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Multicenter Clinical and Functional Evidence Reclassifies a Recurrent Non-canonical *Filamin C* Splice-altering Variant

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Abstract

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Background: Truncating variants in Filamin C (*FLNC*) can cause arrhythmogenic cardiomyopathy (ACM) through haploinsufficiency. Non-canonical splice-altering variants may contribute to this phenotype.

Objective: To investigate the clinical and functional consequences of a recurrent *FLNC* intronic variant of uncertain significance (VUS), c.970–4A>G.

Methods: Clinical data in 9 variant heterozygotes from 4 kindreds were obtained from 5 tertiary healthcare centers. We used *in silico* predictors and functional studies with peripheral blood and patient-specific induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Isolated RNA was studied by reverse transcription polymerase chain reaction (RT-PCR). iPSC-CMs were further characterized at baseline and following nonsense-mediated decay (NMD) inhibition, using quantitative PCR (qPCR), RNA-seq, and cellular electrophysiology. American College of Medical Genetics and Genomics (ACMG) criteria were used to adjudicate variant pathogenicity.

Results: Variant heterozygotes displayed a spectrum of disease phenotypes, spanning mild ventricular dysfunction with palpitations, to severe ventricular arrhythmias requiring device shocks or progressive cardiomyopathy requiring heart transplantation. Consistent with *in silico* predictors, the c.970–4A>G *FLNC* variant activated a cryptic splice acceptor site, introducing a 3-bp insertion containing a premature termination codon. NMD inhibition upregulated aberrantly spliced transcripts by qPCR and RNA-seq. Patch clamp studies revealed irregular spontaneous action potentials, increased action potential duration, and increased sodium late current in proband-derived iPSC-CMs. These findings fulfilled multiple ACMG criteria for pathogenicity.

Conclusion: Clinical, *in silico*, and functional evidence support the prediction that the intronic c.970–4A>G VUS disrupts splicing and drives ACM, enabling reclassification from VUS to pathogenic.

Keywords

Arrhythmogenic cardiomyopathy; arrhythmia; splicing; nonsense-mediated decay; functional genetics

Introduction

Filamin C is an actin-binding protein encoded by *FLNC* that is expressed in cardiac and skeletal muscle. Variants in *FLNC* have been associated with autosomal dominant hypertrophic cardiomyopathy [MIM: 617047] and distal [MIM: 614065] and myofibrillar [MIM: 609524] skeletal myopathy. More recently, truncating *FLNC* variants have been shown to cause a particularly severe and highly penetrant form of arrhythmogenic cardiomyopathy (ACM) in the absence of skeletal muscle phenotypes[1]. ACM encompasses a spectrum of cardiomyopathies, including arrhythmogenic right ventricular cardiomyopathy, and biventricular and arrhythmogenic dilated cardiomyopathy (DCM)[1–3]. Most of the truncating *FLNC* variants reported to date have been nonsense variants or function-altering variants in canonical splice sites. Splice-disrupting intronic variants outside the 2-bp canonical splice sites are an increasingly recognized cause of inherited cardiomyopathies and arrhythmia syndromes[4–6]. These variants may cause frameshifts or inclusion of

premature termination codons, which may converge on the molecular mechanism of haploinsufficiency identified in previous studies of *FLNC* truncating variants[3,7].

A challenge in precision medicine is interpreting the pathogenicity of variants discovered in disease-associated genes. The American College of Medical Genetics and Genomics (ACMG) has provided a framework to interpret variants that integrates multiple variant features, including clinical phenotyping, variant frequency, *in silico* tools, and functional assays[8]. These criteria result in categorical classifications of variants ranging from benign to pathogenic; however, the majority of variants do not have sufficient evidence for benign or pathogenic classification, and therefore fall into the nebulous category of Variant of Uncertain Significance (VUS)[9]. This presents an increasing clinical problem as VUS's cannot be used to guide clinical management, but they can raise alarm for providers and patients, especially in genes such as *FLNC* that are associated with malignant ventricular arrhythmias[10]. Accordingly, we sought to investigate a *FLNC* VUS (c.970–4A>G) that is located near a canonical splice acceptor site and has been reported in 4 separate families worldwide. By combining clinical phenotyping with computational and functional characterization of the variant using patient peripheral blood and patient-derived induced-pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), we provide multiple lines of evidence to reclassify this recurring variant from VUS to pathogenic within the ACMG framework.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request. All data generated from previous studies used in this analysis received IRB/REC approval. The research reported in this paper adhered to guidelines included in the Helsinki Declaration as revised in 2013. Full methods are available in the Supplemental Methods.

Results

Clinical Phenotypes.

We studied 9 c.970–4A>G variant heterozygotes from 4 unique families collected as part of the *FLNC* ACM Registry[1]. Pedigrees are presented in Figure 1.

Family A (U.S.A.).

The proband was a female who first presented with fatigue and palpitations associated with premature ventricular contractions (PVCs) that were first documented during pregnancy at the age of 33. At the time she had a cardiac workup that showed multifocal PVCs with no evidence of ACM by cardiac MRI or echo. Family history was notable for sudden unexplained death of both her mother at the age of 42 and maternal grandfather at the age of 60. Both deaths occurred during mild to moderate physical activity. Following a gap in care and recurrence of symptoms, the patient was referred for additional management 7 years later. Repeat imaging showed normal left ventricular (LV) ejection fraction (LVEF; 54%) and right ventricular (RV) ejection fraction (RVEF; 57%) with mild LV (end-diastolic diameter 5.3 cm) and RV (3.9 cm) dilation; there was no late gadolinium enhancement

(LGE) (Table 1). An exercise stress test showed ventricular ectopy at rest that increased with exercise and episodes of non-sustained ventricular tachycardia (NSVT) of multiple ventricular morphologies (Figure 2). The tentative clinical diagnosis was catecholaminergic polymorphic ventricular tachycardia (CPVT), and genetic testing with an arrhythmia panel was negative (genes in Supplementary Table I).

Clinical evaluation of the proband's 2 brothers showed that they both had PVCs with multiple morphologies. The brother had NYHA Class I HF with a preserved LVEF (55%) with borderline dilation of the LV (5.6 cm) at baseline. Holter monitor recorded PVCs (4597), ventricular couplets (121), and a 4-beat run of NSVT during a 24-hour period. Cardiac MRI showed a normal LVEF (57%) and RVEF (52%) along with segmental LGE of the mid-inferoseptum at the RV insertion. Cardiac MRI of the second brother revealed a normal LVEF (61%), reduced RVEF (45%), and borderline dilation of the LV (5.5 cm) and RV; no LGE was noted by MRI. Combining these findings lead to the reassignment of the clinical diagnosis to ACM in all siblings. Repeat genetic testing with a cardiomyopathy gene panel identified the intronic *FLNC* variant c.970–4A>G, which was classified as a VUS (Supplementary Table I)[11]. This variant was present in all 3 phenotype-positive siblings. The variant was absent in the father, suggesting that the mother was a variant heterozygote. Together, the 3 siblings have 9 children who are currently phenotypically normal and have not completed genetic testing.

Family B (Australia).

The proband was a female diagnosed with ACM at 11 years of age (Table 1). She first presented with dyspnea and NYHA Class III heart failure. A baseline echo showed severe LV dilation (7.0 cm), mild mitral regurgitation, and depressed LVEF (27%). A 24-hour ambulatory monitor showed PVCs and a single instance of a ventricular couplet. Panel sequencing of 105 genes revealed the *FLNC* VUS and an additional VUS in *MYLK2* (c.1360G>T; p.Val454Leu) (all genes tested listed in Supplementary Table I). She had a family history of DCM in both her maternal and paternal grandfathers. The paternal grandfather was diagnosed with DCM in his 7th decade of life and the maternal grandfather died of DCM complications in his 5th decade of life – further details are unknown. Neither her mother or sister carried the *FLNC* variant and were both healthy; the father was unaffected and declined completion of genetic testing. The proband experienced progressive contractile dysfunction that necessitated heart transplantation at age 12. The histology report post-transplant identified nuclear hyperchromasia and enlargement without inflammation or fibrosis, ultimately interpreted as DCM of uncertain etiology. She has remained healthy after successful transplantation.

Family C (Netherlands I).

The familial proband was a male diagnosed with DCM at age 59 (Table 1). Cardiac MRI revealed a severely reduced LVEF (30%), mildly reduced RVEF (45%), and non-specific myocardial fibrosis. He had a negative ischemic evaluation by coronary angiography. Genetic testing revealed the *FLNC* c.970–4A>G VUS and a missense VUS in *TTN*. The proband's sister suffered a cardiac arrest at the age of 50 but declined genetic testing. His father was phenotype-negative and did not carry the *FLNC* variant. His mother died

of unspecified cardiac causes and is an obligate carrier. A maternal aunt and cousin were genotype-positive for the *FLNC* variant but were cared for by different providers. Due to local ethical and privacy concerns, they were unable to be contacted to obtain prior testing results or to perform additional phenotyping. The proband has since received an implanted cardioverter defibrillator and has received multiple appropriate shocks for ventricular arrhythmias.

Family D (Netherlands II).

The proband was a male diagnosed with ACM at 41 years of age. He presented with heart failure and severally depressed LVEF (15 %) with diffuse hypokinesia of the left ventricle by echo and PVCs (Table 1). Subsequent MRI evaluation revealed depressed LVEF (35%), RVEF (43%) and late gadolinium enhancement (3% of LV). With treatment LVEF improved to 45% at the age of 45 years. Genetic testing with a cardiomyopathy panel revealed the *FLNC* VUS c.970–4A>G variant along with another VUS in *FLNC*, c.7399C>T/p.Arg2467Cys, a VUS in *TTN*, c.71310C>G/p.Asp23770Glu, and a VUS in *MYBPC*, c.1471G>A/p.Val491Met. Family history noted maternal heart failure and an enlarged heart in the father; however, the proband is estranged from his family, precluding the possibility of variant segregation within the family.

Variant Characteristics.

The *FLNC* variant c.970–4A>G is located adjacent to the canonical splice acceptor site of exon 6, within the rod 1 functional domain. The frequency of this variant is $5.35 * 10^{-5}$ (15 alleles) in the gnomAD database (v2.1.1)[12]. Given the proximity to the canonical splice acceptor site, the impact on splicing was assessed with *in silico* tools. SpliceAI is an in silico tool that calculates a probability, from 0 to 1, of a genetic variant to introduce or weaken a splice acceptor or donor site. The algorithm also outputs a predicted location of those splice sites with respect to the variant. In this case, the algorithm strongly predicts (0.88) that a competing splice acceptor will be gained 1 nucleotide downstream, and the canonical splice acceptor lost 4 nucleotides downstream. The molecular consequence would be a 3-bp insertion of intronic sequence coincidentally containing a premature termination codon (PTC). Both SpliceAI (Figure 3A) and AlaMut (Supplemental Figure I) predicted the activation of a cryptic splice acceptor site 3 nucleotides upstream of the canonical acceptor site (Figure 3B). Based on this prediction and strong evidence of *FLNC* haploinsufficiency as a disease mechanism, we sought to investigate the effects of the variant on splicing in human peripheral blood through illegitimate transcription[13]. Sanger sequencing of the RT-PCR product from isolated peripheral blood RNA from the Family C proband revealed the presence of a 3 base pair insertion (Figure 3C; primers in Supplemental Table II). This in-frame insertion is predicted to introduce a premature truncation (UAG stop codon).

Transcriptional Studies of Patient-derived iPSC-CMs.

Peripheral blood mononuclear cells were obtained from the USA proband, reprogrammed into iPSCs, and differentiated into iPSC-CMs (see Materials and Methods). Extensive characterization of the iPSCs and iPSC-CMs are presented in Supplemental Figures II–V and Supplemental Table III (karyotyping, immunohistochemistry, differentiation protocol, and cardiac differentiation efficiency). The cells were confirmed to be heterozygous for the

intronic variant. Analysis of the splice junction was completed using RT-PCR with RNA isolated from the patient and control iPSC-CMs (Figure 4A; primers in Supplemental Table II). Unexpectedly, we observed no difference in mobility of the RT-PCR amplicons between the patient and healthy control; Sanger sequencing of the patient line showed a normal splice junction between exons 5 and 6 (Figure 4B).

This result was at variance with the illegitimate transcription result above, so we further tested the idea that the aberrantly spliced variant was degraded by non-sense mediated decay (NMD). NMD is a conserved cellular process by which PTCs in nascent transcripts are recognized and degraded in the transcriptional milieu[14]. This homeostatic process can nevertheless precipitate pathology when degraded PTC-containing transcripts are associated with a haploinsufficient disease. Chemical tools, such as cycloheximide (CHX), may be used to inhibit this machinery and implicate it in disease through functional studies. After exposure to CHX (Figure 4C), the patient iPSC-CM RT-PCR amplicons showed the emergence of a 3-bp in-frame insertion on sequence analysis that was confirmed by TA cloning to represent otherwise degraded variant transcript (Figures 4D and 4E). Figure 4F shows experiments with qPCR displaying increased abundance of *FLNC* transcript in patient vs control lines after treatment with cycloheximide ($p < 0.01$, N=3), presumably by decreasing degradation of variant transcript. The significantly higher increase in the patient *FLNC* transcripts suggests a recovery of aberrantly spliced transcript compared to the mild upregulation observed in the control, consistent with global dysregulation of transcript expression after CHX treatment. To further investigate the transcriptional alterations in the patient-derived iPSC-CMs, we performed RNA-seq of cells treated with vehicle DMSO or CHX and visualized alignments using Sashimi plots (Figure 4G). We observed minimal aberrant splicing in the vehicle control that could not be appreciated by Sanger sequencing alone (25 aberrant reads vs 852 canonical reads) and increased aberrant splicing after treatment with CHX (321 aberrant reads vs 1740 canonical reads).

Cellular Electrophysiological Investigations.

To determine whether aberrant splicing led to arrhythmogenic behavior, we explored the electrophysiologic properties of the patient iPSC-CMs compared to a healthy control iPSC-CMs. Spontaneous action potentials of each line were recorded by manual patch-clamp at day 35 of differentiation. Multiple differentiations of the healthy control line (N=3) featured regular, spontaneous depolarizations (Figure 5A); in contrast, independent differentiations of the patient line (N=3) featured both long spontaneous depolarizations and irregularity of spontaneous depolarizations, including early after depolarizations (Figure 5B).

To explore the basis of arrhythmogenicity, we recorded action potentials. Action potential duration was significantly longer in patient vs healthy control lines (Supplemental Figure VI). Sodium late current has been implicated as a terminal manifestation of aberrations in the PDGFRA signaling axis[15], a signaling pathway previously invoked in the study of other FLNC truncating variants[16]. Indeed, we observed large late currents in proband iPSC-CMs compared to those recorded in population control iPSC-CMs (Supplemental Figure VI). Quantifications and measurements for each individual differentiation are provided in Supplemental Figure VII.

ACMG Variant Reclassification.

The variant c.970–4A>G was previously classified as a VUS in ClinVar (ClinVar: 432231[11]) following application of the PP3 criterion alone. We implemented evidence based on the investigations above to reclassify this variant to Pathogenic as follows[8]. Cosegregation of the phenotype with variant heterozygote status supported the implementation of PP1 at the moderate level (cosegregation of disease in multiple affected family members in a gene definitively known to cause the disease). Functional data in 2 separate assays from 2 independent heterozygotes supported the application of PS3 (well-established functional studies supportive of a damaging effect) following electrophysiological assays and PVS1 (null variant in a gene where loss-of-function is a known mechanism of disease) following transcriptional studies. The multiple concordant deleterious splicing predictions supported application of PP3 (multiple supporting lines of computational evidence). Given the frequency of 5.35e-5 (15 alleles) in gnomAD, we did not apply variant frequency criteria based on recommendations for highly penetrant variants in ARVC and DCM[17]. Altogether, these data support variant reclassification to pathogenic (Figure 5).

Discussion

Here we report the clinical phenotypes and probable molecular mechanisms associated with a heterozygous non-canonical splice-altering *FLNC* variant, c.970–4A>G, identified in 4 unrelated kindreds. We show that the variant presents with a range of severity, including early-onset heart failure necessitating heart transplantation in a pediatric patient and life-threatening arrhythmic events, to mild ventricular dysfunction and symptomatic ventricular ectopy during exercise. Using patient lymphocytes and a patient-specific iPSC-CM model, we demonstrate aberrant splicing consistent with computational predictions. Furthermore, we discover that the resulting in-frame premature termination codon is a substrate for NMD, and that the iPSC-CMs recapitulate the arrhythmogenicity observed in patients. Using the ACMG criteria, we were able to fulfill multiple criteria to reclassify this variant to pathogenic. This work highlights the utility of international clinical and functional collaboration to address recurrent VUS.

Variants in *FLNC* have been associated with many cardiac phenotypes, including DCM[18], ARVC[19], and restrictive cardiomyopathy[20]. One extensive study of *FLNC* variants in patients with familial DCM found variants in 2.2% of affected individuals[21]. Further studies from the *FLNC* ACM Registry defined the natural history of variant carriers, with 49% meeting criteria for DCM, 25% meeting criteria for ARVC, and 3% arrhythmogenic left-dominant cardiomyopathy[1]. Beyond selected cohorts, studies of *FLNC* loss-of-function variants in a large biobank have also supported an increased risk of ACM among unselected individuals carrying such variants[22]. Our clinical findings of predominant LV dysfunction and ventricular arrythmias are consistent with previous reports of truncating *FLNC* variants that were highly arrhythmogenic and favored the LV in contrast to ACM presentations with different genetic substrates[3]. An interesting observation of our clinical case series is that heterozygotes of the c.970–4A>G variant appear to have less LGE than those harboring other truncating variants. Genetic testing will be critically important to help guide these interventions based on genotype, especially for those with minimal structural disease by

MRI upon initial clinical evaluation. Collectively, the findings in these studies support the idea that arrhythmias and sudden death may often be the first manifestation of a genetic cardiomyopathy[23]. This is especially relevant for related individuals of genotype-positive patients in our 4 families, who may benefit from completing genetic testing and detailed clinical phenotyping.

Our work highlights the utility of functional assays in disease-relevant systems to illuminate molecular mechanisms. We show consistent results with both secondary (peripheral blood) and pseudo-primary tissue (iPSC-CMs) from 2 different patients harboring the same variant. The degree of NMD is different between the 2 tissues, with the variant allele completely degraded in the iPSC-CMs. This result may closely resemble the biology in the native cardiomyocyte and contribute to haploinsufficiency provoking the phenotype. NMD serves as regulatory machinery to degrade transcripts containing premature termination codons, and may be a confounder when not explored *in vitro* splicing assays[24]. In our study, we could only identify the aberrantly spliced transcript in venous blood, but not in iPSC-CMs without CHX. Cell-type specific influence of NMD may contribute to divergent outcomes of splicing among different assays (e.g. venous blood, minigene, primary tissue)[4]. Due to this confounding, the yield of splice-altering variants may be underestimated. Future studies should consider inhibition of NMD before ruling out pathogenic variation in haploinsufficient disease. These assays may be applied to other intronic variants in *FLNC* that may be unrecognized as causes of ACM through non-canonical aberrant splicing. Moving beyond transcriptional defects, the arrhythmogenicity of the patient-derived iPSC-CMs provides preliminary evidence to link aberrant splicing to an electrophysiologic property. The presence of early afterdepolarizations observed in the spontaneous action potentials may provide a point of initiation for severe ventricular arrhythmias. We observe increased sodium late current and increased action potential duration in these cells; however, we do not observe extended QT intervals among patients, suggesting an opportunity for future investigation.

An interesting aspect of this variant is the relatively high frequency of this variant in gnomAD (allele count 15; allele frequency $5.35 * 10^{-5}$)[12]. This is a rare, but not ultra-rare frequency which does not clearly lend itself to classification as benign or pathogenic[17]. A characteristic related to the frequency is the recurrence of this variant in geographically distinct families. This may reflect a mutational hotspot adjacent to this exon (the variant c.970–5A>G is also observed in gnomAD 8 times but annotated as likely benign in ClinVar). The natural history of this variant among the 4 families is characterized by phenotypic heterogeneity, with some heterozygotes experiencing mild ectopy, while others have experienced aborted cardiac death. Although cosegregation is weakened by 2 putatively healthy variant heterozygotes in Family C, we cannot exclude the possibility of limited phenotype given the inability to access additional clinical data; we therefore cannot distinguish between clinically unaffected (incomplete penetrance) and unknown phenotype[25].

Limitations.

Both functional assays are limited as they do not capture human primary tissue. As above, we are currently unable to account for the mechanisms underpinning variable penetrance. For certain individuals that are purportedly unaffected, we cannot exclude the possibility they have subclinical disease that could be detected if comprehensive clinical evaluation was performed or will develop clinical signs and symptoms in the future. Another limitation is that variable expressivity among individuals carrying the FLNC variant remains unexplained. One possibility is that the degree of NMD may have varied between individuals, partially accounting for the differences in phenotypic severity. Other possibilities include clinical and polygenic modifiers that were not ascertained in this study. Generation of isogenic controls from healthy control and patient derived lines will be critical to implicate the molecular mechanisms underlying this variant; however, these studies are outside the scope of the current reclassification effort.

Conclusions.

Current ACM guidelines now take into account the results of genetic testing, including the 2019 HRS Consensus Statement[2] and the Padua Criteria[26]. Reclassification of this variant will allow for genetic screening of at-risk family members thereby eliminating the need for continued follow-up in genotype-negative individuals and justifying the need for closer follow and risk reduction strategies in genotype-positive individuals. Inspection of the 4 kindreds in this report demonstrates that several individuals who are at-risk but currently unaffected may directly benefit from our reclassification efforts. Together, this work demonstrates the utility of international collaboration and data sharing in VUS reclassification, and sheds light on an interesting molecular mechanism of a splice-altering variant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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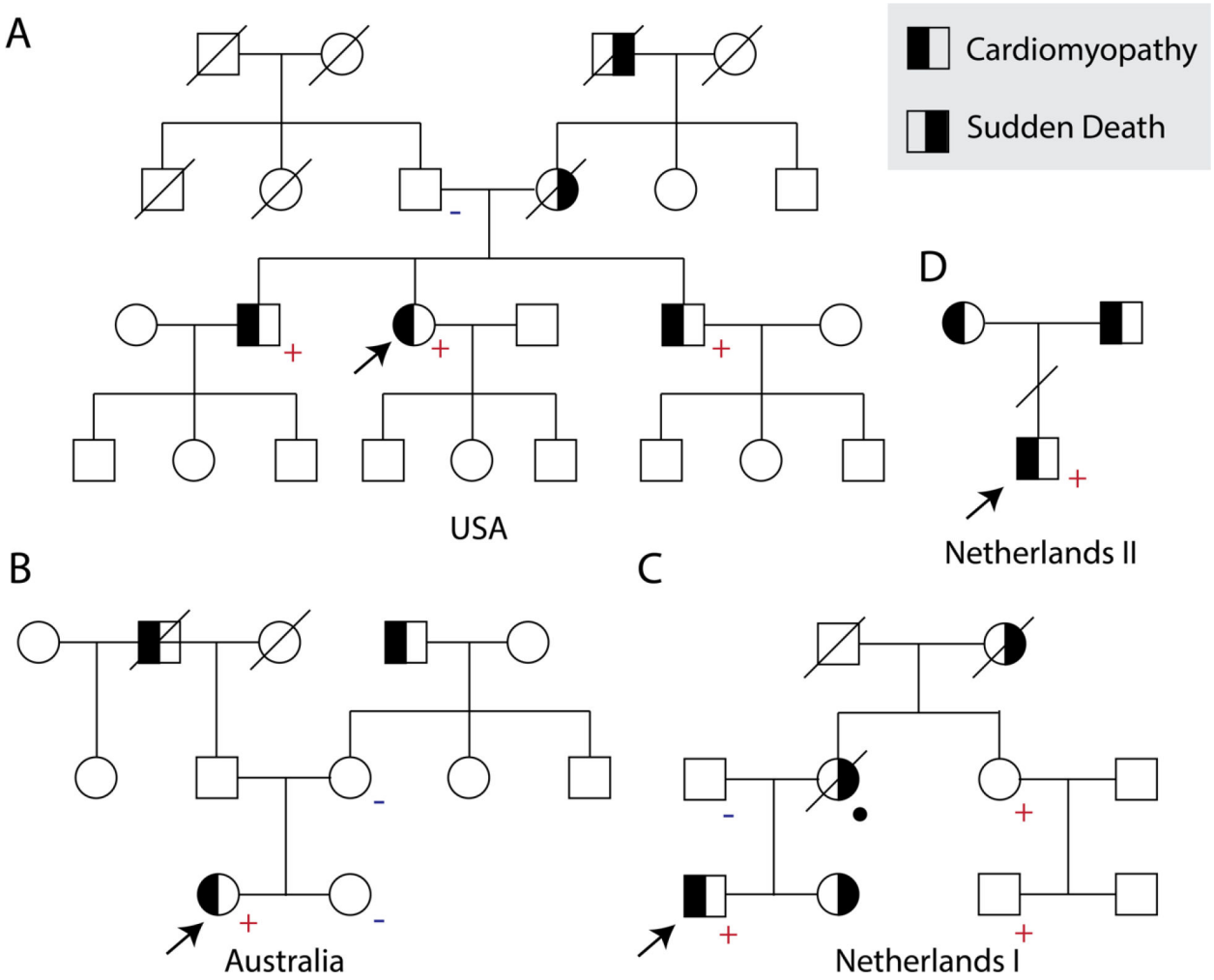

Figure 1. Family pedigrees.

A) 4-generation pedigree of variant heterozygotes cared for by USA centers. All siblings of proband generation are heterozygous for the variant and have a history of ACM. The mother died at 42 of sudden death following the diagnosis of an unknown arrhythmia, and the maternal grandfather suddenly died at the age of 60 one year after a negative ischemic evaluation.

B) 3-generation pedigree of a young proband followed at an Australian center. The index patient had early onset DCM requiring heart transplant. Her mother and sister were both negative for the variant. Family history was otherwise remarkable for both paternal and maternal grandfathers with diagnosed DCM.

C) 3-generation pedigree from Netherlands. The proband presented later in life with severe DCM. The mother and maternal grandmother both died suddenly. A maternal aunt and child were variant heterozygotes in apparently normal health, who were unable to be contacted for additional phenotyping.

D) 2-generation pedigree of additional family from the Netherlands. The proband presented in mid-adulthood with DCM. Both parents had a history of cardiomyopathy, but further were precluded by estrangement from his family.

+ indicates genetic testing and heterozygous status for c.970–4A>G. – indicates wildtype status. • indicates obligate heterozygote. + or – absent when genetic testing was not completed. Arrow indicates proband within each family. Dash line across relationships indicates estrangement.

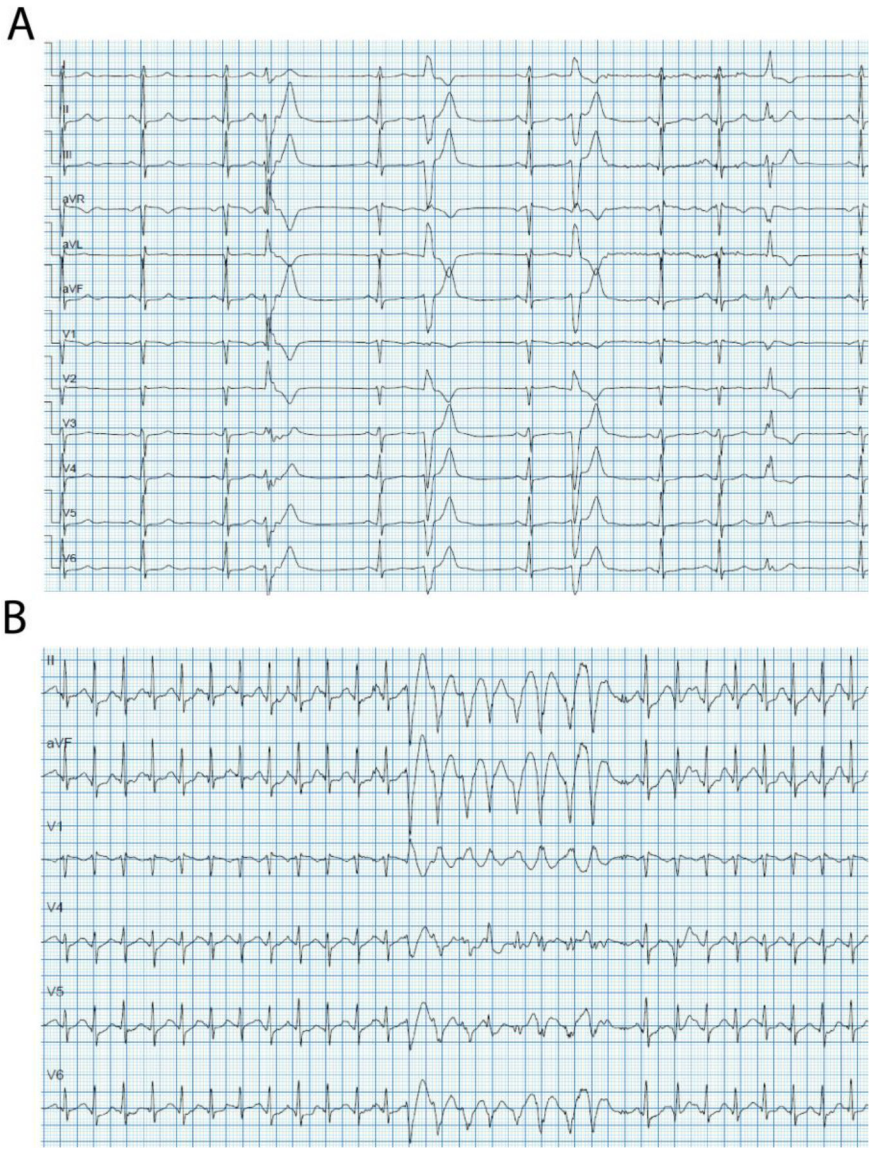

Figure 2. Exercise stress test of USA proband.

A) Proband ECG (Family 1) at rest prior to exercise. Late-coupled PVCs (3 morphologies). B) Proband ECG at Stage 3 of stress test with an 8-beat episode of relatively monomorphic NSVT.

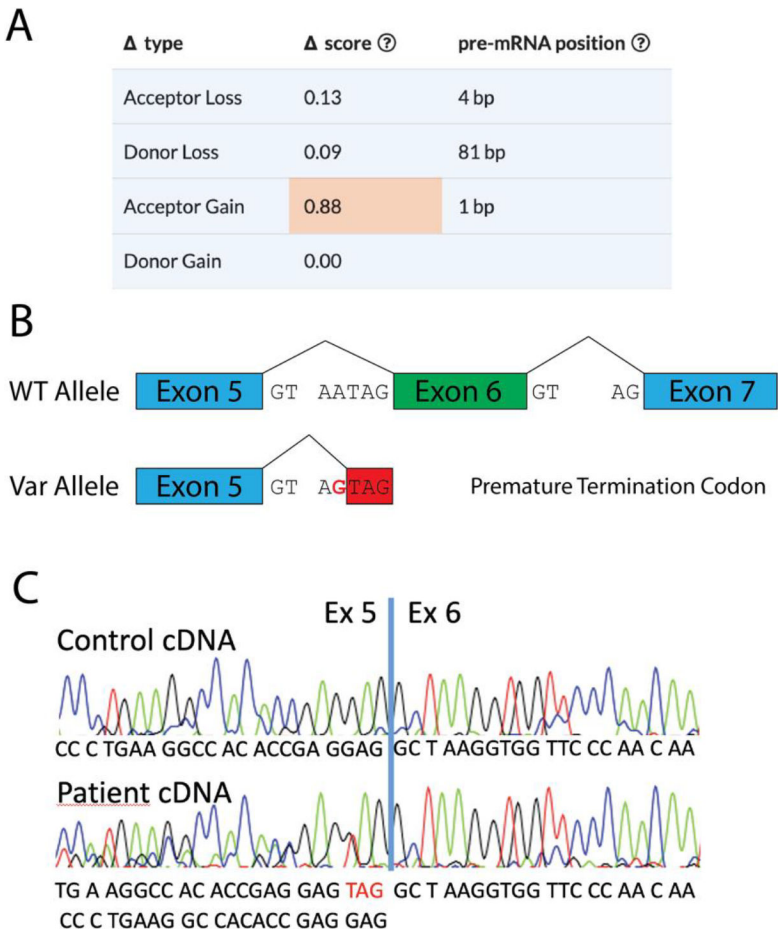

Figure 3. ***FLNC*** **c.970–4A>G Variant Characteristics.**

A) SpliceAI scores for the *FLNC* c.970–4A>G. Strongly predictive of the introduction of a competing splice donor site immediately adjacent to the variant.

B) Schematic of the functional consequences for variant heterozygotes. Competing splice site introduces a 3-bp premature termination codon into the aberrantly spliced exon.

C) Sanger trace of cDNA analysis of peripheral blood obtained from the proband of Family C (The Netherlands I). Trace shows introduction of the predicted premature termination codon in one transcript.

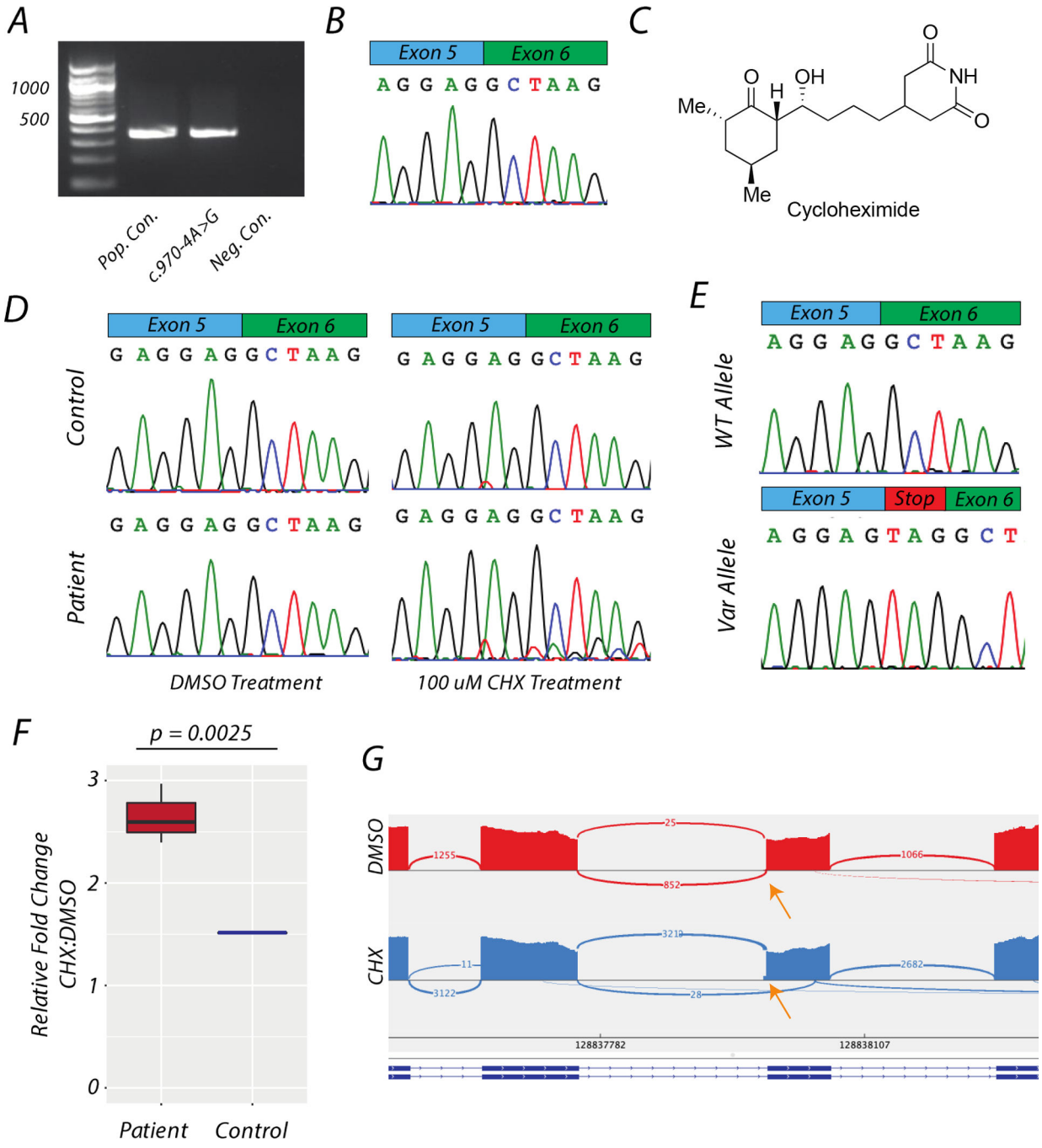

Figure 4. ***FLNC*** **c.970–4A>G iPSC-CM transcriptional investigations.**

A) iPSC-CM RNA was reverse transcribed into cDNA and amplified by PCR. Gel shows amplified DNA from a healthy control (Pop. Con.), USA proband, and a negative control (Neg. Con.) without RNA input to RT-PCR.

B) Sanger sequencing result of the gel extracted band from USA proband iPSC-CM RT-PCR product. Sequence shows only normal splicing of exon 5 to exon 6.

C) Chemical structure of cycloheximide, a natural product inhibitor of nonsense-mediated decay.

D) Sanger sequencing of RT-PCR products from patient and control lines treated with DMSO or 100 μM cycloheximide (CHX).
E) TA cloning of gel extracted bands from 100 μM CHX treatment in panel E. Identification of aberrantly spliced transcript degraded in absence of NMD inhibition.
F) Relative fold change of transcript abundance as determined by qPCR. Two-tailed t-test.
G) Sashimi plot visualization of RNA-seq data in patient cell line. Low level indel incorporation is observed after vehicle treatment, which is markedly upregulated after inhibition of NMD.

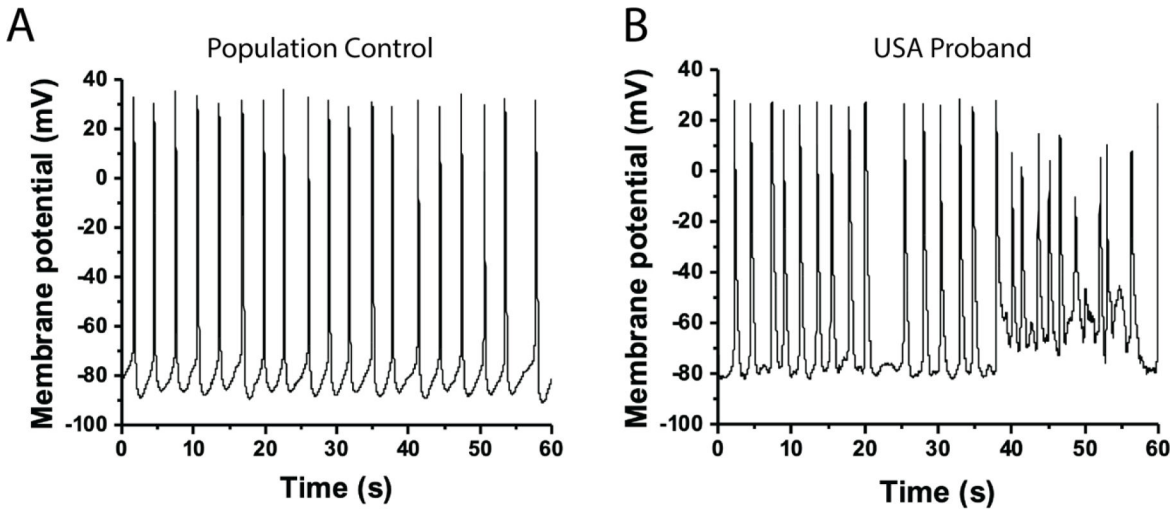

Figure 5. Spontaneous action potentials of *FLNC* c.970–4A>G proband and healthy population control derived iPSC-CMs.

A) Example spontaneous action potentials measured in control iPSC-CMs.

B) Example spontaneous action potentials in *FLNC* proband iPSC-CMs.

Table 1.

Clinical Phenotypes of c.970–4A>G Probands.

Covariate	USA	Australia	Netherlands I	Netherlands II
Sex	Female	Female	Male	Male
Age at Dx	41	11	59	41
Hypertension	Yes	No	No	No
NYHA Class	I	III	II	II
Symptoms	Nausea	Dyspnea	Dyspnea	Dyspnea
ECG (base eval)	Sinus	Sinus	Sinus	Sinus
Echo				
LVEDD, cm	-	7	-	6.8
LVEF, %	-	27	-	15
MRI				
LVEF, %	54	8	30	35
RVEF, %	57	-	44	43
LGE	-	-	Non-specific fibrosis	3% LV
Arrhythmias				
Atrial	SVT	-	-	-
Ventricular	PVCs, NSVT	PVCs, NSVT	VF	PVCs