The role of genetics in inherited cardiomyopathies

Sophie Hespe

BMedSci

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Statement of originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Name: Sophie Hespe

Signature:

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Abstract

While the role of genetics in inherited cardiomyopathies has been recognised as a diagnostic tool for decades, utility of genetics as a prognostic and/or therapeutic tool is relatively new. This thesis takes a gene-specific approach to demonstrate the role genetics can play beyond diagnosis and family screening, and demonstrates gaps and limitations of our knowledge at present in this highly dynamic field.

A review of the utility of genetics in diagnosis, prognosis, and clinical management in inherited cardiomyopathies was conducted. This identified genotype-specific disease sub-groups with strong evidence supporting the use of genetics in clinical management and highlighting that at present the spectrum of clinical utility is not reflected in current guidelines.

A case series of *FLNC* truncating variants is presented and confirms a distinct left-sided arrhythmogenic cardiomyopathy phenotype with high risk of severe cardiac outcomes including end-stage heart failure and sudden cardiac death. This presents an exciting example of insights that can be gleaned from better understanding genotype-specific disease sub-groups, with benefit for patient management and outcomes.

A cohort of patients with heterozygous *ALPK3* loss-of-function variants is described and highlights potential issues that should be considered before this mechanism is definitively associated as a monogenic cause of hypertrophic cardiomyopathy. This includes variable expression with potential for considerable incomplete penetrance, as well as challenges identified due to transcript evolution and correction.

Classification of inherited cardiomyopathies to specific gene and variant type is increasing the value of genetic testing beyond family screening and quickly becoming an important tool in improving diagnosis and management of patients. Although, there are significant gaps in current knowledge which need to be addressed, and limitations due to the rapidly evolving nature of the field.

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List of Abbreviations and Acronyms

ACM	Arrhythmogenic Cardiomyopathy
ACMG	American College of Medical Genetics and Genomics and Association of Molecular Pathologists
AF	Atrial Fibrillation
ALVC	Arrhythmogenic Left Ventricular Cardiomyopathy
ARVC	Arrhythmogenic Right Ventricular Cardiomyopathy
AV	Atrioventricular
BrS	Brugada Syndrome
CCD	Cardiac Conduction Disease
ClinGen	Clinical Genome Resource
CM	Inherited Cardiomyopathies
CMR	Cardiac Magnetic Resonance Imaging
CPVT	Catecholaminergic Polymorphic Ventricular Tachycardia
DCM	Dilated Cardiomyopathy
ECG	Electrocardiogram
EF	Ejection Fraction
*FLNC*tv	*FLNC* Truncating Variants
gnomAD	Genome Aggregation Database
HCM	Hypertrophic Cardiomyopathy
HCVD GCEP	Hereditary Cardiovascular Disorders Gene Curation Expert Panel
ICD	Implantable Cardioverter-Defibrillator
IVCB	Intraventricular Conduction Block
L	Pathogenic
LBBB	Left Bundle Branch Block
LGE	Late Gadolinium Enhancement
LOF	Loss-of-Function
LP	Likely Pathogenic
LQTS	Long QT Syndrome
LV	Left Ventricle
LVEDDi	Left Ventricle End Diastolic Diameter Indexed
LVEDVi	Left Ventricle End Diastolic Volume Indexed
LVEF	Left Ventricle Ejection Fraction
LVESDi	Left Ventricle End Systolic Diameter Indexed
LVH	Left Ventricle Hypertrophy
LVNC	Left Ventricular Non-Compaction Cardiomyopathy
NMD	Nonsense Mediated Decay
NSVT	Non-Sustained Ventricular Tachycardia
NYAH	New York Heart Association
RBBB	Right Bundle Branch Block
RCM	Restrictive Cardiomyopathy
RV	Right Ventricle

RVEDVi	Right Ventricle End Diastolic Volume Indexed
RVEF	Right Ventricle Ejection Fraction
SADS	Sudden Arrhythmic Death Syndrome
SCD	Sudden Cardiac Death
SQTS	Short QT Syndrome
SVC	Superior Vena Cava
SVT	Supraventricular Tachycardia
VA	Ventricular Arrhythmia
VT	Ventricular Tachycardia
VUS	Variant of Uncertain Significance

Publications and Presentations

Publications

1. Stafford F, Krishnan N, Richardson E, Butters A, **Hespe S**, Burns C, Gray B, Medi C, Nowak N, Isbister JC, Raju H, Richmond D, Ryan MP, Singer E, Sy RW, Yeates L, Bagnall RD, Semsarian C, Ingles J. The role of genetic testing in diagnosis and care of inherited cardiac conditions in a specialised multidisciplinary clinic. *Genome Med*. 2022;14(1):145. doi:10.1186/s13073-022-01149-0

Presentations

Chapters and work arising from this thesis have been presented in the following forums.

Oral

1. **Hespe S**. Cardiac patients with *FLNC* truncating variants: a distinct phenotype. *St Vincent's Campus Research Symposium*, Sydney AUS, February 2023

Poster

2. **Hespe S**, Yeates L, Krishnan N, Isbister JC, Duflou J, Puranik R, Bagnall RD, Semsarian C, Gray B, Ingles J. Patients with filamin-c (*FLNC*) truncating variants attending a specialized cardiac genetic clinic. *St Vincent's Campus Research Symposium*, Sydney AUS, February 2023

3. **Hespe S**, Yeates L, Krishnan N, Isbister JC, Duflou J, Puranik R, Bagnall RD, Semsarian C, Gray B, Ingles J. Patients with filamin-c (*FLNC*) truncating variants attending a specialized cardiac genetic clinic. Sydney Cardiovascular Symposium, Sydney AUS, December 2022

4. **Hespe S**, Yeates L, Krishnan N, Isbister JC, Duflou J, Puranik R, Bagnall RD, Semsarian C, Gray B, Ingles J. Patients with filamin-c (*FLNC*) truncating variants attending a specialized cardiac genetic clinic. Human Genetics Society of Australasia Annual Scientific Meeting, Perth AUS, November 2022

Chapter 1

Chapter 1: Introduction and Background: The role of genetic testing in management and prognosis of individuals with inherited cardiomyopathies (Review)

Preface

This chapter is a review of the current evidence of the role of genetics in inherited cardiomyopathies. This chapter establishes the foundation of this thesis, with the overall aim of further clarifying the current role of genetics in inherited cardiomyopathies by gene-specific examples.

Aims:

1. Describe the current role of genetics in inherited cardiomyopathies
    a. Identify examples of gene-specific disease subgroups, with evidence for clinical utility
    b. Describe current clinical utility of genetic results
    c. Identify gaps and limitations of genetic results

This chapter will be submitted for publication. Author contributions to this paper are outlined in the ensuing author contribution statement.

Author contribution statement

Hespe S, Gray B, Puranik R, Peters S, Sweeting J, Ingles J. The role of genetic testing in management and prognosis of individuals with inherited cardiomyopathies.

The Individual roles of co-authors are listed below:

Task	**Role of co-author**
Developing the concept of the study	SH, JI
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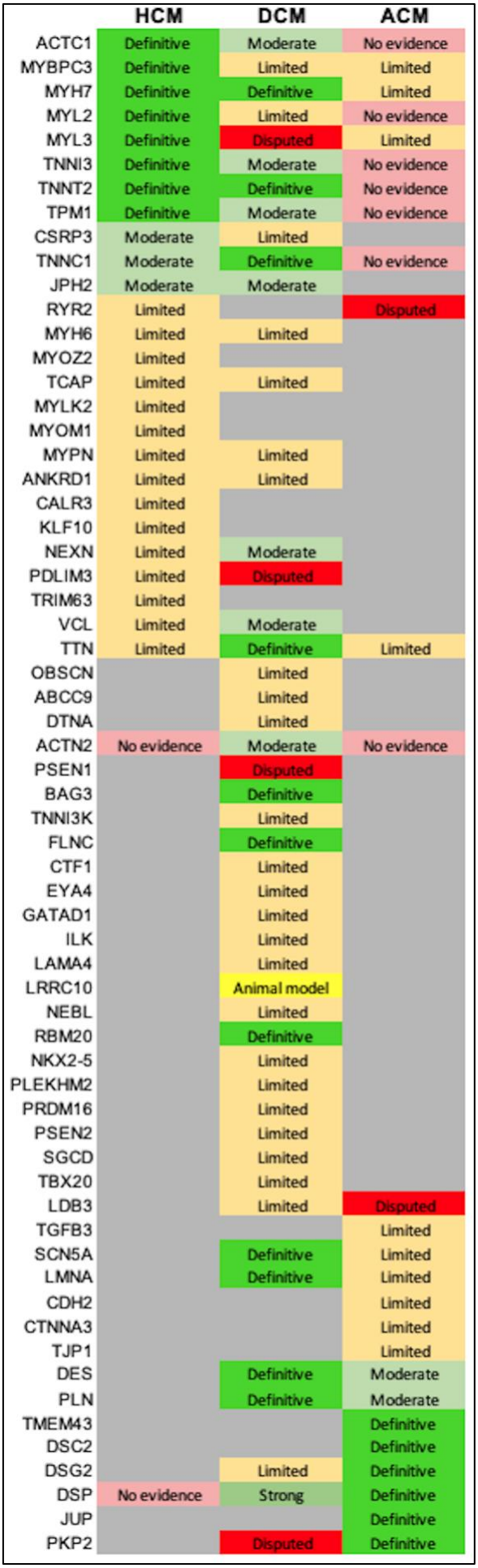
Introduction

Inherited cardiomyopathies (CM) are a heterogeneous group of heart muscle conditions including arrhythmogenic cardiomyopathy (ACM), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM) and left ventricular non-compaction cardiomyopathy (LVNC). They are typically inherited in an autosomal dominant pattern and show marked clinical and genetic heterogeneity due to incomplete penetrance and variable expressivity.[1] Disease classification has traditionally been based on clinical characteristics, though this does not always align with genotype. For example, individuals with pathogenic truncating variants in *DSP* can present with apparent features of ACM or DCM,[2] and variants in *SCN5A* are associated with both DCM and inherited arrhythmia syndrome cross-over phenotypes.[3] Furthermore, several cardiomyopathy genes associated with severe or arrhythmic presentations now have specific management recommendations. This has highlighted the deficiencies in focusing solely on phenotype-derived differentiation to “genotype-associated syndromes”.[4] While there are well described challenges of genetic testing, understanding the role of genotype in patient management is increasingly required. Here we describe the current state of genetic testing in the diagnosis, management and prognosis of individuals with inherited cardiomyopathies. We take a gene-by-gene approach, describing the evidence for genotype-phenotype association and future directions.

Clinical diagnosis of inherited cardiomyopathies

The marked phenotypic heterogeneity among inherited cardiomyopathies means reaching a clinical diagnosis can, at times, be a challenge. HCM, the most common inherited cardiomyopathy, is characterised by asymmetric or less commonly, concentric hypertrophy of the left ventricle (LV)(≥15mm) in the absence of loading conditions, usually with normal

or increased systolic function and normal or reduced chamber size.[5] DCM is typically characterized by the dilation of both ventricles (or the LV in isolation) with associated impaired systolic function (defined by an ejection fraction of <50% in the LV), with the exclusion of reversible causes of LV dysfunction and/or dilation.[6,7] ACM results in progressive fibro-fatty replacement of the right and left ventricular myocardium.[8,9] Classical arrhythmogenic right ventricular cardiomyopathy (ARVC), a sub-type of ACM, is diagnosed by the fulfilment of two major, or one major and two minor, or four minor criteria from different categories of the 2010 ARVC Task Force Criteria.[10] The six categories include; global or regional dysfunction and structural alterations, tissue characterization of wall, repolarization abnormalities, depolarization/conduction abnormalities, arrhythmias, and family history. More recently in 2020, the Padua criteria for ACM were developed to encompass left sided and biventricular disease, however these are yet to be validated and incorporated into guidelines.[11] For the purpose of this review, ACM describes ARVC, arrhythmogenic left ventricular cardiomyopathy (ALVC) and biventricular disease. LVNC is defined by deep trabeculations in the myocardium of the LV with right ventricular (RV) involvement in 22-38% of cases.[12] The LVNC phenotype has been demonstrated to be more prevalent than previously known,[13] and is often a physiological finding, for example in pregnancy or with endurance exercise.[14] LVNC is pathological when occurring in association with another cardiomyopathy, arrhythmias or as part of a syndrome.[13] RCM is characterized by the non-dilation of the left or right ventricle with diastolic dysfunction, which can be heterogeneous in aetiology, from acquired to inherited.[15]



	HCM	DCM	ACM
ACTC1	Definitive	Moderate	No evidence
MYBPC3	Definitive	Limited	Limited
MYH7	Definitive	Definitive	Limited
MYL2	Definitive	Limited	No evidence
MYL3	Definitive	Disputed	Limited
TNNI3	Definitive	Moderate	No evidence
TNNT2	Definitive	Definitive	No evidence
TPM1	Definitive	Moderate	No evidence
CSRP3	Moderate	Limited	
TNNC1	Moderate	Definitive	No evidence
JPH2	Moderate	Moderate	
RYR2	Limited		Disputed
MYH6	Limited	Limited	
MYOZ2	Limited		
TCAP	Limited	Limited	
MYLK2	Limited		
MYOM1	Limited		
MYPN	Limited	Limited	
ANKRD1	Limited	Limited	
CALR3	Limited		
KLF10	Limited		
NEXN	Limited	Moderate	
PDLIM3	Limited	Disputed	
TRIM63	Limited		
VCL	Limited	Moderate	
TTN	Limited	Definitive	Limited
OBSCN		Limited	
ABCC9		Limited	
DTNA		Limited	
ACTN2	No evidence	Moderate	No evidence
PSEN1		Disputed	
BAG3		Definitive	
TNNI3K		Limited	
FLNC		Definitive	
CTF1		Limited	
EYA4		Limited	
GATAD1		Limited	
ILK		Limited	
LAMA4		Limited	
LRRC10		Animal model	
NEBL		Limited	
RBM20		Definitive	
NKX2-5		Limited	
PLEKHM2		Limited	
PRDM16		Limited	
PSEN2		Limited	
SGCD		Limited	
TBX20		Limited	
LDB3		Limited	Disputed
TGFB3			Limited
SCN5A		Definitive	Limited
LMNA		Definitive	Limited
CDH2			Limited
CTNNA3			Limited
TJP1			Limited
DES		Definitive	Moderate
PLN		Definitive	Moderate
TMEM43			Definitive
DSC2			Definitive
DSG2		Limited	Definitive
DSP	No evidence	Strong	Definitive
JUP			Definitive
PKP2		Disputed	Definitive

Figure 1. **Gene-disease classifications.** Clinical validity of genes curated via ClinGen Gene Curation Expert Panels for hypertrophic cardiomyopathy (HCM), arrhythmogenic cardiomyopathy (ACM) and dilated cardiomyopathy (DCM).

Cardiac genetic testing

Genetic testing for inherited cardiomyopathies is a two-step process; firstly, sequencing data for known disease-associated genes is analysed to identify a genetic variant in the index case (proband). If the genetic variant has sufficient evidence to be considered causative of disease, then it will be classified as likely pathogenic or pathogenic (LP/P).[16] A variant with insufficient or conflicting evidence for pathogenicity will be considered a variant of uncertain significance and cannot be used for cascade testing in the family. If a LP/P variant is identified, then cascade genetic testing can be offered to at-risk relatives to test for the presence or absence of this genetic variant, and to assess whether they are at risk of developing disease.

Inherited cardiomyopathies show marked genetic heterogeneity, with a large number of implicated genes and disease mechanisms. The challenges with variant classification have been well described,[17] but recent efforts to systematically evaluate gene-disease relationships have simplified variant curation to a smaller list of clearly disease-causing genes. Gene curation involves evaluation of genetic and experimental data for each gene-disease relationship, and includes work done by both ClinGen and PanelApp.[18,19] ClinGen gene curations have now been completed for HCM, DCM and ACM (Figure 1).[18,20–22]

While historically family cascade genetic testing has been considered as the key utility of genetic testing for inherited cardiomyopathies, there is increasing evidence to support the role of genotype in more precisely guiding diagnosis, management decisions and understanding prognosis.[23] In the next section we review the current evidence for the role of genetic testing in guiding prognosis and management of individuals with inherited cardiomyopathies and LP/P variants in genes definitively associated with disease (Table 1).

Table 1: ClinGen assessed definitive disease-causing genes and their most commonly associated phenotype

Disease	Gene	Variant type	Clinical phenotype
ACM	*DSC2*	Any, mostly biallelic	Right sided ACM with systolic dysfunction
	DSG2	Any	Classical right sided ACM with higher risk of heart failure
	DSP	Any	Left dominant/Biventricular ACM/DCM with higher arrhythmic risk (Carvajal Syndrome [truncating autosomal recessive] – with woolly palmoplantar keratoderma)
	JUP	Mostly truncations, few missense	Classical right sided ACM (Naxos Syndrome [truncating autosomal recessive] – with woolly palmoplantar keratoderma)
	PKP2	Any	Classical right sided ACM
	TMEM43	Missense – p.S358L	Sever biventricular ACM with high risk of sudden cardiac death and heart failure
DCM	*BAG3*	Mostly truncations	Early on-set, heart failure-oriented DCM
	DES	Any	Desminopathy – Cardiac and skeletal myopathy
	FLNC	Truncating Missense	Arrhythmic DCM/ left dominant ACM HCM
	LMNA	Any	DCM with conduction disease and higher arrhythmic risk
	PLN	Truncating	DCM with higher risk for malignant ventricular arrhythmias
	RBM20	Truncation & missense	Aggressive DCM; young onset, higher arrhythmic risk and mortality
	SCN5A	Any	Arrhythmic (including supraventricular arrhythmias) DCM
	TNNC1	Missense	Classical DCM
	TTN	Truncation	Classical DCM with higher arrhythmic risk
DCM & HCM	*MYH7*	Any	HCM & DCM
	TNNT2	Any	HCM & DCM
HCM	Sarcomere positive	Any	HCM

Abbreviations: ACM, arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy.

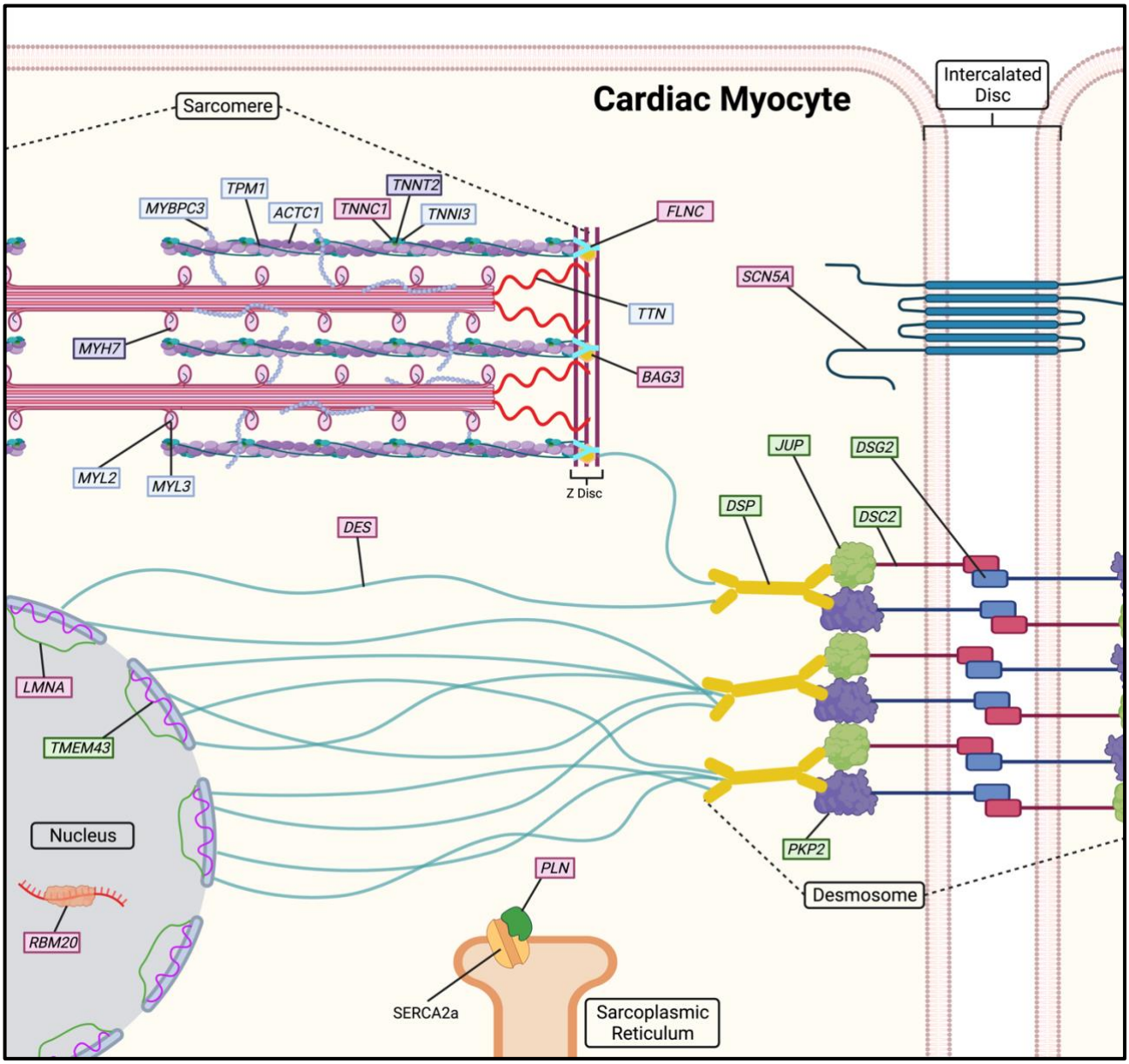


Figure 2. Main structural proteins of the cardiac myocyte encoded by genes definitively associated with the inherited cardiomyopathies. Arrhythmogenic cardiomyopathy genes in green, dilated cardiomyopathy genes in pink, dilated and hypertrophic cardiomyopathy in purple, and hypertrophic cardiomyopathy in blue. Created with BioRender.com.

Arrhythmogenic cardiomyopathy

ARVC was first described as a disease of the desmosome, and at present many definitive ACM genes code for proteins that form part of the cardiac desmosome (Figure 2). Cardiac desmosomes are structures that function to maintain cardiomyocyte adhesion. The pathogenesis of desmosomal ACM is understood as the loss of adhesion between myocytes causing detachment, inflammation and cell death, followed by fibrofatty replacement.[9] As

ACM is currently diagnosed using the 2010 Taskforce criteria,[10] many ALVC and biventricular cases do not meet criteria.[24]

Desmocollin-2 (DSC2)

DSC2 encodes a desmosomal protein responsible for mediating cardiac myocyte adhesion, and accounts for approximately 1-15% of ACM cases where a LP/P variant is identified.[25] The gene was first implicated in a cohort study of 77 probands with ACM where two heterozygous variants in four probands were identified.[26] However, since this cohort study, variants have more often been associated in a biallelic state.[27,28] The phenotype is typically classical right-sided ACM, though left sided disease has also been described.[29]

Desmoglein-2 (DSG2)

DSG2 encodes a specific calcium-dependent cadherin which functions as part of the desmosome to mediate cell-to-cell adhesion.[30] It is a causative gene for ACM and accounts for approximately 2-20% of cases where a LP/P variant is identified,[25,31] with a higher prevalence in the East Asian subpopulation (15.3%).[32]

In the French national ARVC registry, LP/P variants in *DSG2* were associated with greater adverse heart failure-related outcomes, as compared to those with variants in *PKP2,* another important ACM gene.[33] Of 118 patients, 27 (23%) had LP/P variants in *DSG2* and 91 (77%) in *PKP2*. They showed that those with pathogenic *DSG2* variants had significantly more LV dysfunction at age of diagnosis. In addition, they demonstrated significantly higher risk and frequency of cardiac transplant and heart failure-related death as compared to those with variants in *PKP2*. The authors concluded that assessment for early heart failure treatment and careful hemodynamic surveillance should be implemented for *DSG2* carriers.

The homozygous *DSG2* founder variant (p.Phe531Cys), first reported in Fuwai, China, has been shown to lead to a predominantly heart failure phenotype. Of 118 probands described in a series of ARVC cases in China, eight had the homozygous variant. Family screening showed 100% penetrance of ACM for those homozygous for the variant, with heart failure from a young age and bi-ventricular involvement common.[32]

Desmoplakin (DSP)

DSP encodes desmoplakin, a protein that maintains structural integrity in cell to cell adhesion, by anchoring intermediate filaments to the cardiac desmosome.[34] Heterozygous variants have been associated with ALVC, often clinically diagnosed as DCM, and defined by a characteristic sub-epicardial ring-like scar.[35,36] Biallelic variants are associated with Carvajal syndrome, which includes palmoplantar keratoderma, woolly hair, and left-sided ACM.[34] *DSP* accounts for approximately 3-20% of ACM cases where a LP/P variant is identified.[25]

LP/P variants in *DSP* are most often truncating, putative loss-of-function variants. Carriers bear higher arrhythmic risk than those with variants in other desmosomal genes.[37] Truncating *DSP* variants are associated with aggressive arrhythmic phenotypes with a great risk for sudden cardiac death (SCD), even in the absence of severe systolic dysfunction and it has been suggested patients may benefit from an implantable cardioverter-defibrillator (ICD).[38] Another study found those with variants in *DSP* had more than 4 times the occurrence of LV dysfunction and HF compared to those with variants in *PKP2.*[39] A large cohort of patients with *DSP* variants (n=107), characterized *DSP*-cardiomyopathy as sporadic myocardial injury with LV fibrosis that precedes systolic dysfunction, and frequent VAs showing a distinct form of ACM, supporting the need for gene-specific approach to risk stratification in these cases.[2] More recently, the largest series of

DSP truncating variants reported to date showed greater lifetime risk of VA for those with truncating variants in the nonsense mediated decay competent regions, compared to those with variants in the last exon, suggesting haploinsufficiency may lead to more severe outcomes.[36]

Plakoglobin (JUP)

JUP, the first gene associated with ACM, encodes a protein important in cell-to-cell adhesion in connecting with intermediate filaments of desmin as part of the desmosome and the actin skeleton in the adherence junctions.[40] Variants in *JUP* account for approximately 0-1% (higher in Naxos, Greece) of ACM cases where a LP/P variant is identified.[25] *JUP* variants are most often biallelic, including homozygous variants, and cause Naxos disease, a syndromic phenotype of right-sided ACM accompanied by woolly hair and keratosis of the extremities. A comparison study of ACM patients with *PKP2* variants and biallelic *JUP* variants showed that clinical disease expression did not significantly differ.[41] Autosomal dominant variants have also been reported in cases of right sided ACM without syndromic features.[42]

Plakophilin 2 (PKP2)

PKP2 encodes a protein that aids cell-to-cell adhesion through lateral stabilization and intracellular signalling through the regulation of multiple molecular pathways.[43] LP/P variants in *PKP2* are the most common cause of classical right sided ACM, accounting for approximately 20-46% of cases where a LP/P variant is identified.[25]

PKP2 variants, specifically truncating variants[44] have been reported to have lower penetrance and better prognosis than individuals without LP/P *PKP2* variants in multiple studies.[39,45,46] A recent study in a Polish cohort of 56 ACM patients (28 with *PKP2* variants)

showed patients carrying a *PKP2* variant were younger at diagnosis, had higher LV ejection fraction (LVEF), and were less likely to present with heart failure symptoms, have LV involvement and death or cardiac transplant.[46] Reports of SCD and VA without overt structural disease have been associated with LP/P *PKP2* truncating variants, highlighting arrhythmic risk prior to onset of structural features.[47,48] However, studies using healthy population data have shown that identification of LP/P *PKP2* variants as secondary findings showed limited incidence of overt disease.[49,50]

Transmembrane protein 43 (TMEM43)

TMEM43 encoding transmembrane protein 43 is thought to have a role in the maintenance of nuclear envelope structure[51] as well as an element of an adipogenesis pathway, though its function is not fully understood.[52] One missense variant in *TMEM43* (p.S358L), identified in a founder population in Newfoundland, Canada causes an ACM with a high incidence of VA and severe heart failure.[53] The p.S358L variant is highly penetrant[52] and accounts for approximately 0-2% of ACM cases (higher in in Newfoundland) where a LP/P variant is identified.[25]

A cohort study from 15 families with 285 p.S358L carriers and 154 unaffected individuals describes the severe phenotype.[53] Affected males were hospitalized four times more often than affected females and died at a younger age; the median age of death in affected males was 45 years. Holter monitoring was the most clinically useful test with ventricular ectopy occurring early and frequently in the affected group. In a long term study of 24 families, with 148 variant carriers and 148 matched controls (males and females >30 years of age), those with a primary prophylactic ICD showed greater survival than controls without the p.S358L variant.[54] Another study of 80 p.S358L carriers found high-level exercise in the year before ICD implantation conveyed a 9.1 times increased risk of appropriate ICD discharge, as

compared to those that exercised at a moderate level.[55] Evidence from patients with the p.S358L variant highlight one of the few examples where a specific genetic variant may guide management of patients, including lifestyle changes and ICD implantation for VA risk.

Dilated cardiomyopathy

The genetic architecture of heritable DCM is highly heterogenous with >100 genes historically associated with disease and encoding a wide range of proteins with different functions; ion channels, transcription factors, as well as desmosomal, nuclear and, sarcomeric proteins.[56] Many of these genes have been linked to DCM in the context of syndromic of muscular disease. Recently, the ClinGen DCM Gene Curation Expert Panel evaluated 51 genes associated with a predominantly isolated DCM phenotype (non-syndromic) and identified 19 genes with definitive or strong evidence.[21]

BCL2-associated athanogene 3 (BAG3)

BAG3 encodes a co-chaperone of heat shock proteins that localize to the Z-disc.[57] It has been identified as a causative gene for DCM and accounts for approximately 1-5% of familial DCM cases.[56]

A Canadian study investigating 21 patients from four families with *BAG3* nonsense variants found *BAG3* carriers had a worse prognosis including a younger age at disease onset compared to DCM patients without *BAG3* variants (37 versus 48 years; p=0.037).[57] A large multicentre DCM cohort reported 129 individuals with a truncating *BAG3* variant with high penetrance in carriers >40 years (80%), low VA event rate (1.5%) and high rate of adverse heart failure events, including cardiac transplant, left ventricular assist device, or heart failure-related death in 27%.[58]

Desmin (DES)

DES encodes a protein that interconnects the Z-discs to maintain structures of the sarcomere.[59] It has been identified as a causative gene for DCM and accounts for approximately 1-2% of familial DCM cases.[56,60] LP/P variants lead to a highly variable phenotype of cardiomyopathy and/or skeletal myopathy, often described as a desminopathy.[61]

A meta-analysis of 159 *DES* variant carriers described the desminopathy phenotype as a combination of both skeletal myopathy and cardiac disease, although cases with isolated neurological or cardiac phenotypes have been reported.[62] There was cardiac involvement in 74% of carriers, with 50% having a cardiomyopathy (17% DCM, 13% unspecified cardiomyopathy 12% RCM, 6% HCM, 1% ACM), a high incidence of conduction system disease and arrhythmias. Death was reported in a quarter of *DES* variant carriers (mean age 49 years), with the majority determined to be due to cardiac causes (74%; 48% heart failure 26% SCD). While patients with a desminopathy can have a poor prognosis, there are also a diverse range of clinical phenotypes making clinical decision-making challenging.

Filamin C (FLNC)

FLNC encodes one of three isoforms (gamma) of filamin C, an actin binding protein also involved in sarcomere stability.[63] Missense variants are associated with an HCM and RCM phenotype, while truncating variants are associated with an arrhythmic form of DCM.[64] *FLNC*-associated DCM resembles a *DSP* phenotype with a typical subepicardial ring of fibrosis seen on cardiovascular magnetic resonance imaging (CMR).[35]

One study reported 23 *FLNC* truncating variants in 28 probands. After both genetic and clinical testing of probands and their families, a characteristic phenotype of LV dilation,

systolic dysfunction, myocardial fibrosis, inferolateral T-wave inversion and low QRS voltage, as well as a history of VAs was reported.[65] There were 40 cases of SCD in 21 of the 28 affected families, 97% of probands had a LVEF <55% (LVEF 34±13%). These findings support the recommendation of prophylactic ICD therapy for patients with truncating *FLNC* variants and impaired LV function (LVEF <45%) or documented non-sustained ventricular tachycardia (NSVT) included in recent guidelines.[8]

Lamin (LMNA)

LMNA encodes lamin A/C, a protein that provides stability of the nuclear pore complexes of the nuclear membrane.[66] Patients with *LMNA* variants have been described to have a hypokinetic non-dilated cardiomyopathy with an overt phase of systolic dysfunction associated with conduction disease as well as VAs in familial DCM and represent 10-15% of familial DCM cases.[56,66]

Among DCM patients, desmosomal and *LMNA* gene variant carriers are at greatest risk for SCD and life-threatening ventricular arrhythmias, regardless of the LVEF.[67] In a cohort comprising 183 patients, LP/P variants were identified in 178 patients where 19 had *LMNA* variants. Poor outcomes included all-cause mortality and heart failure-related death, heart transplantation, or destination left ventricular assist device implantation and SCD/sustained ventricular tachycardia/ventricular fibrillation (SCD/VT/VF) were assessed. The authors concluded that genetic results should be implemented into discussions of standardized management of patients with DCM and variants in *LMNA.*

A meta-analysis of published patients with *LMNA* variants evaluated common clinical characteristics and found dysrhythmias, defined by the presence of conduction system disease, supraventricular arrhythmias and VAs; were reported in 71% of patients, with incidence increasing with age.[68] The contribution of SCD to total mortality in patients with

LMNA variants was more than double that of healthy population (46% and 18.9% respectively). A more recent genotype-phenotype association meta-analysis of all DCM found that frequency of VAs, conduction disease, heart transplantation, and SCD was higher in *LMNA*-DCM compared to sarcomeric DCM.[69] A large European cohort study reported individual risk factors for malignant VAs in *LMNA* variant carriers, with NSVT, LVEF <45% at first clinical visit, male sex, and non-missense variants identified as risk factors.[70] Malignant VAs occurred in patients with at least two of these risk factors, with risk factors demonstrating cumulative increased risk per additional risk factor. Need for an ICD among patients with *LMNA* variants, stratifying by these risk factors, is clinically indicated in recent guidelines.[8,71]

Phospholamban (PLN)

PLN encodes phospholamban, a protein that functions by regulating cardiac contractility through modulating sarcoplasmic reticulum calcium ion (Ca^{2+}) sequestration.[72] It is a causative gene for DCM and left dominant ACM, and accounts for approximately 1% of familial DCM cases.[56]

In the DCM genotype-phenotype association meta-analysis previously mentioned, *PLN* carriers showed a higher prevalence of VAs, cardiac transplantation, and SCD compared to sarcomeric variant carriers.[69] A Dutch multicentre cohort study found that patients with a specific founder variant (p.Arg14del) in *PLN* had a greater risk of malignant arrhythmias and end stage heart failure.[73] Using the family tree mortality ratio method among 403 *PLN* p.Arg14del variant carriers, the standardized mortality ratio was 1.7 (95% confidence interval, 1.4–2.0) with significant excess mortality starting from the age of 25 years. In a median follow-up period of 42 months, 55 (19%) individuals had a first episode of malignant VAs and 33 (11%) had an end-stage heart failure event. As a result, the authors concluded

that patients with p.Arg14del variant with LVEF <45% and sustained or non-sustained ventricular tachycardia, are deemed at high-risk of malignant VAs and end-stage heart failure, and increased cardiac screening should be recommended for carriers of this variant.

RNA binding motif protein 20 (RBM20)

RBM20 encodes for RNA binding motif protein 20, an mRNA splicing regulator. It is a causative gene for DCM and accounts for approximately 1-5% of cases where a pathogenic variant is identified.[56]

Loss-of-function variants in *RBM20* disturb Ca^{2+} handling and lead to proarrhythmic Ca^{2+} release from the sarcoplasmic reticulum. Despite this, most LP/P variants leading to DCM are due to missense variants in specific hot-spot regions.[74] A database of *RBM20* variants associated with cardiomyopathy was compared to variants observed in a healthy population, showing two regions in exons nine and 11 that were significantly enriched for cardiomyopathy associated variants. Another study had a cohort of 40 patients with DCM; 18 had LP/P variants in *RBM20* and were compared to 22 DCM patients with causative *TTN* variants.[75] Patients with pathogenic *RBM20* variants had more VAs, despite a similar left ventricular function. Use of Ca^{2+} channel blockers was found to reduce VA burden in a knockout mouse model, though this has not been evaluated in human studies.

Titin (TTN)

TTN encodes titin, a large protein that functions as a molecular spring, specifically for the restoring force of the cardiac sarcomere.[76] Causative variants in *TTN* are commonly truncations highly enriched in the A band, however they are also found in areas of the I band and M band.[78] With numerous isoforms, ‘percent spliced’ (PSI) is calculated and must be above 90% for the variant to be considered causative. It is the most common genetic cause

of familial DCM and accounts for approximately 30-35% of cases where a pathogenic variant is identified.[56] Reports also suggest a role in early onset-atrial fibrillation.[77]

A multicentre study showed DCM caused by *TTN* truncating variants is characterized by frequent arrhythmia and systolic dysfunction.[78] The most common primary endpoint, with independent predictors of male sex and baseline LVEF, was an end stage heart failure event (68%) and malignant arrhythmias occurring in 38% of cases and commonly associated with severe systolic dysfunction (LVEF ≤35%). Another study found individuals with a *TTN* truncating variant and fibrosis had a greater rate of appropriate ICD discharge compared to DCM patients without a genetic diagnosis (HR, 16.6; 95% CI, 3.5-79.3; p<0.001).[79] Having a *TTN* truncating variant, especially in combination with mid-wall fibrosis confirmed with CMR, may in future aid risk stratification and ICD decision-making in patients with DCM. A challenge in this setting has been historical difficulty in interpreting LP/P *TTN* truncating variants, especially given the high prevalence of truncating variants in the general population,[80] and specifically in individuals from non-European ancestry.[81] Furthermore, *TTN* truncating variants are associated with incomplete penetrance and have been identified in high proportions among those with 'acquired' DCM (e.g. pregnancy[82], chemotherapy[83], alcoholic[84]), suggesting a complex interplay between genetic risk and environmental factors in carriers, which requires further study.

Sodium voltage-gated channel, α subunit 5, (SCN5A)

SCN5A encodes the sodium voltage-gated channel α subunit 5, a protein that functions to control in inward current of sodium ions (Na+).[85] It is a causative gene for DCM and accounts for approximately ~1% of familial DCM cases where a monogenic cause is identified.[56]

A multicentre DCM registry including 338 individuals from 289 families, identified variants in *SCN5A* in 15 individuals from five families.[86] *SCN5A* variant carriers had an arrhythmic DCM

phenotype, with 93% experiencing an arrhythmic event; supraventricular arrhythmia, VT, or conduction disease. A recent systematic review demonstrated that multifocal ventricular ectopic beats are the predominant phenotype associated with gain of function variants in *SCN5A*, and that 50% of individuals had DCM in addition to arrhythmias.[87] It remains unclear whether *SCN5A*-DCM is purely arrhythmia driven, or whether certain *SCN5A* variants increase vulnerability to myocardial injury. A promising discovery is that many cases can be successfully treated with sodium channel blockade, and this represents one of the most successful gene-based treatments to date for an inherited cardiomyopathy.[87,88]

Sarcomere genes (non-TTN)

The sarcomere is the basic contractile unit of the cardiomyocyte.[89] Variants in genes encoding proteins of the cardiac sarcomere cause both HCM and DCM.[90] The pathogenesis of sarcomere variants have opposing effects in HCM and DCM. In HCM, LP/P variants enhance contractility and impair relaxation; while in DCM, LP/P variants impair contractility by depletion of mechanical force generation. Variants in non-*TTN* sarcomere genes collectively account for approximately 20-30% of familial DCM.[56]

Sarcomere genes that definitively associate with DCM include *MYH7*, *TNNT2* and *TNNC1*.[21] Variants in these genes cause a classical DCM. A Spanish cohort study of 180 cardiomyopathy families found that a specific variant (p.Arg92Gln) in *TNNT2* had a significantly worse prognosis than other *TNNT2* variants, or indeed other sarcomere genes, with early disease development, high penetrance, high risk of SCD and development of either HCM or DCM.[91]

Hypertrophic cardiomyopathy

The presence of LP/P sarcomere variants in HCM patients, compared to those considered sarcomere negative, has consistently been reported to increase risk of poor clinical outcomes.[92] Sarcomere genes definitively associated with HCM include *MYH7, TNNT2*, myosin binding protein C [*MYBPC3*], cardiac troponin I [*TNNI3*], α-tropomyosin [*TPM1*], myosin essential light chain [*MYL2*], myosin regulatory light chain [*MYL3*], and actin [*ACTC*], and these account for about 30-60% of HCM cases. Sarcomere genes can be further sub-grouped into thick (*MYH7* and *MYBPC3*) and thin (*TNNT2*, *TNNI3*, *TMP1*, and *ACTC*) filaments. LV dysfunction and heart failure is more commonly associated with thin filaments whereas arrhythmic risk in both groups is comparable.[93] The incidence of atrial fibrillation is reported to be higher in *MYH7* variant carriers.[94]

Data from the international Sarcomeric Human Cardiomyopathy Registry showed the presence of a LP/P sarcomere variant was a predictor of adverse outcomes.[95] Of 4591 patients, 2763 had genetic testing, of which 46% had a LP/P variant in a sarcomere gene. Sarcomere positive patients had a significantly earlier onset of adverse outcomes including VA events, heart failure events, atrial fibrillation, stroke, or death, compared to sarcomere negative patients. The authors concluded that sarcomeric status should be considered in prognostic discussions for individuals with HCM.

Among 155 HCM patients with genetic testing, non-benign variants were identified in 98 patients (49% *MYBPC3* and 35% *MYH7*). *MYH7* positive patients had a significantly earlier age of onset of disease and higher frequency of major cardiac events than *MYBPC3* positive patients.[96] Adverse outcomes were seen in patients with multiple rare variants in both *MYH7* and *MYBPC3* including greater risk of cardiac events, leading the authors to conclude that genetic findings should be considered when determining frequency of clinical surveillance and timings of intervention discussions for individuals with HCM. Multiple rare variants in

sarcomeric genes are also associated with earlier onset and more severe cardiac outcomes regardless of an uncertain variant classification.[97]

Current guideline recommendations incorporating genotype

Of 13 guideline or expert consensus statements for management of the cardiomyopathies, there are seven gene specific therapeutic recommendations that have been published from four documents (Table 2).[8,71,98,99] These relate to *PLN, LMNA*, *FLNC,* and *RBM20* genetic variants and are shown in Table 3. An important area of clinical management that is increasingly genotype-guided relates to exercise recommendations, specifically in sports cardiology and the management of athletes.[100] The Sport Cardiology Section of the European Association of Preventive Cardiology's position statement gave recommendations for athletes genotype positive-phenotype negative for ACM, DCM, and HCM (Table 4), where it was recommended that athletes with ACM desmosomal variants should not participate in competitive sport and limit exercise to leisure activities.[101]

Table 2: Genetic recommendations for management in current guideline documents

Year	Document	Gene specific recommendations
2022	2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure	✗
2022	2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death	✓
2021	2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure	✓
2020	2020 AHA/ACC Guideline for the Diagnosis and Treatment of Patients with Hypertrophic Cardiomyopathy	✗
2019	Multimodality imaging in the diagnosis, risk stratification, and management of patients with dilated cardiomyopathies: an expert consensus document from the European Association of Cardiovascular Imaging	✗
2019	2019 HRS Expert Consensus Statement on Evaluation, Risk Stratification, and Management of Arrhythmogenic Cardiomyopathy	✓
2019	The Cardiac Society of Australia and New Zealand Position Statement on the Diagnosis and Management of Arrhythmogenic Right Ventricular Cardiomyopathy (2019 Update)	✗
2018	Genetic Evaluation of Cardiomyopathy—A Heart Failure Society of America Practice Guideline	✓
2017	Comprehensive multi-modality imaging approach in arrhythmogenic cardiomyopathy—an expert consensus document of the European Association of Cardiovascular Imaging	✗
2016	Diagnosis and Management of Hypertrophic Cardiomyopathy – Position Statement	✗
2016	Diagnosis and Management of Familial Dilated Cardiomyopathy – Position Statement	✗
2015	Eligibility and Disqualification Recommendations for Competitive Athletes with Cardiovascular Abnormalities: Task Force 3: Hypertrophic Cardiomyopathy, Arrhythmogenic Right Ventricular Cardiomyopathy and Other Cardiomyopathies, and Myocarditis	✗
2014	2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy	✗

Table 3: Gene-specific guideline recommendations

Document	Guideline
2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death[97]	ICD implantation should be considered in DCM/ hypokinetic non-dilated cardiomyopathy patients with a LVEF, 50% and ≥2 risk factors (syncope, LGE on CMR, inducible sustained monomorphic ventricular tachycardia at programmed electrical stimulation pathogenic variants in *LMNA*, *PLN*, *FLNC*, and *RBM20* genes).
2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure[98]	*LMNA*, *RBM20, PLN* and *FLN*C variants. Higher risk of sudden cardiac death: early indication for primary prevention by ICD implantation should be considered.
2019 HRS Expert Consensus Statement on Evaluation, Risk Stratification, and Management of Arrhythmogenic Cardiomyopathy[8]	Individuals with *PLN* cardiomyopathy & LVEF<45% or NSVT, ICD is reasonable.
	Individuals with *LMNA* and 2 or more of: LVEF<45%, NSVT, male sex – ICD is reasonable.
	Individuals with *FLNC* ACM & LVEF<45% - ICD is reasonable.
	Individuals with *LMNA* & indication for pacing, an ICD with pacing capabilities is reasonable.
Genetic Evaluation of Cardiomyopathy—A Heart Failure Society of America Practice Guideline[71]	In patients with cardiomyopathy and significant arrhythmia or known risk of arrhythmia (*LMNA*), an ICD may be considered before LVEF falls below 35%.

Abbreviations: ICD, implantable cardioverter-defibrillator; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; LGE, late gadolinium enhancement; CMR, cardiac magnetic resonance imaging; NSVT, non-sustained ventricular tachycardia; ACM, arrhythmogenic cardiomyopathy; ESC, European Society of Cardiology; HRS, Heart Rhythm Society.

Table 4: Recommendations for athletes genotype positive-phenotype negative for cardiomyopathies

Recommendations for participation in competitive and leisure time sport in athletes with cardiomyopathies, myocarditis, and pericarditis: position statement of the Sport Cardiology Section of the European Association of Preventive Cardiology (EAPC)[100]	
Associated disease	**Recommendation**
ACM	Athletes who are genetic carriers of pathogenic ACM-associated desmosomal mutations (even in the absence of phenotypic expression of the disease) should not participate in competitive sports. These athletes should be advised to limit their exercise programs to leisure-time activities and remain under clinical surveillance.
DCM	The G+P- athletes should be assessed to exclude the phenotypic and clinical features of DCM (including CMR, exercise test, and 24-h ECG). In the absence of evidence for DCM, these individuals may be allowed to engage in all competitive sports, with the recommendation to undergo a periodical evaluation, at least annually, in order to early detect the phenotypic expression of the disease.
HCM	G+P- individuals should be assessed to exclude the broader phenotypic and clinical features of HCM (with ECG, CMR, exercise test, and 24-h ECG monitoring). In the absence of phenotypic features of HCM, these athletes may be allowed to engage in all competitive sports.

Abbreviations: ACM, arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; G+P-, genotype positive phenotype negative; CMR, cardiac magnetic resonance; ECG, electrocardiogram.

Conclusion

Traditionally, the predominant role of genetic testing for patients with inherited cardiomyopathies has been to identify a variant to allow cascade genetic testing of at-risk family members. However, identifying LP/P variants in definitive cardiomyopathy genes is increasingly associated with additional benefits in clarifying diagnosis and defining management strategies for individuals and family members. Indeed, we have recently shown among patients with inherited cardiomyopathies and arrhythmia syndromes where a variant was identified, 13% had their final diagnosis changed or clarified in a clinically useful way.[23] Furthermore, the role of genetic testing in diagnosis is highlighted by the identification of LP/P variants in inherited cardiomyopathy genes as a cause of apparent sudden unexplained cardiac arrest and death.[47,48] Understanding the underlying aetiology of disease is an important first step in unlocking management and therapeutic value.

Beyond the diagnostic value of genetic testing for inherited cardiomyopathies, research is continuing to demonstrate distinct gene-specific phenotypes that can help understand prognosis and potentially guide clinical management. For instance, for patients with DCM, several non-sarcomeric genes are associated with high arrhythmic risk while sarcomeric genes tend to be associated with classical heart failure associated DCM. Furthermore, with studies more often using larger, multi-centre patient cohorts which provide robust statistical power for distinct genotype-associated phenotypes (Figure 3), it will become increasingly possible to consider genotype more explicitly in clinical management than what is recommended in current guidelines. It is likely we will see genotype play an even more important role in management and even gene-specific therapies in future.

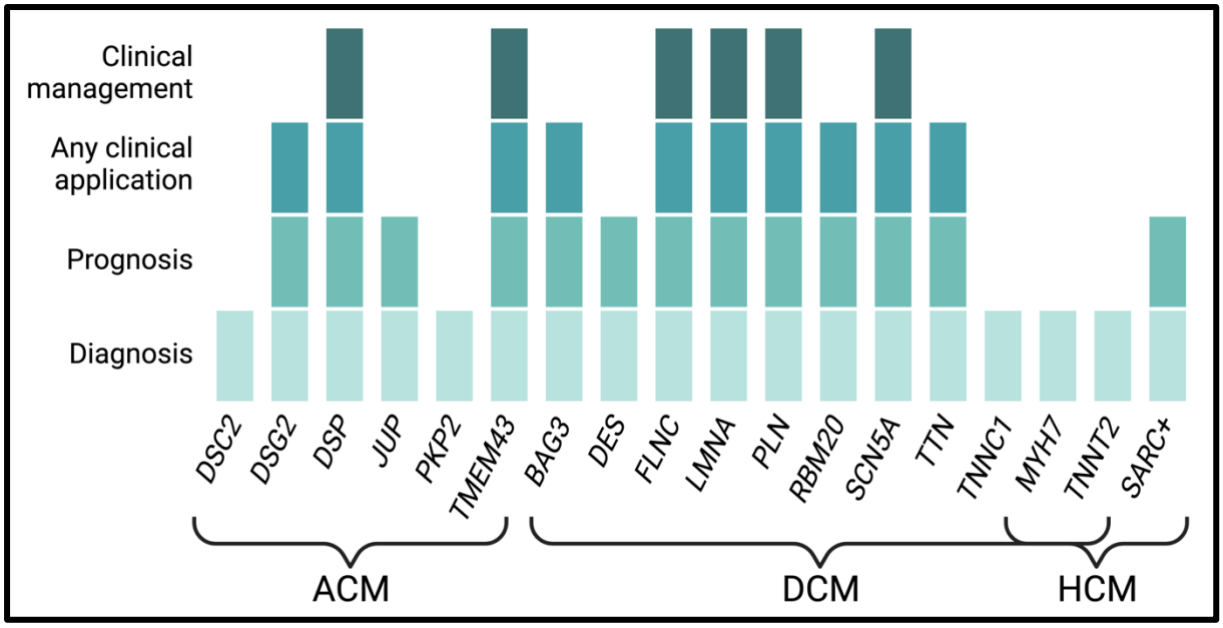


Figure 3. Evidence for the role of genetic testing in diagnosis, prognosis, and clinical management based on current literature.
Abbreviations: ACM, arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy.

In addition to monogenic causes of disease, the role of polygenic variants and non-genetic environmental factors (e.g., exercise, pregnancy, viral infections) and their impact on disease penetrance and expression need to be investigated. A threshold model of ACM pathogenesis has been previously published and describes four types of ACM (autosomal recessive, desmosomal, familial gene elusive, and isolated gene elusive) on a spectrum of mendelian (100% major ACM genes) to multifactorial (combination of genetic modifiers and exercise) aetiology to reach a threshold for ACM onset.[25] This type of approach that considers integrated risk is likely to better explain disease in future.

Rather than sub-grouping all LP/P variants by a single gene, even greater granularity that considers variant type and gene location may have even greater value. For example, studies have shown that *FLNC* missense variants are associated with HCM due to protein misfolding, while truncating variants are associated with DCM due to

haploinsufficiency.[64] Region is important in determining pathogenicity for variants in five sarcomere genes, with hotspots observed to occur more commonly in cases compared to controls,[102] and there has been some suggestion that the of location *MYH7* protein folding could influence disease severity.[103] A recent study found that *DSP* truncating variants occurring in the nonsense mediated decay-competent regions gave a greater lifetime risk of ventricular arrhythmia compared to those with nonsense mediated decay-incompetent truncating variants.[36] Overall, we understand gene region to be important in many ways and this is likely to translate to clinical presentation and outcomes with sufficiently powered cohorts. Indeed, using genotype to help define phenotype is a rapidly evolving space in the field of inherited cardiomyopathies and is likely to expand the role of genetic testing as a means of disease management and for diagnostic and prognostic value.

References

1. McKenna WJ, Judge DP. Epidemiology of the inherited cardiomyopathies. *Nat Rev Cardiol*. 2021;18(1):22-36. doi:10.1038/s41569-020-0428-2

2. Smith ED, Lakdawala NK, Papoutsidakis N, et al. Desmoplakin Cardiomyopathy, a Fibrotic and Inflammatory Form of Cardiomyopathy Distinct From Typical Dilated or Arrhythmogenic Right Ventricular Cardiomyopathy. *Circulation*. 2020;141(23):1872-1884. doi:10.1161/CIRCULATIONAHA.119.044934

3. Wilde AAM, Amin AS. Clinical Spectrum of SCN5A Mutations: Long QT Syndrome, Brugada Syndrome, and Cardiomyopathy. *JACC Clin Electrophysiol*. 2018;4(5):569-579. doi:10.1016/j.jacep.2018.03.006

4. Gray B, Behr ER. New Insights Into the Genetic Basis of Inherited Arrhythmia Syndromes. *Circ Cardiovasc Genet*. 2016;9(6):569-577. doi:10.1161/CIRCGENETICS.116.001571

5. Ommen SR, Mital S, Burke MA, et al. 2020 AHA/ACC Guideline for the Diagnosis and Treatment of Patients With Hypertrophic Cardiomyopathy: Executive Summary: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation*. 2020;142(25):e533-e557. doi:10.1161/CIR.0000000000000938

6. Haas GJ, Zareba KM, Ni H, et al. Validating an Idiopathic Dilated Cardiomyopathy Diagnosis Using Cardiovascular Magnetic Resonance: The Dilated Cardiomyopathy Precision Medicine Study. *Circ Heart Fail*. 2022;15(5):e008877. doi:10.1161/CIRCHEARTFAILURE.121.008877

7. Pinto YM, Elliott PM, Arbustini E, et al. Proposal for a revised definition of dilated cardiomyopathy, hypokinetic non-dilated cardiomyopathy, and its implications for clinical practice: a position statement of the ESC working group on myocardial and pericardial diseases. *Eur Heart J*. 2016;37(23):1850-1858. doi:10.1093/eurheartj/ehv727

8. Towbin JA, McKenna WJ, Abrams DJ, et al. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm*. 2019;16(11):e301-e372. doi:10.1016/j.hrthm.2019.05.007

9. Corrado D, Basso C, Judge DP. Arrhythmogenic Cardiomyopathy. *Circ Res*. 2017;121(7):784-802. doi:10.1161/CIRCRESAHA.117.309345

10. Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the Task Force Criteria. *Eur Heart J*. 2010;31(7):806-814. doi:10.1093/eurheartj/ehq025

11. Corrado D, Perazzolo Marra M, Zorzi A, et al. Diagnosis of arrhythmogenic cardiomyopathy: The Padua criteria. *Int J Cardiol*. 2020;319:106-114. doi:10.1016/j.ijcard.2020.06.005

12. Singh DP, Patel H. Left Ventricular Non-compaction Cardiomyopathy. In: *StatPearls*. StatPearls Publishing; 2022. Accessed October 5, 2022. http://www.ncbi.nlm.nih.gov/books/NBK537025/

13. Ross SB, Jones K, Blanch B, et al. A systematic review and meta-analysis of the prevalence of left ventricular non-compaction in adults. *Eur Heart J*. 2020;41(14):1428-1436. doi:10.1093/eurheartj/ehz317

14. Gati S, Sharma S, Pennell D. The Role of Cardiovascular Magnetic Resonance Imaging in the Assessment of Highly Trained Athletes. *JACC Cardiovasc Imaging*. 2018;11(2 Pt 1):247-259. doi:10.1016/j.jcmg.2017.11.016

15. Muchtar E, Blauwet LA, Gertz MA. Restrictive Cardiomyopathy: Genetics, Pathogenesis, Clinical Manifestations, Diagnosis, and Therapy. *Circ Res*. 2017;121(7):819-837. doi:10.1161/CIRCRESAHA.117.310982

16. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30

17. Bland A, Harrington EA, Dunn K, et al. Clinically impactful differences in variant interpretation between clinicians and testing laboratories: a single-center experience. *Genet Med*. 2018;20(3):369-373. doi:10.1038/gim.2017.212

18. Strande NT, Riggs ER, Buchanan AH, et al. Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. *Am J Hum Genet*. 2017;100(6):895-906. doi:10.1016/j.ajhg.2017.04.015

19. Stark Z, Foulger RE, Williams E, et al. Scaling national and international improvement in virtual gene panel curation via a collaborative approach to discordance resolution. *Am J Hum Genet*. 2021;108(9):1551-1557. doi:10.1016/j.ajhg.2021.06.020

20. Ingles J, Goldstein J, Thaxton C, et al. Evaluating the Clinical Validity of Hypertrophic Cardiomyopathy Genes. *Circ Genom Precis Med*. 2019;12(2):e002460. doi:10.1161/CIRCGEN.119.002460

21. Jordan E, Peterson L, Ai T, et al. Evidence-Based Assessment of Genes in Dilated Cardiomyopathy. *Circulation*. 2021;144(1):7-19. doi:10.1161/CIRCULATIONAHA.120.053033

22. James CA, Jongbloed JDH, Hershberger RE, et al. International Evidence Based Reappraisal of Genes Associated With Arrhythmogenic Right Ventricular Cardiomyopathy Using the Clinical Genome Resource Framework. *Circ Genom Precis Med*. 2021;14(3):e003273. doi:10.1161/CIRCGEN.120.003273

23. Stafford F, Krishnan N, Richardson E, et al. The Role of Genetic Testing in Diagnosis and Care of Inherited Cardiac Conditions in a Specialised Multidisciplinary Clinic. Published online June 13, 2022:2022.02.04.22270485. doi:10.1101/2022.02.04.22270485

24. Corrado D, van Tintelen PJ, McKenna WJ, et al. Arrhythmogenic right ventricular cardiomyopathy: evaluation of the current diagnostic criteria and differential diagnosis. *Eur Heart J*. 2020;41(14):1414-1429. doi:10.1093/eurheartj/ehz669

25. James CA, Syrris P, van Tintelen JP, Calkins H. The role of genetics in cardiovascular disease: arrhythmogenic cardiomyopathy. *Eur Heart J*. 2020;41(14):1393-1400. doi:10.1093/eurheartj/ehaa141

26. Syrris P, Ward D, Evans A, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. *Am J Hum Genet*. 2006;79(5):978-984. doi:10.1086/509122

27. Gerull B, Kirchner F, Chong JX, et al. Homozygous founder mutation in desmocollin-2 (DSC2) causes arrhythmogenic cardiomyopathy in the Hutterite population. *Circ Cardiovasc Genet*. 2013;6(4):327-336. doi:10.1161/CIRCGENETICS.113.000097

28. Brodehl A, Weiss J, Debus JD, et al. A homozygous DSC2 deletion associated with arrhythmogenic cardiomyopathy is caused by uniparental isodisomy. *J Mol Cell Cardiol*. 2020;141:17-29. doi:10.1016/j.yjmcc.2020.03.006

29. Christensen AH, Platonov PG, Jensen HK, et al. Genotype-phenotype correlation in arrhythmogenic right ventricular cardiomyopathy-risk of arrhythmias and heart failure. *J Med Genet*. 2022;59(9):858-864. doi:10.1136/jmedgenet-2021-107911

30. Dieding M, Debus JD, Kerkhoff R, et al. Arrhythmogenic cardiomyopathy related DSG2 mutations affect desmosomal cadherin binding kinetics. *Sci Rep*. 2017;7(1):13791. doi:10.1038/s41598-017-13737-x

31. Celeghin R, Pilichou K. The complex molecular genetics of arrhythmogenic cardiomyopathy. *Int J Cardiol*. 2019;284:59-60. doi:10.1016/j.ijcard.2018.11.004

32. Chen L, Rao M, Chen X, et al. A founder homozygous DSG2 variant in East Asia results in ARVC with full penetrance and heart failure phenotype. *Int J Cardiol*. 2019;274:263-270. doi:10.1016/j.ijcard.2018.06.105

33. Hermida A, Fressart V, Hidden-Lucet F, et al. High risk of heart failure associated with desmoglein-2 mutations compared to plakophilin-2 mutations in arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Eur J Heart Fail*. 2019;21(6):792-800. doi:10.1002/ejhf.1423

34. Favre B, Begré N, Marsili L, van Tintelen JP, Borradori L. Desmoplakin Gene Variants and Risk for Arrhythmogenic Cardiomyopathy. *Circ Genom Precis Med*. 2018;11(9):e002241. doi:10.1161/CIRCGEN.118.002241

35. Augusto JB, Eiros R, Nakou E, et al. Dilated cardiomyopathy and arrhythmogenic left ventricular cardiomyopathy: a comprehensive genotype-imaging phenotype study. *Eur Heart J Cardiovasc Imaging*. 2020;21(3):326-336. doi:10.1093/ehjci/jez188

36. Hoorntje ET, Burns C, Marsili L, et al. Variant Location Is a Novel Risk Factor for Individuals With Arrhythmogenic Cardiomyopathy Due to a Desmoplakin (DSP)

Truncating Variant. *Circ Genom Precis Med*. Published online December 29, 2022:e003672. doi:10.1161/CIRCGEN.121.003672

37. Castelletti S, Vischer AS, Syrris P, et al. Desmoplakin missense and non-missense mutations in arrhythmogenic right ventricular cardiomyopathy: Genotype-phenotype correlation. *Int J Cardiol*. 2017;249:268-273. doi:10.1016/j.ijcard.2017.05.018

38. López-Ayala JM, Gómez-Milanés I, Sánchez Muñoz JJ, et al. Desmoplakin truncations and arrhythmogenic left ventricular cardiomyopathy: characterizing a phenotype. *Europace*. 2014;16(12):1838-1846. doi:10.1093/europace/euu128

39. Bhonsale A, Groeneweg JA, James CA, et al. Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. *Eur Heart J*. 2015;36(14):847-855. doi:10.1093/eurheartj/ehu509

40. Leopoulou M, Mattsson G, LeQuang JA, et al. Naxos disease - a narrative review. *Expert Rev Cardiovasc Ther*. 2020;18(11):801-808. doi:10.1080/14779072.2020.1828064

41. Antoniades L, Tsatsopoulou A, Anastasakis A, et al. Arrhythmogenic right ventricular cardiomyopathy caused by deletions in plakophilin-2 and plakoglobin (Naxos disease) in families from Greece and Cyprus: genotype-phenotype relations, diagnostic features and prognosis. *Eur Heart J*. 2006;27(18):2208-2216. doi:10.1093/eurheartj/ehl184

42. Asimaki A, Syrris P, Wichter T, Matthias P, Saffitz JE, McKenna WJ. A novel dominant mutation in plakoglobin causes arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet*. 2007;81(5):964-973. doi:10.1086/521633

43. Costa S, Cerrone M, Saguner AM, Brunckhorst C, Delmar M, Duru F. Arrhythmogenic cardiomyopathy: An in-depth look at molecular mechanisms and clinical correlates. *Trends Cardiovasc Med*. 2021;31(7):395-402. doi:10.1016/j.tcm.2020.07.006

44. Dries AM, Kirillova A, Reuter CM, et al. The genetic architecture of Plakophilin 2 cardiomyopathy. *Genet Med*. 2021;23(10):1961-1968. doi:10.1038/s41436-021-01233-7

45. Dalal D, James C, Devanagondi R, et al. Penetrance of mutations in plakophilin-2 among families with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *J Am Coll Cardiol*. 2006;48(7):1416-1424. doi:10.1016/j.jacc.2006.06.045

46. Biernacka EK, Borowiec K, Franaszczyk M, et al. Pathogenic variants in plakophilin-2 gene (PKP2) are associated with better survival in arrhythmogenic right ventricular cardiomyopathy. *J Appl Genet*. 2021;62(4):613-620. doi:10.1007/s13353-021-00647-y

47. Ingles J, Bagnall RD, Yeates L, et al. Concealed Arrhythmogenic Right Ventricular Cardiomyopathy in Sudden Unexplained Cardiac Death Events. *Circ Genom Precis Med*. 2018;11(11):e002355. doi:10.1161/CIRCGEN.118.002355

48. Isbister JC, Nowak N, Yeates L, et al. Concealed Cardiomyopathy in Autopsy-Inconclusive Cases of Sudden Cardiac Death and Implications for Families. *J Am Coll Cardiol*. 2022;80(22):2057-2068. doi:10.1016/j.jacc.2022.09.029

49. Carruth ED, Beer D, Alsaid A, et al. Clinical Findings and Diagnostic Yield of Arrhythmogenic Cardiomyopathy Through Genomic Screening of Pathogenic or Likely Pathogenic Desmosome Gene Variants. *Circ Genom Precis Med*. 2021;14(2):e003302. doi:10.1161/CIRCGEN.120.003302

50. Lacaze P, Sebra R, Riaz M, et al. Genetic variants associated with inherited cardiovascular disorders among 13,131 asymptomatic older adults of European descent. *NPJ Genom Med*. 2021;6(1):51. doi:10.1038/s41525-021-00211-x

51. L B, H O. LUMA interacts with emerin and influences its distribution at the inner nuclear membrane. *Journal of cell science*. 2008;121(Pt 4). doi:10.1242/jcs.019281

52. Merner ND, Hodgkinson KA, Haywood AFM, et al. Arrhythmogenic right ventricular cardiomyopathy type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. *Am J Hum Genet*. 2008;82(4):809-821. doi:10.1016/j.ajhg.2008.01.010

53. Hodgkinson KA, Connors SP, Merner N, et al. The natural history of a genetic subtype of arrhythmogenic right ventricular cardiomyopathy caused by a p.S358L mutation in TMEM43. *Clin Genet*. 2013;83(4):321-331. doi:10.1111/j.1399-0004.2012.01919.x

54. Hodgkinson KA, Howes AJ, Boland P, et al. Long-Term Clinical Outcome of Arrhythmogenic Right Ventricular Cardiomyopathy in Individuals With a p.S358L Mutation in TMEM43 Following Implantable Cardioverter Defibrillator Therapy. *Circ Arrhythm Electrophysiol*. 2016;9(3):e003589. doi:10.1161/CIRCEP.115.003589

55. Paulin FL, Hodgkinson KA, MacLaughlan S, et al. Exercise and arrhythmic risk in TMEM43 p.S358L arrhythmogenic right ventricular cardiomyopathy. *Heart Rhythm*. 2020;17(7):1159-1166. doi:10.1016/j.hrthm.2020.02.028

56. Pérez-Serra A, Toro R, Sarquella-Brugada G, et al. Genetic basis of dilated cardiomyopathy. *Int J Cardiol*. 2016;224:461-472. doi:10.1016/j.ijcard.2016.09.068

57. Chami N, Tadros R, Lemarbre F, et al. Nonsense mutations in BAG3 are associated with early-onset dilated cardiomyopathy in French Canadians. *Can J Cardiol*. 2014;30(12):1655-1661. doi:10.1016/j.cjca.2014.09.030

58. Domínguez F, Cuenca S, Bilińska Z, et al. Dilated Cardiomyopathy Due to BLC2-Associated Athanogene 3 (BAG3) Mutations. *J Am Coll Cardiol*. 2018;72(20):2471-2481. doi:10.1016/j.jacc.2018.08.2181

59. Hnia K, Ramspacher C, Vermot J, Laporte J. Desmin in muscle and associated diseases: beyond the structural function. *Cell Tissue Res*. 2015;360(3):591-608. doi:10.1007/s00441-014-2016-4

60. Taylor MRG, Slavov D, Ku L, et al. Prevalence of desmin mutations in dilated cardiomyopathy. *Circulation*. 2007;115(10):1244-1251. doi:10.1161/CIRCULATIONAHA.106.646778

61. Clemen CS, Herrmann H, Strelkov SV, Schröder R. Desminopathies: pathology and mechanisms. *Acta Neuropathol*. 2013;125(1):47-75. doi:10.1007/s00401-012-1057-6

62. van Spaendonck-Zwarts KY, van Hessem L, Jongbloed JDH, et al. Desmin-related myopathy. *Clin Genet*. 2011;80(4):354-366. doi:10.1111/j.1399-0004.2010.01512.x

63. Song S, Shi A, Lian H, Hu S, Nie Y. Filamin C in cardiomyopathy: from physiological roles to DNA variants. *Heart Fail Rev*. 2022;27(4):1373-1385. doi:10.1007/s10741-021-10172-z

64. Verdonschot JAJ, Vanhoutte EK, Claes GRF, et al. A mutation update for the FLNC gene in myopathies and cardiomyopathies. *Hum Mutat*. 2020;41(6):1091-1111. doi:10.1002/humu.24004

65. Ortiz-Genga MF, Cuenca S, Dal Ferro M, et al. Truncating FLNC Mutations Are Associated With High-Risk Dilated and Arrhythmogenic Cardiomyopathies. *J Am Coll Cardiol*. 2016;68(22):2440-2451. doi:10.1016/j.jacc.2016.09.927

66. Captur G, Arbustini E, Bonne G, et al. Lamin and the heart. *Heart*. 2018;104(6):468-479. doi:10.1136/heartjnl-2017-312338

67. Gigli M, Merlo M, Graw SL, et al. Genetic Risk of Arrhythmic Phenotypes in Patients With Dilated Cardiomyopathy. *J Am Coll Cardiol*. 2019;74(11):1480-1490. doi:10.1016/j.jacc.2019.06.072

68. van Berlo JH, de Voogt WG, van der Kooi AJ, et al. Meta-analysis of clinical characteristics of 299 carriers of LMNA gene mutations: do lamin A/C mutations portend a high risk of sudden death? *J Mol Med (Berl)*. 2005;83(1):79-83. doi:10.1007/s00109-004-0589-1

69. Kayvanpour E, Sedaghat-Hamedani F, Amr A, et al. Genotype-phenotype associations in dilated cardiomyopathy: meta-analysis on more than 8000 individuals. *Clin Res Cardiol*. 2017;106(2):127-139. doi:10.1007/s00392-016-1033-6

70. van Rijsingen IAW, Arbustini E, Elliott PM, et al. Risk factors for malignant ventricular arrhythmias in lamin a/c mutation carriers a European cohort study. *J Am Coll Cardiol*. 2012;59(5):493-500. doi:10.1016/j.jacc.2011.08.078

71. Hershberger RE, Givertz MM, Ho CY, et al. Genetic Evaluation of Cardiomyopathy-A Heart Failure Society of America Practice Guideline. *J Card Fail*. 2018;24(5):281-302. doi:10.1016/j.cardfail.2018.03.004

72. MacLennan DH, Kranias EG. Phospholamban: a crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol*. 2003;4(7):566-577. doi:10.1038/nrm1151

73. van Rijsingen IAW, van der Zwaag PA, Groeneweg JA, et al. Outcome in phospholamban R14del carriers: results of a large multicentre cohort study. *Circ Cardiovasc Genet*. 2014;7(4):455-465. doi:10.1161/CIRCGENETICS.113.000374

74. Parikh VN, Caleshu C, Reuter C, et al. Regional Variation in RBM20 Causes a Highly Penetrant Arrhythmogenic Cardiomyopathy. *Circulation: Heart Failure*. 2019;12(3):e005371. doi:10.1161/CIRCHEARTFAILURE.118.005371

75. van den Hoogenhof MMG, Beqqali A, Amin AS, et al. RBM20 Mutations Induce an Arrhythmogenic Dilated Cardiomyopathy Related to Disturbed Calcium Handling. *Circulation*. 2018;138(13):1330-1342. doi:10.1161/CIRCULATIONAHA.117.031947

76. LeWinter MM, Granzier H. Cardiac titin: a multifunctional giant. *Circulation*. 2010;121(19):2137-2145. doi:10.1161/CIRCULATIONAHA.109.860171

77. Vissing CR, Rasmussen TB, Dybro AM, et al. Dilated cardiomyopathy caused by truncating titin variants: long-term outcomes, arrhythmias, response to treatment and sex differences. *J Med Genet*. 2021;58(12):832-841. doi:10.1136/jmedgenet-2020-107178

78. Akhtar MM, Lorenzini M, Cicerchia M, et al. Clinical Phenotypes and Prognosis of Dilated Cardiomyopathy Caused by Truncating Variants in the TTN Gene. *Circ Heart Fail*. 2020;13(10):e006832. doi:10.1161/CIRCHEARTFAILURE.119.006832

79. Di Marco A, Brown PF, Bradley J, et al. Improved Risk Stratification for Ventricular Arrhythmias and Sudden Death in Patients With Nonischemic Dilated Cardiomyopathy. *J Am Coll Cardiol*. 2021;77(23):2890-2905. doi:10.1016/j.jacc.2021.04.030

80. Akinrinade O, Koskenvuo JW, Alastalo TP. Prevalence of Titin Truncating Variants in General Population. *PLoS One*. 2015;10(12):e0145284. doi:10.1371/journal.pone.0145284

81. Haggerty CM, Damrauer SM, Levin MG, et al. Genomics-First Evaluation of Heart Disease Associated With Titin-Truncating Variants. *Circulation*. 2019;140(1):42-54. doi:10.1161/CIRCULATIONAHA.119.039573

82. Ware JS, Li J, Mazaika E, et al. Shared Genetic Predisposition in Peripartum and Dilated Cardiomyopathies. *N Engl J Med*. 2016;374(3):233-241. doi:10.1056/NEJMoa1505517

83. Garcia-Pavia P, Kim Y, Restrepo-Cordoba MA, et al. Genetic Variants Associated With Cancer Therapy-Induced Cardiomyopathy. *Circulation*. 2019;140(1):31-41. doi:10.1161/CIRCULATIONAHA.118.037934

84. Ware JS, Amor-Salamanca A, Tayal U, et al. Genetic Etiology for Alcohol-Induced Cardiac Toxicity. *J Am Coll Cardiol*. 2018;71(20):2293-2302. doi:10.1016/j.jacc.2018.03.462

85. Li W, Yin L, Shen C, Hu K, Ge J, Sun A. SCN5A Variants: Association With Cardiac Disorders. *Front Physiol*. 2018;9:1372. doi:10.3389/fphys.2018.01372

86. McNair WP, Sinagra G, Taylor MRG, et al. SCN5A mutations associate with arrhythmic dilated cardiomyopathy and commonly localize to the voltage-sensing mechanism. *J Am Coll Cardiol*. 2011;57(21):2160-2168. doi:10.1016/j.jacc.2010.09.084

87. Peters S, Thompson BA, Perrin M, et al. Arrhythmic Phenotypes Are a Defining Feature of Dilated Cardiomyopathy-Associated SCN5A Variants: A Systematic Review. *Circ Genom Precis Med*. 2022;15(1):e003432. doi:10.1161/CIRCGEN.121.003432

88. Mann SA, Castro ML, Ohanian M, et al. R222Q SCN5A mutation is associated with reversible ventricular ectopy and dilated cardiomyopathy. *J Am Coll Cardiol*. 2012;60(16):1566-1573. doi:10.1016/j.jacc.2012.05.050

89. Pettinato AM, Ladha FA, Hinson JT. The Cardiac Sarcomere and Cell Cycle. *Curr Cardiol Rep*. 2022;24(6):623-630. doi:10.1007/s11886-022-01682-9

90. Frazier AH, Ramirez-Correa GA, Murphy AM. Molecular mechanisms of sarcomere dysfunction in dilated and hypertrophic cardiomyopathy. *Prog Pediatr Cardiol*. 2011;31(1):29-33. doi:10.1016/j.ppedcard.2010.11.006

91. Ripoll-Vera T, Gámez JM, Govea N, et al. Clinical and Prognostic Profiles of Cardiomyopathies Caused by Mutations in the Troponin T Gene. *Rev Esp Cardiol (Engl Ed)*. 2016;69(2):149-158. doi:10.1016/j.rec.2015.06.025

92. Yotti R, Seidman CE, Seidman JG. Advances in the Genetic Basis and Pathogenesis of Sarcomere Cardiomyopathies. *Annu Rev Genomics Hum Genet*. 2019;20:129-153. doi:10.1146/annurev-genom-083118-015306

93. Coppini R, Ho CY, Ashley E, et al. Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. *J Am Coll Cardiol*. 2014;64(24):2589-2600. doi:10.1016/j.jacc.2014.09.059

94. Lee SP, Ashley EA, Homburger J, et al. Incident Atrial Fibrillation Is Associated With MYH7 Sarcomeric Gene Variation in Hypertrophic Cardiomyopathy. *Circ Heart Fail*. 2018;11(9):e005191. doi:10.1161/CIRCHEARTFAILURE.118.005191

95. Ho CY, Day SM, Ashley EA, et al. Genotype and Lifetime Burden of Disease in Hypertrophic Cardiomyopathy: Insights from the Sarcomeric Human Cardiomyopathy Registry (SHaRe). *Circulation*. 2018;138(14):1387-1398. doi:10.1161/CIRCULATIONAHA.117.033200

96. Mathew J, Zahavich L, Lafreniere-Roula M, et al. Utility of genetics for risk stratification in pediatric hypertrophic cardiomyopathy. *Clin Genet*. 2018;93(2):310-319. doi:10.1111/cge.13157

97. Burns C, Bagnall RD, Lam L, Semsarian C, Ingles J. Multiple Gene Variants in Hypertrophic Cardiomyopathy in the Era of Next-Generation Sequencing. *Circ*

Cardiovasc Genet. 2017;10(4):e001666. doi:10.1161/CIRCGENETICS.116.001666

98. Zeppenfeld K, Tfelt-Hansen J, de Riva M, et al. 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Eur Heart J*. 2022;43(40):3997-4126. doi:10.1093/eurheartj/ehac262

99. McDonagh TA, Metra M, Adamo M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021;42(36):3599-3726. doi:10.1093/eurheartj/ehab368

100. Castelletti S, Gray B, Basso C, et al. Indications and utility of cardiac genetic testing in athletes. *Eur J Prev Cardiol*. 2022;29(12):1582-1591. doi:10.1093/eurjpc/zwac080

101. Pelliccia A, Solberg EE, Papadakis M, et al. Recommendations for participation in competitive and leisure time sport in athletes with cardiomyopathies, myocarditis, and pericarditis: position statement of the Sport Cardiology Section of the European Association of Preventive Cardiology (EAPC). *Eur Heart J*. 2019;40(1):19-33. doi:10.1093/eurheartj/ehy730

102. Walsh R, Mazzarotto F, Whiffin N, et al. Quantitative approaches to variant classification increase the yield and precision of genetic testing in Mendelian diseases: the case of hypertrophic cardiomyopathy. *Genome Med*. 2019;11(1):5. doi:10.1186/s13073-019-0616-z

103. Homburger JR, Green EM, Caleshu C, et al. Multidimensional structure-function relationships in human β-cardiac myosin from population-scale genetic variation. *Proc Natl Acad Sci U S A*. 2016;113(24):6701-6706. doi:10.1073/pnas.1606950113

Chapter 2

Chapter 2: Patients with filamin-c (FLNC) truncating variants attending a specialized cardiac genetic clinic

Preface to the chapter

As noted in chapter 1, genotype-specific disease subgroups can guide and improve clinical management. This chapter presents results of a case study of patients attending a specialised clinic with *FLNC* truncating variants addressing the aim: *'To identify examples of gene-specific disease subgroups, with evidence for clinical utility.'* This chapter will be submitted for publication. Author contributions to this paper are outlined in the ensuing author contribution statement.

Author contribution statement

Hespe S, Yeates L, Krishnan N, Isbister JC, Duflou J, Puranik R, Bagnall RD, Semsarian C, Gray B*, Ingles J*. Patients with filamin-c (*FLNC*) truncating variants attending a specialized cardiac genetic clinic.
*(*Dr Gray and A/Prof Ingles contributed equally to this work)*

The Individual roles of co-authors are listed below:

Task	**Role of co-author**
Developing the concept of the study	SH, JI, BG
Patient review	LY, JD, CS, BG, JI
Acquisition & compilation of case data	SH, LY, JI
Drafting the manuscript	SH
Review & critical comments on manuscript	All Authors

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Disclosures

None.

Introduction

FLNC encodes for filamin C, a protein expressed in Z-discs of cardiac and skeletal muscle, involved in intracellular signalling and mechanical stabilization.[1] Variants in *FLNC* can cause diverse phenotypes with skeletal (myofibrillar or distal myopathy) and/or cardiac (hypertrophic, restrictive, dilated and arrhythmogenic cardiomyopathies) manifestations. Missense variants that interfere with dimerization and folding of the protein lead to protein aggregates in the sarcomere and have been associated with hypertrophic and/or restrictive cardiomyopathy phenotypes (Figure 1).[2,3] *FLNC* presents one of the first examples in cardiac genetics to highlight the value of better characterization of genotype-specific disease sub-groups, with evidence to support genotype-guided recommendations in disease guidelines, specifically stating those with an *FLNC*tv and left ventricular ejection fraction (LVEF) <50% at increased risk of ventricular arrhythmia and for consideration of an implantable cardioverter defibrillator.[44]

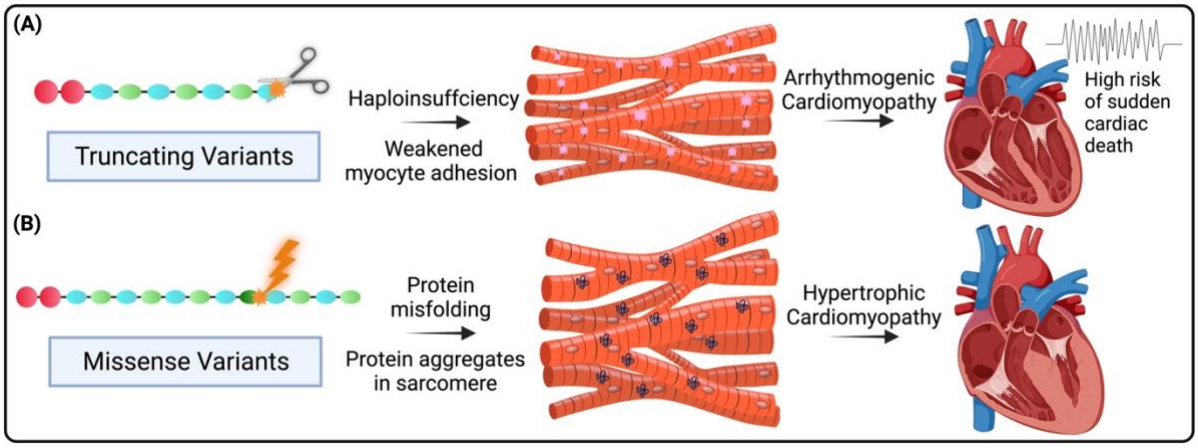


Figure 1. Mechanism of *FLNC* variants. Graphic of understood pathomechanism of variant types; (A) truncating variants causing arrhythmogenic cardiomyopathy (ACM), while (B) missense variants cause hypertrophic and restrictive cardiomyopathies (HCM/RCM). Created with BioRender.com.

FLNC is highly constrained for loss of function variation,[5] with *FLNC* truncating variants (*FLNC*tv) that are predicted to undergo nonsense mediated decay and result in haploinsufficiency recently implicated in arrhythmogenic cardiomyopathy (ACM) and dilated cardiomyopathy (DCM),[1,2,6] due to weakening of the myocyte adhesion. We present our experience of managing families with *FLNC*tv at the specialized Genetic Heart Disease Clinic at Royal Prince Alfred Hospital in Sydney.

Methods

Those attending a specialized multidisciplinary genetic heart disease clinic at Royal Prince Alfred Hospital, who had a likely pathogenic or pathogenic truncating variant in *FLNC*, and provided written informed consent were included. Approximately 1700 unrelated patients have been evaluated in this clinic, including genetic testing, since 2002. This included at-risk relatives attending for clinical screening where the proband was deceased and post-mortem genetic testing had been performed. We reviewed medical records, including clinical history, family history and genetic test reports. Cardiac investigations included echocardiogram, electrocardiogram (ECG) and cardiac magnetic resonance (CMR) imaging. Genetic variants from both research and clinical genetic reports were reviewed, and those with putative loss of function variants, i.e., truncating variants such as insertions or deletions leading to a frameshift or nonsense variants were included (Figure 2). Variants were classified according to the American College of Medical Genetics and Genomics and Association of Molecular Pathologists standards for variant interpretation.[7] Approval was granted by Sydney Local Health District Human Research Ethics Committee and participants, or their next of kin, provided written informed consent (NSW Hearts ethics, AGHDR ethics, SCD family ethics, Genetic Determinants ethics).

*FLNC*tv in ClinVar (date: August 2022) and gnomAD v2.1 (date: August 2022) were extracted and plotted on a gene topology figure along with our case variants. Exons were defined by ensembl transcript: ENST00000325888.13; protein domains by uniprot Q14315 FLNC_HUMAN.

Results

Gene topology

Location of *FLNC*tv along the gene for cases including 120 variants in ClinVar and our 7 reported cases is shown in Figure 2. variants showing exons, defined in ensembl transcript: ENST00000325888.13; protein domains, defined in uniprot Q14315 FLNC_HUMAN; and reported truncating variants in cases (our cohort and ClinVar reports) shown above the gene; and controls (gnomADv2.1, n=42) shown below the gene.

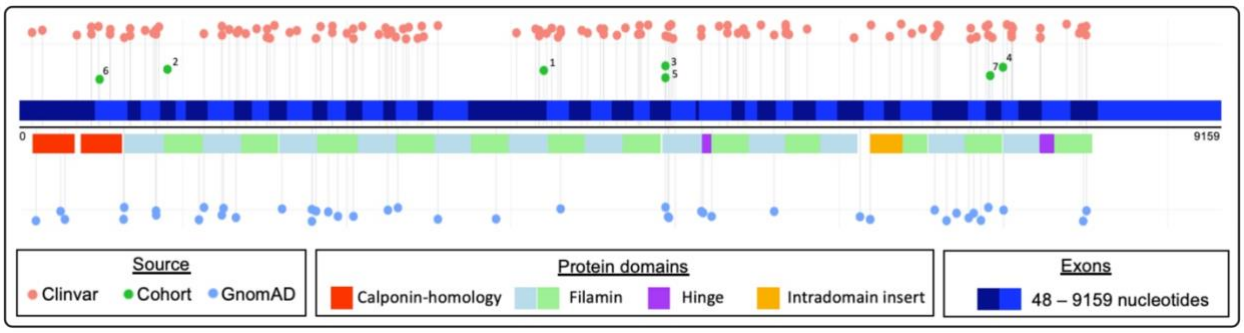


Figure 2. Gene topology of *FLNC* truncating variants (Cases and controls). Gene topology of *FLNC* showing exons, defined in ensembl transcript: ENST00000325888.13; protein domains, defined in uniprot Q14315 FLNC_HUMAN; and reported truncating variants in cases (our cohort and ClinVar reports) shown above the gene; and controls (gnomADv2.1) shown below the gene.

Cases

Case 1: Patient 1 suffered a sudden cardiac death (SCD) aged 38 years while attending to her child. An initial post-mortem examination was performed by a forensic pathologist and cause of death was determined to be unascertained and due to possible cardiac arrhythmia. Macroscopically, the heart showed no abnormalities with the anterior left ventricular (LV) wall measuring 13mm, lateral 10mm and posterior 9mm in thickness. The interventricular septum measured 11mm in thickness and the anterior right ventricular (RV) wall 4mm. Microscopically, sections from the RV and LV showed no significant abnormalities. Post-mortem research-based exome sequencing identified a likely pathogenic deletion resulting in a frameshift and a premature stop codon in *FLNC* c.4000_4015del, p.Ala1334Profs*6, and a missense variant of uncertain significance in *KCNH2* c.2274G>T, p.Gly925Val. The *FLNC* variant is absent in the genome aggregation database (gnomAD) and has not been reported in literature. Post-mortem cardiac histology sections were re-examined by an expert forensic pathologist blinded to the genetic result, who identified areas of fibrosis with fatty infiltration within the LV free wall and septum and a limited chronic inflammatory cell infiltrate in a subendocardial band type distribution in some sections. Possible healing of LV myocardial infarction was noted in a single focus area shown by advanced tissue granulation. There was general hypertrophy of the LV myocytes but with no disarray or unusual nuclear morphology and minimal changes in the RV. Expert cardiac pathologist review concluded that this was a possible case of LV dominant ACM. The family history revealed a first-degree relative who died suddenly aged 42 years with post-mortem evaluation reporting the cause of death as pulmonary oedema but no additional information is available.

Case 2: Patient 2 presented during pregnancy at 27 years of age. She was asymptomatic and underwent cardiac assessment after her father suffered a SCD at 43 years playing cricket, 7 years after a diagnosis of DCM. Initial assessment showed impaired LV function and non-sustained ventricular tachycardia (NSVT) on Holter monitoring. She developed episodes of recurrent ventricular tachycardia (VT) leading to a caesarean section at 33 weeks and subsequently underwent insertion of an implantable cardioverter defibrillator (ICD). She experienced complications with an ICD lead failure which required lead extraction. During her 2nd pregnancy there were further complications from the extraction with superior vena cava (SVC) obstruction, requiring venoplasty initially leading to a hospital admission at 26 weeks of pregnancy until delivery at 32 weeks, followed by SVC repair and reconstruction. She later developed systolic dysfunction, with an LV ejection fraction (LVEF) of 15% and was stabilized on medical therapy. At 40 years of age, she developed worsening heart failure (New York Heart Association; NYHA class III-IV symptoms), leading to an acute hospital admission for inotropic support. Her repeat echocardiogram showed deterioration in LV function (LVEF 30%), with heart failure medications up-titrated and consideration for cardiac transplant. She responded well to medical therapy, stabilizing with improved systolic function (LVEF 42%), NYHA status (Class II), and one run of NSVT over the following 3 years. Research-based exome sequencing identified a likely pathogenic deletion resulting in a frameshift and a premature stop codon in *FLNC,* c.1134_1135delGT, p.Ala380Profs*19. The variant is absent in gnomAD and has not been reported in literature.

Case 3: Patient 3 presented for clinical screening at 29 years due to strong family history of DCM. Initial cardiac assessment revealed normal sinus rhythm with mild ST depression in the lateral leads on ECG. Her echocardiogram showed cardiac

dimensions at the upper limit of normal; LV end diastolic dimension indexed (LVEDDi) 32.7mm/m^2, LV end systolic diameter (LVESD) 23.5mm/m^2, and low normal systolic function (LVEF 52%). Over the next 8 years she remained asymptomatic with serial imaging demonstrating mildly dilated LV with mildly reduced systolic function (LVEF ~46%), leading to a diagnosis of DCM. At 38 years of age she underwent CMR imaging demonstrating normal LV size with mild-moderate impairment of systolic function (LVEF 45%), normal RV size with mild impairment of systolic function (RV ejection fraction, RVEF 49%), and typical basal-mid septal mid-wall late gadolinium enhancement (Figure 3 A&B).[8] Research-based exome sequencing identified a pathogenic insertion resulting in a frameshift and a premature stop codon in *FLNC, c.4926_4927insACGTCACA*, p.Val1643Thrfs*26. The variant has been reported twice in ClinVar, and seen once in gnomAD. There is a positive family history of SCD with her mother dying in her early fifties with DCM and her brother dying at age 26 years from progressive heart failure and DCM, but DNA is not available for cascade genetic testing.

Case 4: Patient 4 presented with general lethargy at 35 years of age and was referred for cardiac investigations. 24-hour Holter monitor showed significant burden of ~10,000 multifocal ventricular ectopic beats (11% total beats) and 12-lead ECG demonstrating the origin of the predominant PVC to be basal-mid inferior LV (superior axis and positive laterally). CMR imaging demonstrated a severely dilated LV (LVEDVi 132ml/m^2) with mildly reduced systolic function (LVEF 46%), as well as mildly dilated RV (RV end diastolic volume, indexed; RVEDVi 120ml/m^2) with low normal systolic function (RVEF 51%), and no late gadolinium enhancement. Research-based exome sequencing identified a pathogenic insertion resulting in a frameshift and a premature stop codon in *FLNC,* c.7496_7497insTGCT, p.Gln2499Hisfs*46. The variant has been

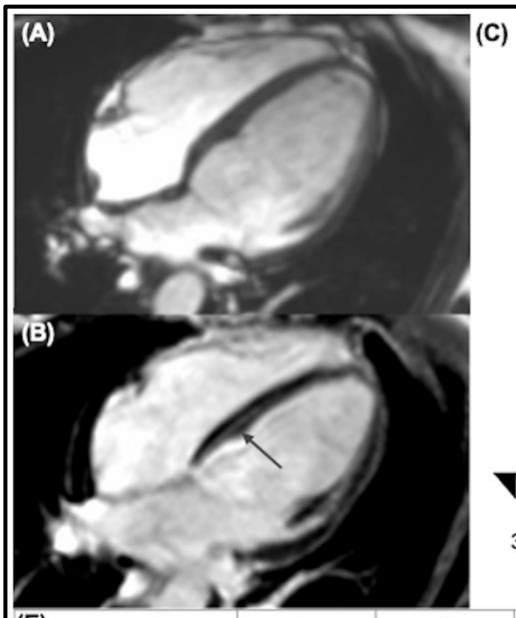
reported once in ClinVar and is absent in gnomAD. The patient's mother has a diagnosis of mild DCM, with bigeminy and couplets on a 24-hour Holter monitoring. Four years after initial presentation, patient 4 has decreased ectopic burden on Holter monitoring (~2000 beats) and further reduction in systolic function (LVEF 38%) and dilation (LVEDVi 136ml/m^2).

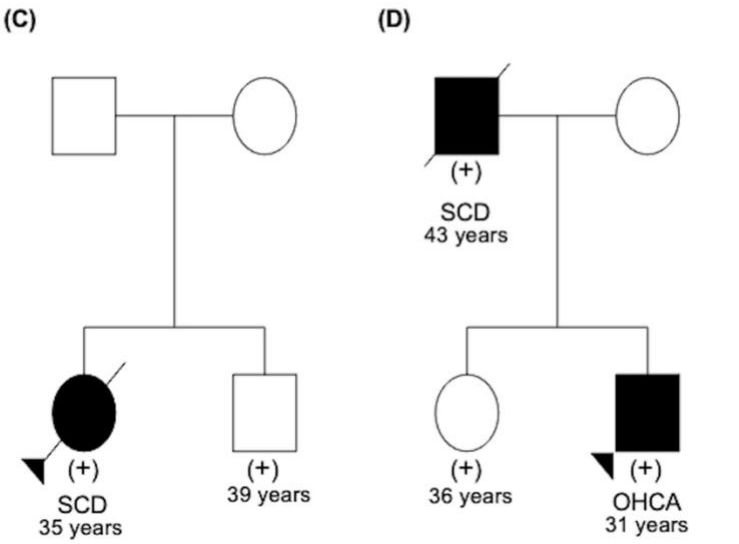
Case 5: Patient 5 died of SCD aged 35 years at work. Post-mortem examination revealed moderate LV dilation (45mm), limited small foci of myocardial replacement fibrosis in the LV free wall, and a minor degree of non-transmural fatty infiltration of the outflow tract. The post-mortem investigation concluded that these changes may be DCM, albeit without significant enlargement and no evidence of congestive heart failure. Post-mortem exome sequencing identified the same variant as case 3, a pathogenic insertion resulting in a frameshift and premature stop codon in *FLNC* c.4926_4927insACGTCACA, p.Val1643Thrfs*26. The patient's asymptomatic sibling underwent cascade genetic testing and was found to carry the same variant (Figure 3C). Comprehensive cardiac investigations showed no clear evidence of cardiomyopathy, however CMR imaging showed a limited focus of late-gadolinium enhancement (LGE) on the RV side of the basal septum.

Case 6: Patient 6 presented at 31 years following an out of hospital cardiac arrest. Prolonged cardiopulmonary resuscitation (60-70 minutes), 11 automatic external defibrillator shocks and 6 mg of adrenaline was required to achieve return of spontaneous circulation and multiorgan dysfunction resulted. Echocardiogram revealed normal LV size and wall thickness but global impairment of LV systolic function which persisted for the duration of admission (lowest LVEF of 13%). At discharge the principal diagnosis was ventricular fibrillation with secondary acute kidney injury, hypoxic brain injury, ischemic bowel, ischemic hepatitis and anaemia.

There was uncertainty regarding the underlying aetiology of the arrest and whether the LV dysfunction was the primary cause or the result of prolonged resuscitation efforts. Research based exome sequencing identified a likely pathogenic nonsense variant resulting in a premature stop codon in *FLNC* c.617G>A p.Trp206Ter. This variant is absent in gnomAD, and has not been reported previously. Family history (Figure 3D) identified the patient's father suffered a SCD at 43 years, with cause determined to be myocarditis at post-mortem. Analysis of post-mortem DNA showed he also carried the *FLNC*tv. Further, cascade genetic testing of the patient's sister identified she carries the familial variant, though all cardiac investigations are within normal parameters at age of 35 years.

Case 7: Patient 8 presented at 17 years of age with delayed development and syndromic features consistent with Noonan's syndrome. Clinical trio whole exome sequencing was performed and a pathogenic *de novo* variant in *LZTR1* explaining the clinical diagnosis was identified. In addition, a paternally inherited likely pathogenic deletion resulting in a frameshift and a premature stop codon in *FLNC,* c.7399del, p.Arg2467Alafs*62 was reported. The variant is absent in gnomAD and has not been reported in literature. Subsequent cardiac investigations were performed in both the patient and her father, and all showed normal cardiac parameters. Ongoing clinical screening every 12 months was advised until the patient reaches 20 years old, then every 2-3 years if investigations remain unremarkable.





(E)

	1	2	3	4	5	6	7
Variant	p.Ala1334fs*	p.Ala380fs*	p.Val1643fs*	p.Gln2499fs*	p.Val1643fs*	p.Trp206*	p.Arg2467Alafs*
Classification	Likely Pathogenic	Likely Pathogenic	Pathogenic	Pathogenic	Pathogenic	Likely Pathogenic	Likely Pathogenic
Age of onset	38	27	29	35	35	31	NA
Major cardiac event	SCD	SCD	SCD	No	SCD	OHCA	No
Ventricular Arrhythmia	Yes (SCD)	Yes (VT)	Yes (SCD)	Yes (VEB burden)	Yes (SCD)	Yes (OHCA)	No
Fibrofatty infiltration	Yes (PM)	No	Yes (CMR-LGE)	No	Yes (PM)	No	No
LV dilation	Yes	Yes	Yes	Yes	Yes	No	No
Reduced systolic function	Yes	Yes	Yes	Yes	No	Yes	No
Cardiac phenotype	Yes	Yes	Yes	Yes	Yes	Yes	No

Figure 3. Clinical and genetic overview of cases. (A) 4 Chamber cine cardiac magnetic resonance (CMR) imaging view of case 3. (B) 4 Chamber view with contrast CMR of case 3 showing late gadolinium enhancement of the mid-wall from basal to mid septum. (C) Family pedigree of case 5 (D) Family pedigree of case 6. (E) Table of cardiac clinical outcomes seen in each family. Variant classifications are from ACMG criteria PVS1 and PM2 for likely pathogenic variants, with the addition of PS4_M for the pathogenic variants.

Abbreviations: Sudden cardiac death (SCD), Out of hospital cardiac arrest (OHCA), Ventricular tachycardia (VT), Ventricular ectopic beat (VEB), Post-mortem (PM), Cardiac magnetic resonance late gadolinium enhancement (CMR-LGE)**.**

Discussion

We report a series of patients attending a specialized cardiac genetic clinic with a putative loss of function *FLNC*tv. The phenotype is characterized by left-sided ACM in the presence of fibro-fatty infiltration of the myocardium with high risk of severe cardiac outcomes including end-stage heart failure and SCD (Figure 3E).[1] This phenotype is congruent with the understood pathomechanism of *FLNC*tv, i.e., truncation of the protein, leading to putative loss of function of the allele causing haploinsufficiency (Figure 1A). Filamin-C crosslinks with actin filaments at the Z-disc between sarcomeres. Insufficient production of Filamin-C leads to weakened myocyte adhesion at the molecular level, resulting in thinning and dilation of the myocardium and loss of myocytes with fibro-fatty replacement.[6] In comparison, in-frame deletions and missense variants likely cause misfolding of filamin-C, with deposition of large protein aggregates within the sarcomere at the Z-disc, resulting in larger fiber diameter of the myocardium and an HCM or RCM phenotype.[3]

Of the 7 families identified, 6 had primary cardiac phenotypes with frameshift variants predicted to cause nonsense mediated decay. Among those with cardiac phenotypes, proband age at diagnosis ranged 27-35 years and 4/6 were female. Four families experienced SCD of a young relative (age range: 30-43 years). Cardiac imaging was available for 5 affected individuals across 4 families, with left ventricular (LV) dilation and systolic dysfunction reported in all (LV ejection fraction range: 13-46%). LV fibro-fatty infiltration of the myocardium, demonstrated as late gadolinium enhancement on CMR (Figure 3B) or on postmortem histology, was seen in 3 families. For all families, there is marked variability in clinical presentation, from ventricular arrhythmias to end-stage heart failure, as well as examples of non-penetrance.

While existing literature and examples from this case series support *FLNC*tv as a cause of ACM with high risk of ventricular arrhythmia and heart failure, increasing reports highlight these variants identified as secondary genetic findings, i.e., identified in individuals without the ACM phenotype undergoing genetic testing for another purpose.[9,10] However, these cases may not necessarily be benign bystanders, with carriers in the general population potentially at increased risk of ventricular arrhythmia.[10] Non-penetrance was seen in Family 7, where the *FLNC*tv was identified secondary to another identified genetic cause explaining their non-cardiac condition. Further, at-risk family members who had undergone cascade genetic testing in family 5 and 6 demonstrate additional examples of non-penetrance, with those individuals having essentially normal cardiac investigations, including one male with limited focus of septal LGE on CMR imaging. In one large population reference database, gnomAD, truncating variants occur infrequently, with each being individually rare (Figure 2), demonstrating intolerance of *FLNC* to loss of function variation (pLI=1 and LOEUF=0.25).[5] Cases, both from Clinvar and our cohort, show causative variants distributed throughout the gene suggesting gene-wide constraint. Existing literature reports high penetrance of disease (97% at >40 years) and guidelines recommend early ICD implantation in high-risk affected patients.[1] In addition, it is broadly recommended that these families are managed in specialist multidisciplinary clinics including genetic counselling, given the nuances regarding clinical heterogeneity and incomplete penetrance.

Conclusion

FLNC truncating variants cause a distinct and severe cardiac phenotype, characterized by a left-sided ACM phenotype with high risk of severe cardiac

outcomes including end-stage heart failure and SCD. The phenotype is underpinned by a pathomechanism specific to this variant type. Non-penetrance exists, and highlights that other factors modulating disease expression remain incompletely understood. *FLNC*tv present an exciting example of the insights that can be gleaned from better understanding genotype-specific disease sub-groups, with benefit for patient management and outcomes.

References

1. Ortiz-Genga MF, Cuenca S, Dal Ferro M, et al. Truncating FLNC Mutations Are Associated With High-Risk Dilated and Arrhythmogenic Cardiomyopathies. *J Am Coll Cardiol*. 2016;68(22):2440-2451. doi:10.1016/j.jacc.2016.09.927

2. Verdonschot JAJ, Vanhoutte EK, Claes GRF, et al. A mutation update for the FLNC gene in myopathies and cardiomyopathies. *Hum Mutat*. 2020;41(6):1091-1111. doi:10.1002/humu.24004

3. Valdés-Mas R, Gutiérrez-Fernández A, Gómez J, et al. Mutations in filamin C cause a new form of familial hypertrophic cardiomyopathy. *Nat Commun*. 2014;5:5326. doi:10.1038/ncomms6326

4. Towbin JA, McKenna WJ, Abrams DJ, et al. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm*. 2019;16(11):e301-e372. doi:10.1016/j.hrthm.2019.05.007

5. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-443. doi:10.1038/s41586-020-2308-7

6. Agarwal R, Paulo JA, Toepfer CN, et al. Filamin C Cardiomyopathy Variants Cause Protein and Lysosome Accumulation. *Circ Res*. 2021;129(7):751-766. doi:10.1161/CIRCRESAHA.120.317076

7. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30

8. Bp H, A G, A A, et al. Association Between Midwall Late Gadolinium Enhancement and Sudden Cardiac Death in Patients With Dilated Cardiomyopathy and Mild and Moderate Left Ventricular Systolic Dysfunction. *Circulation*. 2017;135(22). doi:10.1161/CIRCULATIONAHA.116.026910

9. McGurk KA, Zheng SL, Henry A, et al. Correspondence on “ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG)” by Miller et al. *Genet Med*. 2022;24(3):744-746. doi:10.1016/j.gim.2021.10.020

10. Carruth ED, Qureshi M, Alsaid A, et al. Loss-of-Function FLNC Variants Are Associated With Arrhythmogenic Cardiomyopathy Phenotypes When Identified Through Exome Sequencing of a General Clinical Population. *Circ Genom Precis Med*. 2022;15(4):e003645. doi:10.1161/CIRCGEN.121.003645

Chapter 3

Chapter 3: Genetic evidence to support heterozygous loss-of-function *ALPK3* variants as a cause of hypertrophic cardiomyopathy

Preface to the chapter

While there is strong evidence of the clinical utility of genetics in inherited cardiomyopathies as noted in chapter 1 and 2, there are still gaps and limitations. This chapter presents the results of an international cohort study of patients with heterozygous *ALPK3* loss-of-function variants addressing the aim: *'To identify the gaps and limitations of genetic results.'* This chapter will be submitted for publication. Author contributions to this paper are outlined in the ensuing author contribution statement.

Author contribution statement

Hespe S, Singer E, Reuter C, Murray B, Jordan E, Chowns J, Peters S, Chahal A, Callewaert B, Gray B, Hershberger R, Landstrom A, Mayers M, McNally E, Semsarian C, Thaxton C, Ware J, Owens A, James C, Parikh V, Bagnall RD, Ingles J. Genetic evidence to support heterozygous Loss-of-function *ALPK3* variants as a cause of hypertrophic cardiomyopathy.

The Individual roles of co-authors are listed below:

Task	**Role of co-author**
Developing the concept of the study	SH, JI
Designing the study protocol	SH
Acquisition and analysis of data	SH, ES, CR, BM, EJ, JC, SP, AC, RDB, JI
Drafting the manuscript	SH
Review & critical comments on manuscript	All Authors

Introduction

Inherited cardiomyopathies are a genetically and phenotypically heterogeneous group of diseases affecting the ventricular myocardium.[1] Severe outcomes can include end-stage heart failure and sudden cardiac death, including among young people. Fewer than half of all patients with an inherited cardiomyopathy will have a causative variant identified following genetic testing, and these variants typically follow an autosomal dominant pattern of inheritance.[1]

In 2016, bi-allelic variants in *ALPK3* were reported as a cause of paediatric cardiomyopathy including hypertrophy (HCM) and dilated cardiomyopathies (DCM), with extracardiac features such as musculoskeletal abnormalities, facial dysmorphism and cleft palate.[2] Very few heterozygous carrier family members from these cases series were shown to express any cardiac phenotype, with only 3/21 carriers from 2 families reported to have HCM.[2–4] More recently, studies have reported heterozygous loss-of-function (LOF) *ALPK3* variants as a cause of HCM, accounting for 1-2% of adult cases based on burden testing.[3,5,6] Further, a locus near *ALPK3* has been associated with both HCM and DCM, with opposing effects in genome-wide association studies indicating common variants in this gene might also play an important role in polygenic contributions to inherited cardiomyopathy.[7]

The reported phenotype among heterozygous adults includes more frequent apical or concentric pattern of hypertrophy and shorter PR interval, compared to sarcomere-positive HCM patients.[5] Further, cases with *ALPK3* LOF variants with other cardiac phenotypes including DCM and ACM have been described, including one homozygote with apparent typical HCM, and a series showing 20% (7/35) of patients had raised serum creatine kinase.[5] Taken together, human genetic evidence reported to date is

mixed and does not provide a clear clinical picture of the natural history of *ALPK3* in a heterozygous LOF state.

ALPK3 is located at ch15q25.3 and has 14 exons encoding 1705 amino acids on the most recently updated transcript (NM_020778.5). The previous transcript (NM_020778.4) encoded 1907 amino acids, which included an additional 201 amino acids preceding the start of exon 1, and one amino acid at the end of exon 14. *ALPK3* encodes the protein α-kinase 3, an essential cardiac pseudokinase that inserts in the nuclear envelope and the sarcomere M-band of cardiac myocytes functioning to aid in myosin-mediated force buffering and sarcomere proteostasis.[8] A recent functional study showed the pathomechanism of *ALPK3* LOF variants as a cause of both DCM and HCM.[8] The DCM mechanism was described as insufficient myomesin-mediated force buffering from mislocalization of myomesins compromising the structural integrity of the myocardium causing thinning and dilation, while HCM was caused by dysregulation of M-band proteins impairing sarcomere proteostasis.

While biallelic *ALPK3* variants have been curated as strong evidence for gene-disease association with an autosomal recessive infant-onset cardiomyopathy,[9] human genetic evidence to support gene curation of heterozygous *ALPK3* variants as a cause of HCM are limited to a few published studies. Here we describe clinical phenotypes and genetic variants identified among patients drawn from international specialised centers and highlight some of the challenges in considering this is a Mendelian, autosomal dominant cause of disease.

Methods

Patient series

A patient series comprising seven institutions in Australia, U.S.A, and the UK was developed. Patients who had a cardiac phenotype and any reported *ALPK3* LOF variant (using either transcript NM_020778.4 or NM_020778.5) were included. All clinical data were de-identified by the contributing site. Human research ethical approval was granted at each site according to local regulations.

Genetic variants

Genetic variants from both research and clinical genetic reports were reviewed, and those with putative LOF variants, i.e., truncating variants such as insertions or deletions leading to a frameshift, nonsense, or splice site variant were included. Baseline variant classifications by the reporting laboratory were recorded, and variant evidence was reviewed using American College of Medical Genetics and Genomics and Association of Molecular Pathologists standards for variant interpretation (ACMG criteria)[10] assuming an autosomal dominant model of inheritance. Variants were classified as pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), or likely benign/benign (LB/B).

Exons were defined for the previous transcript by 5nsembl transcript: ENST00000258888.5 (NM_020778.4), and the new transcript by 5nsembl transcript: ENST00000258888.6 (NM_020778.5). Coding regions of both transcripts were plotted on a gene topology figure aligned by nucleotide position, with ENST00000258888.5 starting at 0 and ending at 10917, and ENST00000258888.6 starting at position 677 and ending at 10914. *ALPK3* LOF variants in ClinVar (date: November 2022) and the

Genome Aggregation Database[11] (gnomAD v2.1; date: November 2022) were extracted and plotted along with our cohort variants (Figure 1).

Clinical characteristics

Clinical data were collected from sites and comprised a minimum dataset including basic demographics, clinical diagnosis and key characteristics, and family history information. Cardiac imaging was reported by each site according to local clinical protocols. A 3-generation family history was collected and included information about the presence of other family members with any cardiac phenotypes and/or major cardiac events such as cardiac transplantation or sudden cardiac arrest.

Results

Patient characteristics

There were 28 patients from 25 families included. Clinical diagnoses of probands included HCM, DCM, arrhythmogenic cardiomyopathy (ACM), idiopathic left ventricular hypertrophy (LVH), or familial sudden arrhythmic death syndrome (SADS). Patients 8, 22, and 28 had *ALPK3* LOF variants (p.Ala181Profs*130, p.Gly134Argfs*30, and Glu148Glnfs*8) reported on the superseded transcript NM_020778.4. After alignment to newer transcript NM_020778.5, these variants precede the start of the coding region of *ALPK3* (Figure 1) and therefore excluded from the clinical analyses.

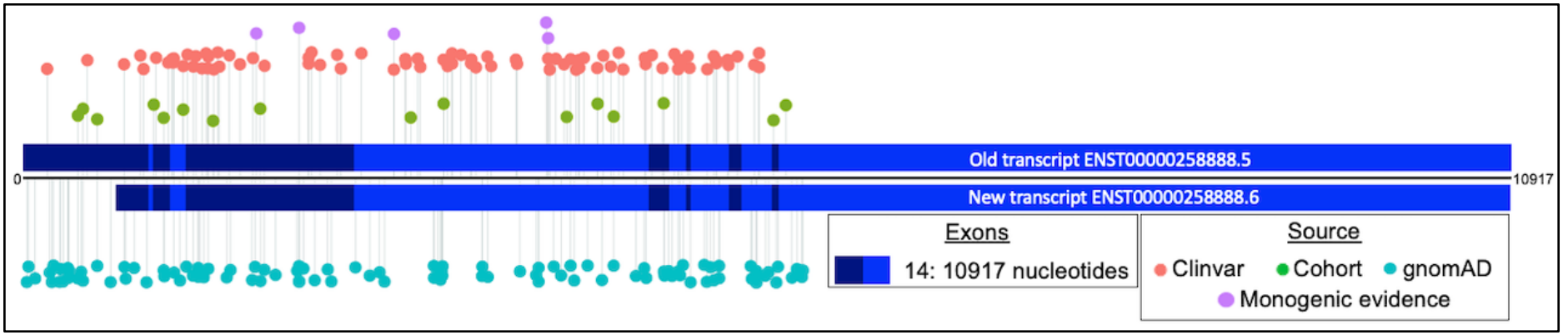


Figure 1. Gene topology of *FLNC* truncating variants (cases and controls). Gene topology of *ALPK3* showing exons, defined for the old transcript by ensembl transcript: ENST00000258888.5 (NM_020778.4), and the new transcript by ensembl transcript: ENST00000258888.6 (NM_020778.5); and reported LOF variants in cases above the gene; our cohort (variants with strong monogenic evidence in purple and the remaining in green) and ClinVar reports (red); and controls (blue) (gnomADv2.1) below the gene.

Clinical phenotype

Table 1 and Supplementary Table I provide clinical summary data for all 28 patients investigated. For the 25 patients with a *ALPK3* LOF variants within the coding region according to the newer transcript (NM_020778.5), 22 (88%) were probands, 20 (80%) were male and the age of diagnosis ranged from 13 to 74 years (median 33 years). Diagnoses included 20 (80%) in HCM grouped diagnoses, comprising 17(68%) with HCM, 1 (4%) with HCM/RCM, 1 (4%) with possible HCM, and 1 (4%) with LVH. There was 1 (4%) with DCM, 3 (12%) with ACM, and 1 (4%) with familial SADS.

There were 4 (16%) patients who had at least 1 episode of atrial arrhythmia including atrial fibrillation or supraventricular tachycardia, while 3 (12%) had non-sustained ventricular tachycardia. There were 5 (20%) with intraventricular conduction block and 2 (8%) had first-degree atrioventricular block. Five (20%) patients had an implantable cardioverter-defibrillator. Those diagnosed with HCM had a mean maximum left ventricle (LV) wall thickness of 20 ± 6mm, with apical, concentric and asymmetric septal hypertrophy patterns reported (Supplementary Table I). Three patients had cardiac magnetic resonance imaging with one patient reported to have late gadolinium enhancement.

Table 1: Characteristics of patients with heterozygous *ALPK3* LOF variants

	Cohort	
	Proportion	**%**
Proband	22/25	88
Male sex	20/25	80
Clinical diagnosis		
HCM Group	20/25	80
HCM	17/25	68
HCM/RCM	1/25	4
Possible HCM	1/25	4
LVH	1/25	4
DCM	1/25	4
ACM	3/25	12
Familial SADS	1/25	4
Age of diagnosis		
<18 years	3/25	12
18-39 years	12/25	48
40-69 years	9/25	36
>70 years	1/25	4
AF/SVT	4/25	16
NSVT	3/25	12
1st degree AV block	2/25	8
IVCB	5/25	20
RBBB	3/25	12
LBBB	0/25	0
Unspecified	2/25	8
ICD	5/25	20

Abbreviations: HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; ACM, arrhythmogenic cardiomyopathy; RCM, restrictive cardiomyopathy; LVH, left ventricular hypertrophy; SADS, sudden arrhythmic death syndrome; AF, atrial fibrillation; SVT, supraventricular tachycardia; NSVT, non-sustained ventricular tachycardia; AV, atrioventricular; IVCB, intraventricular conduction block; RBBB, right bundle branch block; LBBB left bundle branch block; LGE, late gadolinium enhancement; CMR, cardiac magnetic resonance; ICD, implantable cardioverter-defibrillator.

Table 2: Cohort *ALPK3* loss-of-function variant summary. All variants reported on NM_020778.5.

Variant	Exon	Original Lab classification	No. AD Probands	Phenotypes reported	Segregation	Cases with other LP/P variants
*p.Ala181Profs*130*	*Non-coding*	*VUS*	*1*	*DCM*	*0*	*TTR* p.Val142Ile
*p.Gly134Argfs*30*	*Non-coding*	*VUS*	*1*	*DCM*	*0*	-
*p.Glul48Glnfs*8*	*Non-coding*	*VUS*	*1*	*DCM*	*0*	*TTR* p.Val142Ile
del exon 3	3	P	1	HCM	0	-
p.Arg141Serfs*32	3	P	1	LVH	0	-
p.Val166Hisfs*4	4	VUS	1	HCM	0	-
p.Gly262fs*7	5	VUS	1	HCM	0	-
p.Pro378Leufs*38	5	VUS	1	HCM	0	-
p.Thr368Argfs*14	5	LP	1	HCM	1	-
p.Gln473Profs*4	5	LP	1(+1 literature[6^])	HCM	6 literature [6^]	*TTR* p.Val142Ile
p.Asp1077Glufs*13	6	P & VUS	3 (+2 literature[5])	HCM & ACM	1 (HCM)	-
p.Lys1243Argfs*29	6	P	1	ACM	0	-
p.Gly1203Argfs*50	6	P	1	ACM	0	*PKP2* p.Ser837Valfs*94
p.Glu682*	6	VUS	2 (+1 literature[5])	HCM	1	-
p.Arg1059*	6	VUS	2 (+5 literature[5])	HCM	0	-
p.Ser1129Glnfs*5	6	VUS	1	HCM	0	-
p.Gly746Valfs*6	6	VUS	1	HCM	0	-
p.Pro804Alafs*4	6	P	1 (+1 literature[5])	HCM/RCM	0	-
c.4023+5G>C	Intron	VUS	1	Familial SADS	0	-
p.Gln1634Lysfs*19	13	VUS	1	DCM	0	*HFE* p.His63Asp
p.Lys1641*	14	VUS	1	HCM	0	-

Abbreviations: HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; ACM, arrhythmogenic cardiomyopathy; RCM, restrictive cardiomyopathy; LVH, left ventricular hypertrophy; SADS, sudden arrhythmic death syndrome; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance.

^Literature family variant at same position but different protein change p.Gln473Serfs*30[6]

Genetic summary

Supplementary Table II provides genetic summary data for all 28 patients investigated. Case variants included 21 *ALPK3* LOF variants reported in our cohort and 83 LP/P variants reported in ClinVar, while control variants included 102 putative LOF variants from gnomAD (gnomADv2.1; Figure 1). Five case variants from our cohort (n=3) and ClinVar (n=2) lie outside the coding region according to the latest *ALPK3* transcript (NM_020778.5). There were 13 variants identified in patients with HCM and classified as VUS, LP, and P; 2 variants associated with ACM and were classified as P; all 4 variants associated with DCM were classified as VUS; 1 VUS was associated with familial SADS; and 1 variant was associated with both HCM and ACM, and classified as P/VUS and P respectively (Table 2). All 21 coding variants were classified as VUS after re-curation assuming an autosomal dominant of inheritance model (Table 2 & Supplementary Table III).

There were 5 variants that had segregations and/or higher than expected proband counts (p.Thr368Argfs*14, , p.Asp1077Glufs*13, p.Glu682*, p.Arg1059*, and p.Gln473Profs*4). The variant p.Thr368Argfs*14 had 1 proband and segregated to 1 relative with HCM. The variant p.Asp1077Glufs*13 had a proband count of 5 (3 cohort and 2 literature), 4 reported with HCM and 1 reported with ACM, and had 1 segregation with HCM. The variant p.Glu682* had 3 probands (2 cohort and 1 literature) reported with HCM and 1 segregation. The variant p.Arg1059* had a proband count of 7 (2 cohort and 5 literature) all reported with HCM. The variant p.Gln473Profs*4 (c.1417dupC) had 1 proband reported with HCM in our cohort, in addition to a previously published HCM family with 6 segregations reported with the variant p.Gln473Serfs*30 (c.1417delC) with the same protein change of p.Gln473fs.

Table 3: Proportion of heterozygous *ALPK3* loss-of-function variants per exon for cases and controls (Reported on transcript NM_020778.5)

Exon	Cases %(n)			Controls
	Cohort	***ClinVar***	***Total***	**%(n)**
1	0.0 (0)	3.7 (3)	3.0 (3)	4.7 (4)
2	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
3	11.1 (2)	2.5 (2)	4.0 (4)	2.4 (2)
4	5.6 (1)	4.9 (4)	5.1 (5)	3.5 (3)
5	22.2 (4)	24.7 (20)	24.2 (24)	24.7 (21)
6	44.4 (8)	42.0 (34)	42.4 (42)	32.9 (28)
7	5.6 (1)	2.5 (2)	3.0 (3)	3.5 (3)
8	0.0 (0)	4.9 (4)	4.0 (4)	4.7 (4)
9	0.0 (0)	2.5 (2)	2.0 (2)	1.2 (1)
10	0.0 (0)	7.4 (6)	6.1 (6)	7.1 (6)
11	0.0 (0)	1.2 (1)	1.0 (1)	0.0 (0)
12	0.0 (0)	3.7 (3)	3.0 (3)	8.2 (7)
13	5.6 (1)	0.0 (0)	1.0 (1)	2.4 (2)
14	5.6 (1)	0.0 (0)	1.0 (1)	4.7 (4)
Total	100 (18)	100 (81)	100 (99)	100 (85)

Case variants appear to be enriched in exon 6 (cohort and variants reported in ClinVar) compared to controls (gnomAD), accounting for 42.4% of variants in cases compared to 32.9% of variants in controls (Table 3). For all other exons there is no difference in variant proportion between cases and controls. Due to small numbers in many exons, statistical testing of this was not able to be performed.

Discussion

We report an international cohort of patients with 21 unique heterozygous putative LOF *ALPK3* variants, with varying phenotypes of HCM, ACM, DCM, LVH, and familial SADS. This expands on the previously published phenotypes associated with heterozygous *ALPK3* LOF variants, however our data supports HCM as being the most predominant phenotype.[3,5,6] There was a large range in age of diagnosis (13-74

years) with the median age of diagnosis 33 years, younger than previous literature.[5] This could be due to a proportion of the patients having additional contributing factors such as another VUS/LP/P variant (Patient's 1, 4, and 9) or perhaps high exercise load as an elite athlete (Patient 3; Supplementary Table I and II). Clinical diagnosis of HCM requires asymmetric or concentric hypertrophy of the LV ε15mm in the absence of loading conditions, and typically with normal or increased systolic function and normal or reduced chamber size.[12] We showed that those diagnosed with HCM in our cohort had a mean LV thickness of 20 ± 6 mm indicating moderate thickening in line with previous literature.[5]

After excluding the 3 non-coding variants originally classified on the old transcript (NM_020778.4), our phenotype-specific variant reclassification assuming an autosomal dominant pattern of inheritance resulted in all variants reclassified as VUS (Table 2). There is currently insufficient evidence to classify any of these variants higher than VUS using the ACMG criteria. This is due the lack of an established gene-disease relationship for heterozygous LOF variants in *ALPK3*, few published cases, conflicting predictions from *in silco* tools, and the functional mechanism of disease for LOF heterozygous *ALPK3* variants being unknown. We identified 5 variants (p.Thr368Argfs*14, , p.Asp1077Glufs*13,p.Glu682*, p.Arg1059*, and p.Gln473Profs*4) found in exons 5 and 6 (Figure 1) that showed stronger evidence of monogenic/autosomal dominant disease due to segregation with disease and/or increased proband counts.

All LOF case variants (cohort & ClinVar) were spread throughout the gene, with an apparent clustering in the largest exons 5 & 6 (Figure 1). Case variants from both the cohort and ClinVar were found in the last exon, which would be expected to escape nonsense mediated decay and therefore cause disease via an alternative mechanism.

A recent functional study showed that heterozygous *ALPK3* knock out mice developed ventricular hypertrophy beyond 1 year of age, compared to homozygous *ALPK3* knockout mice that developed rapidly progressive cardiomyopathy within the first week of life.[8] Although in gnomAD *ALPK3* is not constrained for LOF in population data, these constraint metrics are based on the old transcript including numerous LOF variants now known to be in the non-coding region. Importantly, there is functional evidence for disease, and closer inspection of potential hot-spot regions could allow better determination of which LOF variants are monogenic and which potentially act as genetic modifiers. These questions remain important to clarify, and will guide use of PVS1 for variant classification. For example, determining whether PVS1 could be downgraded and applied as PVS1_strong for nonsense and frameshift variants more likely to be causative of monogenic disease.[13]

While work to recognise gene regions that might be more likely associated with monogenic disease variants could contribute to explaining the high-rates of non-penetrance among heterozygous LOF *ALPK3* variant carriers, it is important to note that non-penetrance was also observed in variants within this region. For example, one variant, p.Arg1059* located in exon 6 with a proband count of 7 is also observed in gnomAD 6 times. Further, as heterozygous LOF *ALPK3* disease is less severe and later onset compared to biallelic (autosomal recessive) disease, it could be hypothesised that carriers identified in the healthy population harbouring these variants may not necessarily be considered innocent benign bystanders. Indeed, some individuals may have undetected mild or late-onset disease. Biallelic *ALPK3* LOF variants accompany a distinct severe paediatric phenotype, characterized by DCM that evolves to HCM with poor systolic function.[2–4,14] Functional studies support development of both HCM and DCM due to *ALPK3* LOF variants.[8] In the sarcomere,

myomesins function to minimize force imbalances in the M-band by opposing myosins during sarcomere contraction. It is speculated that the insertion of myomesins into the nuclear membrane protects the nucleus by functioning as force buffers in the neonatal period when cardiac contractility must be maintained during cardiomyocyte division and replication. The authors speculated that in the early stages of development, biallelic LOF variants in *ALPK3* can cause the mislocalization of the myomesins at the sarcomere and nuclear envelope impairing cardiac contractility and causing ventricular dilation in this early stage of development. In homozygous and heterozygous *ALPK3* LOF mice, reduced levels of expression of MuRF1, a protein involved in protein turnover that is expressed at highest levels at the end of gestation, which is maintained through life.[8,15] This could explain disease progression of paediatric biallelic *ALPK3* LOF cases and suggests that heterozygous *ALPK3* LOF carriers may still express functional protein, albeit at a reduced level, equating to more mild disease and later onset.

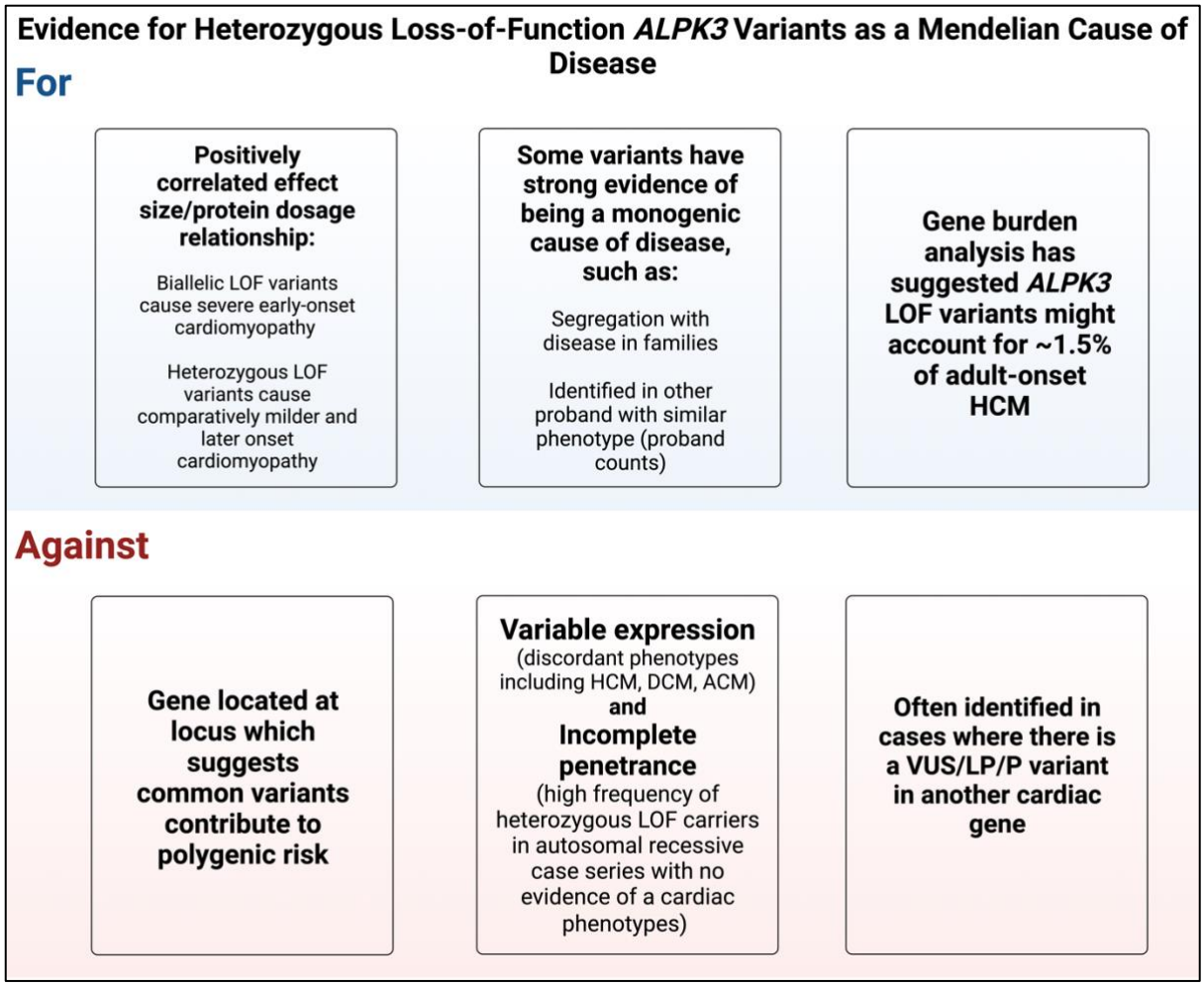


Figure 2. Evidence of heterozygous LOF *ALPK3* variants as a Mendelian cause of disease.

Abbreviations: LOF, loss of function; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; ACM, arrhythmogenic cardiomyopathy; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance; GWAS, genome-wide association study.

Heterozygous LOF *ALPK3* variants have been shown to be enriched in cases compared to controls, and contributing to approximately 1.5% of adult onset HCM from a cohort from genetic testing referrals.[5] It has been suggested that *ALPK3* variants span a spectrum of effect size that is positively correlated with dosage of protein.[16] Where biallelic truncations are associated with severe early onset cardiomyopathy, heterozygous truncations are associated with mild late-onset cardiomyopathy. Further, recent genome-wide association data showed a locus near *ALPK3* as associated with polygenic contributions to HCM and DCM.[7] Critically, this raises

questions in whether there is currently sufficient human genetic evidence to support heterozygous LOF *ALPK3* variants as a monogenic cause of disease (Figure 2). Given the conflicting and discordant evidence, we hypothesise that most heterozygous LOF variants in *ALPK3* are not large effect size (monogenic) variants, with most contributing small to moderate contributions to disease, suggestion other factors may be necessary to "tip" a patient to develop disease. Evidence exists to support some variants as capable of causing autosomal dominant disease and efforts to determine how these variants can be distinguished are needed.

Conclusion

Heterozygous *ALPK3* LOF variants have been reportedly associated with adult-onset HCM. Reported phenotypes are inconsistent, although predominately include mild HCM. Gene curation to weigh up the evidence for a monogenic gene-disease association is currently hampered by limited and conflicting reports. Further, variants reported using a superseded transcript that are now known to be located in a non-coding region add additional challenges to clinicians required to interpret genetic reports. While biallelic *ALPK3* variants are already established to cause an autosomal recessive infant-onset cardiomyopathy, the role of heterozygous LOF *ALPK3* variants remains unclear. Taken together, including evidence we report and available in the literature, we suggest heterozygous LOF variants in *ALPK3* may be monogenic in some cases, but are more likely to be genetic modifiers. Further investigation and research are needed to delineate this gene-disease relationship.

References

1. McKenna WJ, Judge DP. Epidemiology of the inherited cardiomyopathies. *Nat Rev Cardiol*. 2021;18(1):22-36. doi:10.1038/s41569-020-0428-2

2. Almomani R, Verhagen JMA, Herkert JC, et al. Biallelic Truncating Mutations in ALPK3 Cause Severe Pediatric Cardiomyopathy. *J Am Coll Cardiol*. 2016;67(5):515-525. doi:10.1016/j.jacc.2015.10.093

3. Herkert JC, Verhagen JMA, Yotti R, et al. Expanding the clinical and genetic spectrum of ALPK3 variants: Phenotypes identified in pediatric cardiomyopathy patients and adults with heterozygous variants. *Am Heart J*. 2020;225:108-119. doi:10.1016/j.ahj.2020.03.023

4. Jaouadi H, Kraoua L, Chaker L, et al. Novel ALPK3 mutation in a Tunisian patient with pediatric cardiomyopathy and facio-thoraco-skeletal features. *J Hum Genet*. 2018;63(10):1077-1082. doi:10.1038/s10038-018-0492-1

5. Lopes LR, Garcia-Hernández S, Lorenzini M, et al. Alpha-protein kinase 3 (ALPK3) truncating variants are a cause of autosomal dominant hypertrophic cardiomyopathy. *Eur Heart J*. 2021;42(32):3063-3073. doi:10.1093/eurheartj/ehab424

6. Cheawsamoot C, Phokaew C, Chetruengchai W, et al. A Pathogenic Variant in ALPK3 Is Associated With an Autosomal Dominant Adult-onset Hypertrophic Cardiomyopathy. *Circ Genom Precis Med*. 2020;13(6):e003127. doi:10.1161/CIRCGEN.120.003127

7. Tadros R, Francis C, Xu X, et al. Shared genetic pathways contribute to risk of hypertrophic and dilated cardiomyopathies with opposite directions of effect. *Nat Genet*. 2021;53(2):128-134. doi:10.1038/s41588-020-00762-2

8. Agarwal R, Wakimoto H, Paulo JA, et al. Pathogenesis of Cardiomyopathy Caused by Variants in ALPK3, an Essential Pseudokinase in the Cardiomyocyte Nucleus and Sarcomere. *Circulation*. 2022;146(22):1674-1693. doi:10.1161/CIRCULATIONAHA.122.059688

9. Ingles J, Goldstein J, Thaxton C, et al. Evaluating the Clinical Validity of Hypertrophic Cardiomyopathy Genes. *Circ Genom Precis Med*. 2019;12(2):e002460. doi:10.1161/CIRCGEN.119.002460

10. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30

11. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-443. doi:10.1038/s41586-020-2308-7

12. Ommen SR, Mital S, Burke MA, et al. 2020 AHA/ACC Guideline for the Diagnosis and Treatment of Patients With Hypertrophic Cardiomyopathy: Executive Summary: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation*. 2020;142(25):e533-e557. doi:10.1161/CIR.0000000000000938

13. Abou Tayoun AN, Pesaran T, DiStefano MT, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Hum Mutat*. 2018;39(11):1517-1524. doi:10.1002/humu.23626

14. Al Senaidi K, Joshi N, Al-Nabhani M, et al. Phenotypic spectrum of ALPK3-related cardiomyopathy. *Am J Med Genet A*. 2019;179(7):1235-1240. doi:10.1002/ajmg.a.61176

15. Perera S, Holt MR, Mankoo BS, Gautel M. Developmental regulation of MURF ubiquitin ligases and autophagy proteins nbr1, p62/SQSTM1 and LC3 during cardiac myofibril assembly and turnover. *Dev Biol*. 2011;351(1):46-61. doi:10.1016/j.ydbio.2010.12.024

16. Walsh R, Bezzina CR. ALPK3: a full spectrum cardiomyopathy gene? *European Heart Journal*. 2021;42(32):3074-3077. doi:10.1093/eurheartj/ehab415

Supplementary Material

Supplementary Table I. Clinical summary of cohort

Pt	Sex	Dx	Age at diagnosis	Proband	ECG findings	LVH (mm) and pattern		ICD	LGE on CMR	Ancestry	Other phenotype notes
18	*M*	*DCM*	*43*	*Y*	*1st degree AV block & IVCB*	*-*	*-*	*N*	*N*	*African*	*Bicuspid aortic valve*
28	*M*	*DCM*	*80*	*Y*	*LBBB*	*-*	*-*	*N*	*Y*	*European*	*Severe DCM, CRT-P*
8	*M*	*DCM*	*24*	*Y*	*-*	*-*	*-*	*N*	*-*	*African American*	*Severe DCM, heart transplant*
1	M	ACM	13	Y	-	-	-	N	-	-	Severe right sided ACM
2	M	HCM	16	Y	AF/SVT	28	ASH	Y	-	European	-
3	M	Possible HCM	16	Y	-	14	Concentric	N	-	-	Investigated for athletes' heart after syncope
4	M	Familial SADS	20	Y	SVT	-	-	N	N	-	Structurally normal heart
5	M	HCM	22	Y	-	21	-	Y/PPM	-	-	-
6	M	HCM	23	Y	RBBB	30	ASH	Y	-	-	-
7	F	HCM	23	Y	-	20	Apical	N	Y	-	-
9	M	HCM/RCM	25	Y	Abnormal ECG	-	-	N	-	-	Initially diagnosis myocarditis, consistently elevated troponin
10	F	HCM	26	Y	-	21	ASH	N	-	-	Also w/ congenital progressive external ophthalmoplegia (*POLG*, AR)
11	M	HCM	26	Y	-	18	Concentric	N	-	-	-
12	M	HCM	30	Y	RBBB	12	Apical	N	-	European	-
13	M	HCM	31	N	AF, 1st degree AV block	15	Mild concentric	N	-	-	-
14	M	HCM	33	Y	IVCB	19	Apical	N	N	-	-
15	M	HCM	35	Y	NSVT	22	ASH	Y	-	-	-
16	M	HCM	39	Y	IVCB	12	Apical	N	-	European	-
17	M	DCM	42	Y	-	-	-	N	-	-	History of significant ETOH
19	F	ACM	47	Y	VT	-	-	N	-	-	Bi-ventricular ACM
20	F	HCM	50	N	-	23	Concentric	N	-	-	-
21	M	ACM	54	Y	AF	-	-	N	-	-	SCA & bi-ventricular ACM

22	M	HCM	54	Y	NSVT, RBBB	30	-	N	-	African American	
23	M	HCM	58	N	1st degree AV block	17	ASH	N	-	-	-
24	M	HCM	60	Y	AF, NSVT	16	ASH	Y/PPM	-	Eastern European	-
25	F	HCM	60	Y	-	16	ASH	Y	-	European	ASA & myectomy
26	M	LVH	64	Y	-	-	-	N	-	-	Worked up for amyloidosis, Grade 1 PYP
27	M	HCM	74	Y	-	18	Apical	N	-	European	Had LVH 15 years prior to diagnosis

Abbreviations: Pt, patient number; Dx, diagnosis; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; ACM, arrhythmogenic cardiomyopathy; RCM, restrictive cardiomyopathy; LVH, left ventricular hypertrophy; SADS, sudden arrhythmic death syndrome; ECG, electrocardiogram; AF, atrial fibrillation; SVT, supraventricular tachycardia; NSVT, non-sustained ventricular tachycardia; AV, atrioventricular; IVCB, intraventricular conduction block; RBBB, right bundle branch block; LBBB left bundle branch block; ASH, asymmetrical hypertrophy; LGE, late gadolinium enhancement; CMR, cardiac magnetic resonance; ICD, implantable cardioverter-defibrillator; CRT-P, cardiac resynchronisation therapy pacemaker; AR, autosomal recessive; EtOH, ethyl alcohol; SCA, sudden cardiac arrest; ASA, alcohol septal ablation; PYP, pyrophosphate.

Patients with non-coding variants

Supplementary Table II. Genetic summary of cohort

Pt	Dx	*ALPK3* variant c.	*ALPK3* variant p.	Transcript	*ALPK3* variant p. on NM_020778.5	Variant Type	Lab classify-cation	Segre-gation	Other LP/P variants	Other genetic notes
18	*DCM*	*c.541del*	*p.Ala181Profs*130*	*NM_020778.4*	*NA*	*Frameshift*	*VUS*	*-*	*TTR p.Val142Ile (P)*	*-*
28	*DCM*	*c.399dup*	*p.Gly134Argfs*30*	*NM_020778.4*	*NA*	*Frameshift*	*VUS*	*-*	*-*	*VUS: ALPK3 c.5558G>A p.Glu1520Lys*
8	*DCM*	*c.436_440dupCCAGG*	*p.Glul48Glnfs*8*	*NM_020778.4*	*NA*	*Frameshift*	*VUS*	*-*	*TTR p.Val142Ile (P)*	*MYH7 VUS p.Arg798Lys*
1	ACM	c.4214_4219 del	p.Gly1405Argfs*50	NM_020778.4	p.Gly1203Argfs*50	Frameshift	P	1	*PKP2* p.Ser837Valfs*94 (P)	-
2	HCM	c.2044G>T	p.Glu682*	NM_020778.5	p.Glu682*	Nonsense	VUS	1	-	-
3	HCM	c.3987delG	p.Ser1331Glnfs*5	NM_020778.4	p.Ser1129Glnfs*5	Frameshift	VUS	-	-	-
4	Familial SADS	c.4699+5G>C	Intronic	NM_020778.4	Intronic	Splice site	VUS	-	-	*SCN5A* p.Asp2009Glu prev P, now VUS
5	HCM	c.4920C>CT	p.Lys1641*	NM_020778.5	p.Lys1641Ter	Nonsense	VUS	-	-	--
6	HCM	c.3231_3232 ACG>A	p.Asp1077Glufs*13	NM_020778.5	p.Asp1077Glufs*13	Frameshift	VUS	1	-	-
7	HCM	c.2843delG	p.Gly948Valfs*6	NM_020778.4	p.Gly746Valfs*6	Frameshift	VUS	-	-	-
9	HCM/RCM	c.2408dup	p.Pro804Alafs*4	NM_020778.5	p.Pro804Alafs*4	Frameshift	P	-	-	VUS: in *DMD, FLNC, JPH2, SLC22A5*
10	HCM	del exon 3		NM_020778.4			P	-	-	VUS: *BAG3* p.Glu430Lys
11	HCM	c.1709_1719 del	p.Thr570Argfs*14	NM_020778.4	p.Thr368Argfs*14	Frameshift	LP	1	-	-
12	HCM	c.3175C>T	p.Arg1059*	NM_020778.5	p.Arg1059*	Nonsense	VUS	-	-	-
13	HCM	c.2044G>T	p.Glu682*	NM_020778.5	p.Glu682*	Nonsense	VUS	(above)	-	-
14	HCM	c.496_509>del	p.Val166Hisfs*4	NM_020778.5	p.Val166Hisfs*4	Frameshift	VUS	-	-	-
15	HCM	c.1391TG>G	p.Gly464fs*7	NM_020778.4	p.Gly262fs*7	Frameshift	VUS	-	-	-
16	HCM	c.3175C>T	p.Arg1059*	NM_020778.5	p.Arg1059*	Nonsense	VUS	-	-	-

17	DCM	c.5506del	p.Gln1836Lysfs*19	NM_020778.4	p.Gln1634Lysfs*19	Frameshift	VUS	-	*HFE* p.His63Asp (P)	-
19	ACM	c.4332del	p,Lys1445Argfs*29	NM_020778.4	p.L1243Argfs*29	Frameshift	P	1	-	-
20	HCM	c.1709_1719 del	p.Thr570Argfs*14	NM_020778.4	p.Thr368Argfs*14	Frameshift	LP	(above)	-	-
21	ACM	c.3837_3838 del	p.Asp1279Glufs*13	NM_020778.4	p.Asp1077Glufs*13	Frameshift	P	-	-	VUS: *MYL2* p.Lys62*
22	HCM	c.2023dupC	p.Gln675Profs*4	NM_020778.4	p.Gln473Profs*4	Frameshift	LP	-	*TTR* p.Val142Ile (P)	-
23	HCM	c.3231_3232 ACG>A	p.Asp1077Glufs*13	NM_020778.5	p.Asp1077Glufs*13	Frameshift	VUS	(above)	-	-
24	HCM	c.2044G>T	p.Glu682*	NM_020778.5	p.Glu682*	Nonsense	VUS	-	-	-
25	HCM	c.1737del	p.Pro580Leufs*38	NM_020778.4	p.Pro378Leufs*38	Frameshift	VUS	-	-	-
26	LVH	c.1029del	p.Arg343Serfs*32	NM_020778.4	p.Arg141Serfs*32	Frameshift	P	-	-	-
27	HCM	c.3837_3838 del	p.Asp1279Glufs*13	NM_020778.4	p.Asp1077Glufs*13	Frameshift	P	-	-	-

Abbreviations: Pt, patient number; Dx, diagnosis; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; ACM, arrhythmogenic cardiomyopathy; RCM, restrictive cardiomyopathy; LVH, left ventricular hypertrophy; SADS, sudden arrhythmic death syndrome; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance.

Patients with non-coding variants

Supplementary Table III. Gene curation summary (All variants reported on NM_02778.5)

Variant	Exon	Original Lab Classification	No. AD Probands	Phenotypes Reported	Segregation	gnomAD Allele Count	Cases with other LP/P Variants	ACMG	New Classification
*p.Ala181Profs*130*	*Non-coding*	*VUS*	*1*	*DCM*	*0*	*-*	*TTR p.Val142Ile*	*NA*	*NA*
*p.Gly134Argfs*30*	*Non-coding*	*VUS*	*1*	*DCM*	*0*	*-*	*-*	*NA*	*NA*
*p.Glul48Glnfs*8*	*Non-coding*	*VUS*	*1*	*DCM*	*0*	*-*	*TTR p.Val142Ile*	*NA*	*NA*
del exon 3	3	P	1	HCM	0	0	-	PM4, PM2	VUS
p.Arg141Serfs*32	3	P	1	LVH	0	0	-	PM2	VUS
p.Val166Hisfs*4	4	VUS	1	HCM	0	1	-	PM2	VUS
p.Gly262fs*7	5	VUS	1	HCM	0	0	-	PM2	VUS
p.Pro378Leufs*38	5	VUS	1	HCM	0	1	-	PM2	VUS
p.Thr368Argfs*14	5	LP	1	HCM	1	0	-	PM2	VUS
p.Gln473Profs*4	5	LP	1(+1 literature^)	HCM	6^	1	TTR p.Val142Ile	PM2, PP1_M	VUS
p.Asp1077Glufs*13	6	P & VUS	3 (+2 literature)	HCM & ACM	1 (HCM)	0	-	PM2, PS4_M, PP1	VUS
p.Lys1243Argfs*29	6	P	1	ACM	0	1	-	PM2	VUS
p.Gly1203Argfs*50	6	P	1	ACM	0	0	PKP2 p.Ser837Valfs*94	PM2, BP5	VUS

p.Glu682*	6	VUS	2 (+1 literature)	HCM	1	1	-	PM2, PS4_M, PP1	VUS
p.Arg1059*	6	VUS	2 (+5 literature)	HCM	0	6	-	PS4_M	VUS
p.Ser1129Glnfs*5	6	VUS	1	HCM	0	0	-	PM2	VUS
p.Gly746Valfs*6	6	VUS	1	HCM	0	0	-	PM2	VUS
p.Pro804Alafs*4	6	P	1 (+1 literature)	HCM/RCM	0	0	-	PM2, PS4_M	VUS
c.4023+5G>C	Intron	VUS	1	Familial SADS	0	0	-	PM2	VUS
p.Gln1634Lysfs*19	13	VUS	1	DCM	0	0	HFE p.His63Asp	PM2	VUS
p.Lys1641*	14	VUS	1	HCM	0	4	-		VUS

Abbreviations: HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; ACM, arrhythmogenic cardiomyopathy; RCM, restrictive cardiomyopathy; LVH, left ventricular hypertrophy; SADS, sudden arrhythmic death syndrome; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance.

^Literature family variant at same position but different protein change p.Gln473Serfs*30[6]

Non-coding variants on NM_020778.5

Chapter 4

Chapter 4: Discussion and conclusions

Overall summary of findings

While the role of genetics in inherited cardiomyopathies has been recognised as a diagnostic tool for decades, the utility of a genetic result as a prognostic and/or therapeutic tool is a relatively new concept. The work outlined in this thesis has taken a gene-specific approach to demonstrate the role genetics can play beyond diagnosis and family screening, specifically in highlighting the gaps and limitations in our knowledge in this highly dynamic and quickly evolving field.

Inherited cardiomyopathies are a genetically and phenotypically heterogenous group of diseases. The umbrella term “inherited cardiomyopathies” consist of five phenotypically different diseases; arrhythmogenic cardiomyopathy (ACM), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM) and left ventricular non-compaction cardiomyopathy (LVNC). Within each cardiomyopathy classification there can be differing phenotypes. For example, the variable presentations of ACM which can include classical arrhythmogenic right ventricular cardiomyopathy (ARVC) or the more recently acknowledged variations with bi-ventricular involvement and/or left sided ACM. These distinctions are proving important, with differing natural history and outcomes. As described in Chapter 1, there are many genes from numerous gene pathways implicated in development of inherited cardiomyopathies, offering insight into gene-specific phenotypic differences. The current model of clinical management based solely on a clinical diagnosis is broad and can be too non-specific. Understanding the genetic basis of the disease has the ability to provide granularity regarding the

pathomechanism, with the possibility of predicting disease progression/prognosis and hence guiding management.

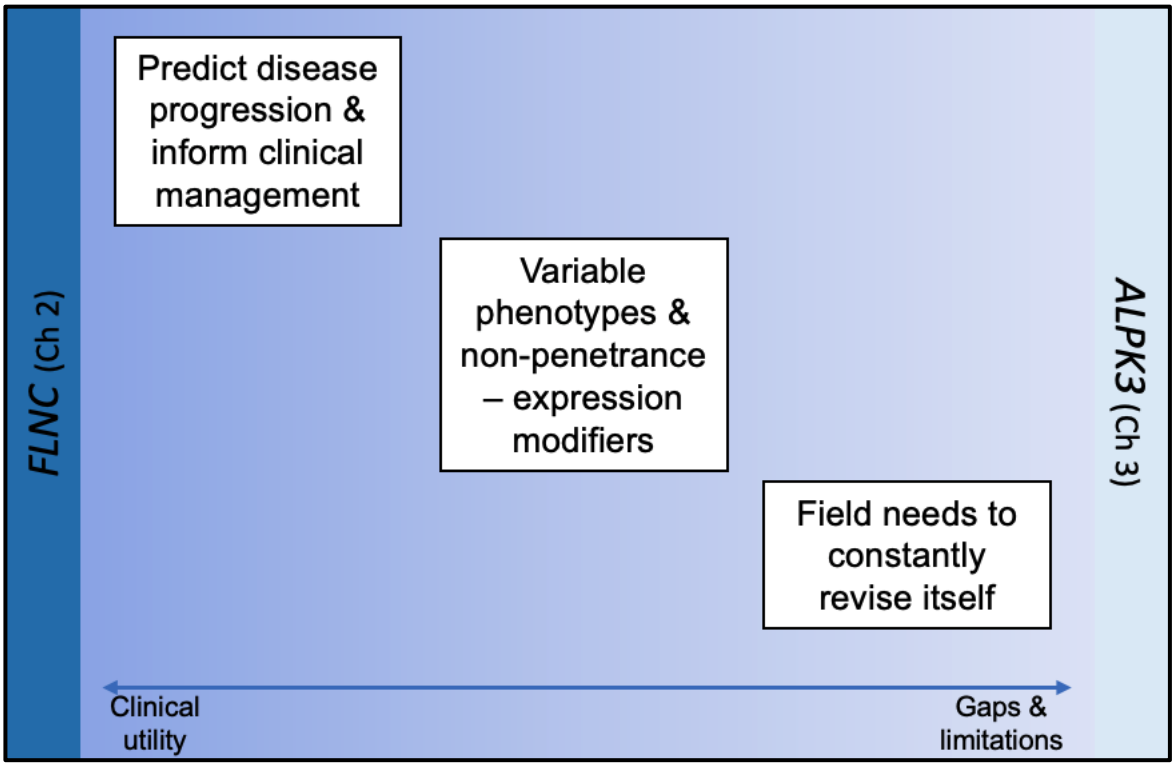


Figure 1: Summary of the outcomes of this research

Figure 1 shows the key results from this thesis and how they directly impact the clinical utility of genetic testing in *FLNC* and *ALPK3*-associated cardiomyopathy. A spectrum of the clinical utility of genetic testing is represented, and our results show the clinical significance genetic testing can have beyond family screening, as well as demonstrating limitations and gaps of knowledge in this area.

In the clinical setting, inherited cardiomyopathy families have unique management needs, including treatment and interventions to ensure good symptomatic control and sudden cardiac death (SCD) prevention for affected patients. In addition, there is need for periodic clinical assessment of family members and to provide the option for cascade genetic testing in cases where a causative variant is identified in the proband. Cascade testing can refine those requiring ongoing clinical surveillance through identifying those without the causative variant, thereby releasing them from lifelong

surveillance. The role of genetic testing as a tool for screening of at-risk family members is well established, however for the affected individuals the broader spectrum of clinical utility is not fully appreciated in current clinical practice.

Genetics can inform clinical manifestation

We highlight some of these important roles of genetic testing for inherited cardiomyopathies, including highlighting the distinct phenotype of severe ACM/DCM with high risk for ventricular arrhythmias and SCD in patients with *FLNC* truncating variants reported previously.[1] This demonstrates an exciting example of the insights that can be gleaned from better understanding genotype-specific disease sub-groups, with benefits for patient management and outcomes using precision medicine (Chapter 2). These findings support previous functional research, suggesting that the pathophysiological mechanism of *FLNC* truncating variants is loss of function resulting in haploinsufficiency.[2] Identifying a *FLNC* truncating variant in an individual can guide clinical management. Specifically, allowing stratification for risk of ventricular arrhythmias, thereby optimising patient selection for preventative treatments and interventions such as prophylactic implantable cardioverter-defibrillators in at-risk individuals. This example shows knowing the finer details of variant type, i.e., missense versus loss of function, can have clinical utility.

Current clinical practice is not utilising the full potential of genetics

(A)

Disease	Diagnostic	Prognostic	Therapeutic
LQTS	+++	+++	++
CPVT	+++	+	–
BrS	+	+	–
CCD	+	+	+
SQTS	+/–	–	–
AF	–	+	–
HCM	+++	++	+
ACM/ARVC	+	+/–	–
DCM	+/–	–	–
DCM + CCD	++	++	+
LVNC	+	–	–
RCM	+	+	+

(B)

Disease	Diagnostic	Prognostic	Therapeutic
Arrhythmia syndromes			
Long QT syndrome	+++	+++	+++
CPVT	+++	+	+
Brugada syndrome	+	+	+
Progressive cardiac conduction disease	+	+	+
Short QT syndrome	+	+	+
Sinus node disease	–	+	–
Atrial fibrillation	–	+	–
Early repolarization syndrome	–	–	–
Cardiomyopathies			
Hypertrophic cardiomyopathy	+++	++	++
Dilated cardiomyopathy	++	+++	++
Arrhythmogenic cardiomyopathy	+++	++	++
Left ventricular non-compaction	+	+	–
Restrictive cardiomyopathy	+	+	+
Congenital heart disease			
Syndromic CHD	+++	+	–
Non-syndromic CHD	+	–	–
Familial CHD	++	–	–

Figure 2: (A) Impact of genetic testing for the proband from consensus statement published in 2011; HRS/EHRA expert consensus statement on the state of genetic testing for channelopathies and cardiomyopathies[3] (B) Updated consensus statement published in 2022; European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) Expert Consensus Statement on the State of Genetic Testing for Cardiac Disease[4].

Abbreviations: LQTS, long QT syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; BrS, Brugada syndrome; CCD, cardiac conduction disease; SQTS, short QT syndrome; AF, atrial fibrillation; HCM, hypertrophic cardiomyopathy; ACM, arrhythmogenic cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; LVNC, left ventricular non-compaction cardiomyopathy; RCM, restrictive cardiomyopathy

In Chapter 1 of this thesis summarises studies adequately powered to show genotype-specific disease sub-groups with strong evidence supporting the use of genetic evidence in clinical management in many other genes like; *DSP*, *TMEM43*, *LMNA*, *PLN*, and *SCN5A*. Figure 2 highlights the evidence for clinical utility of genetic testing, in terms of diagnosis, prognosis and therapeutic utility. Figure 2A is from the genetic testing consensus statement published in 2011[3] while Figure 2B is from the updated consensus statement recently published in 2022.[4] Since it can take time for adequately powered studies and subsequent validation of findings in additional cohorts, translation of these findings to clinical guideline recommendations can take time. Indeed, current guidelines for clinical management of inherited cardiomyopathies largely do not reflect the emerging advances in the field (Chapter 1).

Our team recently reported an 18-year clinic experience recognising that 13% of patients attending a specialized multidisciplinary genetic heart disease clinic had their final diagnosis changed or clarified in a clinically useful way due to cardiac genetic testing.[5] Yet, there are currently only five gene-specific recommendations from two of eleven clinical guidelines or expert consensus statements focused on clinical management of cardiomyopathies (Chapter 1). Specifically, for this study I contributed case data of individuals originally diagnosed with DCM where the genetic results showed examples of clinical utility after clarification and changing diagnosis (Figure 3). For example, clarification of diagnosis for patients with *TTN* truncating variants had clinical utility supported by studies presented in Chapter 1. Additionally, the diagnosis was changed in patients with *FLNC* truncating variants, similar to that described in Chapter 2. We show the need for even more research that is focused on identifying and validating these genotype-specific findings, so that patients worldwide can receive the highest standard of care and avoid poor clinical outcomes. Bridging the gap

between experts (specialist in cardiac genetics) and the general cardiologist is important in ensuring rapid translation of research findings into gene-specific recommendations

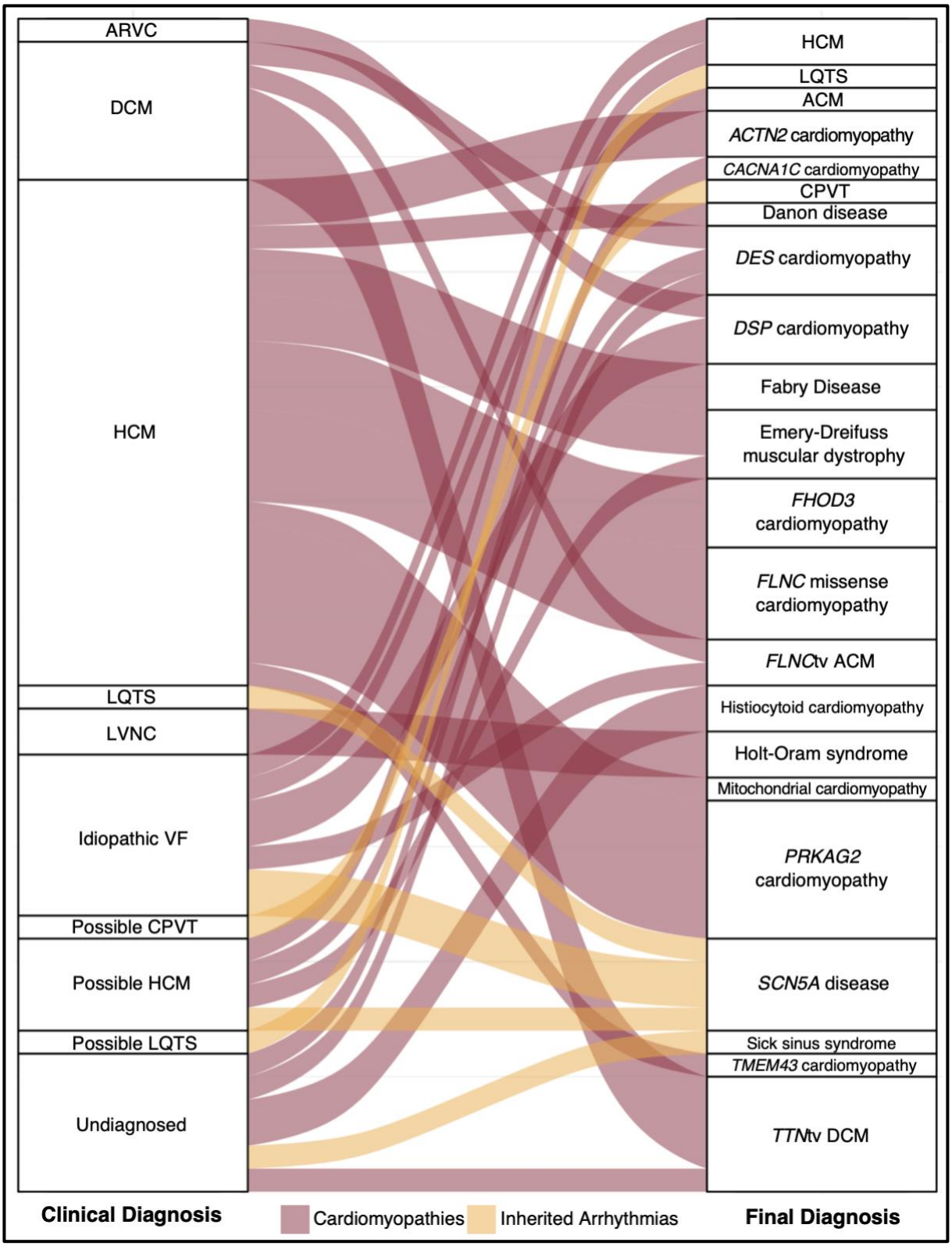


Figure 3: Impact of genomics in clarifying or changing the overall diagnosis (Figure from work by our team that I contributed to[5]).

Baseline phenotype of ARVC included those who met the 2010 Task Force Criteria. *Abbreviations:* ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; LVNC, left-ventricular non-compaction; CPVT, catecholaminergic polymorphic ventricular tachycardia; ACM, arrhythmogenic cardiomyopathy

Limitations and gaps in genetics

That being said, the role of genetics in inherited cardiomyopathies is not clear cut, our research highlights a grey area due to variable expression and incomplete penetrance. For example, we observed non-penetrance in individuals with *FLNC* truncating variants (Chapter 2) and also inconsistent phenotypes of individuals with heterozygous loss of function *ALPK3* variants (Chapter 3). This work forms a basis for additional, potentially conflicting evidence for the monogenic role of heterozygous loss of function *ALPK3* variants in HCM to be used in determining clinical validity. Investigating the factors modulating variable expression of monogenic disease will form the basis of future research, where the role of common variants (measured using polygenic risk scores) and non-genetic environmental factors (e.g., exercise, pregnancy, viral infections) and their impact on disease penetrance and expression likely play an important role.

As well as the issues of variable expression and incomplete penetrance in modulating disease, the field is also highly dynamic whereby new research can contradict and/or dispute the validity of previous findings. This demonstrates the importance of considering how to ensure clinical practice keeps up with new evidence and findings. In my recent experience as a biocurator for the NIH-funded Clinical Genome Resource (ClinGen) Hereditary Cardiovascular Disorders Gene Curation Expert Panel (HCVD GCEP), I participated in the re-curation of genes associated with HCM. Gene curation is the evaluation of genetic and experimental evidence supporting or contradicting a gene-disease relationships and applying one of the following classifications; "Definitive," "Strong," "Moderate," "Limited," "No Reported Evidence," or "Conflicting Evidence."[6] This project involved the re-curation of *CALR3*, a protein belonging to a family of Ca^{2+} binding chaperones localised mainly in the endoplasmic and

sarcoplasmic reticulum, which was previously curated as having limited gene-disease association in the initial curation effort. My re-curation found new literature showing genetic evidence that contradicts this as a potential HCM-associated gene. This included non-segregation, high frequency of probands with another disease-causing variant and non-significant case/control correlations.[7] Further, experimental evidence published more recently has now shown there is no expression in myocardial tissue and normal cardiac function in mice knockout studies.[8] The final result was to revise the original assertion from limited gene-disease association to 'disputed', providing an excellent example of the highly dynamic nature of our understanding of the genetic architecture of inherited cardiomyopathies. My contributions to the HCVD-GCEP will include authorship on the HCM re-curation manuscript to be prepared over the coming months.

While, there are accepted processes in place for the curation and re-curation of genes, there is likewise need for regular reclassification of variants. Literature suggests that in patients with cardiomyopathies, that 35% of variants reclassified would have changed medical management recommendations.[9] We found among those with heterozygous loss of function *ALPK3* variants*,* the identification of a new gene transcript that reallocated most of exon 1 to non-coding sequence led to downgrading of three variants to likely benign or benign (Chapter 3). However, in practice the implementation of regular re-curation of individual variants is a huge undertaking. At present, clinical laboratories do not necessarily have capacity to take this on in any systematic way, therefore clinicians need to have a more active role in this. We have adopted an approach of performing an expedited review of variants for patients attending clinic appointments for follow-up. This includes evaluating gnomAD frequency, updated ClinVar assertions, changes to the family history, ensuring no

changes to clinical validity of the gene, which requires minimal time but serves as a mechanism for identifying those variants requiring more detailed discussion.

Conclusions

In conclusion, this work has identified the value of genetic testing for inherited cardiomyopathies, showing gene and variant-specific disease as a means of improving diagnosis and management, that is only just beginning to be reflected in clinical practice. We highlight the gaps in knowledge and the limitations of this highly dynamic field. My work has applicability to clinical recommendations, and will form the basis of future research into genotype-specific disease subgroups, with the ultimate goal of improving patient outcomes in individuals and families with inherited cardiomyopathies.

References

1. Ortiz-Genga MF, Cuenca S, Dal Ferro M, et al. Truncating FLNC Mutations Are Associated With High-Risk Dilated and Arrhythmogenic Cardiomyopathies. *J Am Coll Cardiol*. 2016;68(22):2440-2451. doi:10.1016/j.jacc.2016.09.927

2. Agarwal R, Paulo JA, Toepfer CN, et al. Filamin C Cardiomyopathy Variants Cause Protein and Lysosome Accumulation. *Circ Res*. 2021;129(7):751-766. doi:10.1161/CIRCRESAHA.120.317076

3. Ackerman MJ, Priori SG, Willems S, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm*. 2011;8(8):1308-1339. doi:10.1016/j.hrthm.2011.05.020

4. Wilde AAM, Semsarian C, Márquez MF, et al. European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) Expert Consensus Statement on the State of Genetic Testing for Cardiac Diseases. *Heart Rhythm*. 2022;19(7):e1-e60. doi:10.1016/j.hrthm.2022.03.1225

5. Stafford F, Krishnan N, Richardson E, et al. The role of genetic testing in diagnosis and care of inherited cardiac conditions in a specialised multidisciplinary clinic. *Genome Med*. 2022;14(1):145. doi:10.1186/s13073-022-01149-0

6. Strande NT, Riggs ER, Buchanan AH, et al. Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. *Am J Hum Genet*. 2017;100(6):895-906. doi:10.1016/j.ajhg.2017.04.015

7. Verhagen JMA, Veldman JH, van der Zwaag PA, et al. Lack of evidence for a causal role of CALR3 in monogenic cardiomyopathy. *Eur J Hum Genet*. 2018;26(11):1603-1610. doi:10.1038/s41431-018-0208-1

8. Ikawa M, Tokuhiro K, Yamaguchi R, et al. Calsperin is a testis-specific chaperone required for sperm fertility. *J Biol Chem*. 2011;286(7):5639-5646. doi:10.1074/jbc.M110.140152

9. VanDyke RE, Hashimoto S, Morales A, Pyatt RE, Sturm AC. Impact of variant reclassification in the clinical setting of cardiovascular genetics. *J Genet Couns*. 2021;30(2):503-512. doi:10.1002/jgc4.1336