figure 2).

Ultrastructural analysis of LV tissue of affected patient II:1 (DNFDC057) using TEM (Figures 4A and 4B) revealed largely normal sarcomere structures, although abnormal Z-discs were noted. In LV tissue from individual II:2 and II:1 (DNFDC057), the Z-discs appeared less compact and diffuse along the
Z-disc axis (Figures 4C and 4D). In contrast to MFM cases, no intracellular aggregates were seen.

**IMMUNOHISTOCHEMICAL ANALYSIS OF FLNC AND ARRHYTHMOGENIC-ASSOCIATED PROTEINS.** Abnormalities in the distribution and expression of desmosomal and gap junction proteins have been reported in cardiac tissue of patients with arrhythmogenic cardiomyopathy (26). Because some of the clinical (SCD, ventricular arrhythmia) and structural (RV involvement, fibrofatty infiltration) features in our FLNC patients mirrored features of arrhythmogenic cardiomyopathy, we used immunohistochemistry to characterize selected protein distribution in the LV myocardial tissue in affected individual II:2 of family DNFDC057 (Figure 5).

Immunohistochemistry staining for the desmosomal protein desmoplakin and SAP97, a membrane-associated guanylate kinase involved in trafficking sodium and potassium channel subunits to the cell surface, revealed overall decreased signal intensity for both of these proteins compared with healthy control tissues (Figure 5), agreeing with previous studies that showed decreased expression of these proteins in ARVC (27). Additionally, staining for GSK3β revealed that this protein retained its cytoplasmic distribution and did not translocate to the intercalated discs, which has been shown previously to occur in classical ARVC (28). Last, staining for the desmosomal protein plakoglobin and the major cardiac gap junction protein Cx43 (normally located in the intercalated discs) revealed these proteins to have immunoreactive signals similar in intensity and distribution to the healthy control tissues, although the expression of these proteins is usually reduced at cardiac cell-cell junctions in classical ARVC (26,27). Immunohistochemical staining of FLNC revealed no significant intracellular protein aggregates in cardiac myocytes (Figure 6).

Desmosomal and gap junction protein abnormalities have similarly been previously reported in the
buccal mucosa of patients with ARVC (26), which led us to also perform immunostaining of buccal mucosa smears obtained from affected patients DNFD057 II:1 and II:2 (Figure 7). Our buccal mucosa cell staining results were generally concordant with the LV myocardial immunostaining of patient II:2, including diminished signal intensity for desmoplakin and SAP97, and signal intensity near normal levels for plakoglobin. However, unlike the normal levels of Cx43 observed in the LV myocardial immunostaining, staining for Cx43 was found to be reduced in the buccal mucosa cells.

**DISCUSSION**

In a cohort of 319 DCM families, prospectively enrolled in the Familial Cardiomyopathy Research Registry at the University of Colorado Hospital and Azienda Sanitaria Universitaria Integrata of Trieste Hospital, we identified 7 families (2.2%) harboring 6 different FLNC truncation variants distributed across the FLNC gene. These truncation carriers had a prominent arrhythmogenic phenotype characteristic of arrhythmogenic DCM, family history of SCD, frequent RV involvement, and displayed no clinical signs of...
skeletal muscle involvement. As we previously re-
ported, arrhythmogenic DCM patients have a higher
risk for life-threatening ventricular arrhythmias and
SCD, in particular when a family history of SCD is pre-
sent, highlighting the importance of early identi-
fication of patients carrying FLNC truncation variants (13).

Filamins are large cytoskeletal actin cross-linking
proteins that stabilize the actin filament networks
and link them to the cell membrane by binding
transmembrane proteins and ion channels (29).
FLNC encodes a large protein (2,725 amino acids)
primarily expressed in the cardiac and skeletal
muscle that interacts with sarcomeric proteins in the
Z-disc and the sarcolemma (6). Mutations in
FLNC were initially reported to cause MFM (6), while car-
diac involvement may have been noted, but not
studied extensively. A study of German families with
the p.Trp2710* founder mutation reported that 8 of
31 patients had LV hypertrophy, atrial
flutter, and
right bundle branch block (30). More recent reports
support FLNC involvement in a spectrum of cardio-
mypathies, including HCM, RCM, and DCM, where
arrhythmias, cardiac conduction disease, and SCD
were also described (7,8) in the absence of skeletal
muscle pathology (7–12). Similarly, our FLNC trun-
cation carriers also exhibited no clinical evidence of
skeletal muscle abnormalities. Our current report
provides additional evidence of an arrhythmogenic
DCM phenotype likely caused by FLNC truncation
variants, including LV dysfunction, RV involvement,
severe arrhythmias, and conduction disease.

All variants identified in our study are expected to
lead to a truncated or absent FLNC protein. Our anal-
ysis of FLNC transcripts revealed that most (87.0%)
were wildtype, suggestive of a haploinsuf
fi
ce
ncy model. This agrees with our previous investigation
reporting that explanted heart tissue from a FLNC
truncation carrier had reduced levels of FLNC protein
compared with healthy control samples by Western
blot, lending support for a haploinsufficiency mecha-
nism of FLNC pathology (9). We also previously found
that a reduction in flncb (ortholog of human FLNC)
RNA expression in zebra
fi
sh results in structural and
functional cardiac abnormalities (9), further support-
ing the theory that reduced FLNC expression may
result in the observed cardiac dysfunctions.

We additionally found irregular and thickened
Z-discs in the tissue of FLNC truncation carriers
(Figures 4A to 4D). Similarly, in our flncb MO knock-
down zebrafish model, the cardiac muscle ultrastruc-
ture displayed prominent Z-disc disarray and
malformations, and in some instances the Z-disc was
absent (9). Surprisingly, our current study did not find
cytoplasmic protein aggregates in the heart, which
have been described previously in FLNC-associated patients with MFM (in muscle biopsies) as well as HCM and RCM patients (7,8). In these cases, accumulations of protein aggregates are believed to result from the inability of FLNC to dimerize and cross-link with actin at the C-terminal end of FLNC. The absence of FLNC protein aggregates in our study and in the series of Ortiz-Genga et al. (12), is again more in line with a haploinsufficiency model.

The highly arrhythmogenic phenotype and the pattern of biventricular subepicardial fibrosis and fatty infiltration seen in 2 siblings from family DNFDC057 is more reminiscent of features previously described specifically in left-dominant arrhythmogenic cardiomyopathy and PLN R14del cardiomyopathy (31) rather than classical ARVC. For example, reduced junctional signal for desmoplakin, seen in both the heart and buccal mucosa in siblings from family DNFDC057, is more closely linked with biventricular involvement. Similarly, in the distribution of cell-cell junction proteins in the heart and buccal mucosa of these patients are more typical of left-dominant arrhythmogenic cardiomyopathy than classical ARVC. Interestingly, we also observed reduced signal for the membrane-associated guanylate kinase protein SAP97 in both the heart and buccal mucosa in 2 siblings from kindred DNFDC057. Such reduced SAP97 signal has been implicated in abnormal trafficking of ion channel proteins involved in channeling sodium and potassium ions, specifically related to regulating the \( \lambda \text{Na} \) and \( \lambda \text{K} \) currents, which help maintain normal cardiac ventricular resting membrane action potential (27). This may be relevant to the highly arrhythmogenic phenotype seen in our patients: reduced junctional signals for plakoglobin and Cx43 and translocation of GSK3\( \beta \) to cell-cell junctions are all features consistently seen in classical ARVC, but were not seen in our patients. Finally, although we have previously shown that buccal mucosa cells exhibit changes similar to those seen in the hearts of ARVC patients (24), studies here are the first to directly compare buccal cells and myocardium from the same patients. These results add further credence to the idea that changes in the heart of complex familial arrhythmia syndromes may also be seen in the buccal mucosa.

**STUDY LIMITATIONS.** Our study may be limited by the low frequency of FLNC truncations, the inability to perform extensive segregation analysis due to small family sizes, and an incomplete availability of DNA samples from biological members of FLNC truncation families. Efforts to recruit additional family members, especially reportedly affected individuals, have not been fruitful to date. In addition, cardiac tissue was only available from 1 family (2 siblings), which hinders our ability to systematically evaluate the effects of different variants on cardiac cellular structure and function. The presence of a variant in another DCM-related gene (SCN5A) in combination with the FLNC truncation variant in patient TSFDC043 I:1 presents an additional confounding variable. Although all patients are routinely examined for muscle wasting, rigidity, muscle strength, and coordination, invasive skeletal muscle studies that may reveal more subtle pathology, such as electromyography and muscle biopsy, were not performed. Additional phenotypic characterization, such as with contrast-enhanced cardiac magnetic resonance, was also not done in our population. Finally, future studies are needed to elucidate how FLNC truncation variants lead to cardiomyocyte dysfunction and cardiac muscle disease.

**CONCLUSIONS**

Our report provides new evidence that FLNC truncation variants are associated with a severe arrhythmogenic DCM phenotype in the absence of overt skeletal muscle disease. FLNC should be included in DCM genetic testing panels, particularly when arrhythmias complicate the presenting phenotype. Additionally, patients with FLNC truncation variants should be clinically monitored for arrhythmias and considered for implantable cardioverter-defibrillators. Histological and ultrastructural analysis of heart muscle showed no protein aggregates as described in MFM, but instead showed biventricular subepicardial fibrofatty infiltration, Z-disc abnormalities, and redistribution of cell-cell junction proteins. We theorize that haploinsufficiency of FLNC (9) may disrupt its normal functions of cross-linking actin filaments, connecting subsarcolemmal sarcomere Z-discs to the cell membrane and integrins, and connecting actin to cell-cell adhesion junctions in intercalated discs, to result in interference with the desmosomal/cell-cell junction pathway and manifest as a phenotypically arrhythmogenic cardiomyopathy.

**ACKNOWLEDGMENTS** The authors thank the families who contributed to the project by participating in this study, as well as Keona Begay for assistance in editing the FLNC map and other figures.

**ADDRESS FOR CORRESPONDENCE:** Dr. Matthew Taylor, Cardiovascular Institute and Adult Medical Genetics Program, University of Colorado Denver, 12700 East 19th Avenue, Room P15-8022, Aurora, Colorado 80045. E-mail: matthew.taylor@ucdenver.edu.
COMPETENCY IN MEDICAL KNOWLEDGE: As the number of genes associated with DCM continues to increase, differences in clinical presentations are becoming recognized. Patients with FLNC truncation variants should be clinically monitored for arrhythmias and considered for implantable cardioverter-defibrillator devices.

TRANSLATIONAL OUTLOOK: DCM is a common cause of heart failure and life-threatening arrhythmias, and it is frequently caused by gene mutations. The whole spectrum of genetic determinants of DCM is still unknown: however, recently, a novel disease gene known to cause muscular dystrophy, filamin C, has been reported in human dilated cardiomyopathy and zebrafish models. Here, we report that FLNC truncating variants are not rare, are associated with a cardiac-restricted phenotype, are characterized by a high risk of life-threatening ventricular arrhythmias, and are a pathological cellular phenotype affecting the cell–cell adhesion structures, which partially overlaps with ARVC.

REFERENCES


APPENDIX

For expanded Methods and Results sections, as well as supplemental figures and tables, please see the online version of this paper.