The Burden of Dilated Cardiomyopathy Variants in an Adult Cardiac Transplant Bank

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Abstract

- >4,500 heart transplants are performed in the U.S. each year, with ~90% in recipients over 18 years old.[1] Of these, most common causes are ischemic heart failure (IHF)[2] and dilated cardiomyopathy (DCM),[3] but the genetic bases of each are incompletely understood.
- Whole genome sequencing (WGS) was performed in 520 adult end-stage heart failure (HF) and 164 adult nonfailing cardiac samples. A novel variant classification pathway (CVI) was compared with an established pathway (FKN), using ACMG guidelines, to examine 57 genes.
- 34.8% of adult DCM samples had at least one pathogenic or likely pathogenic (P/LP) variant. Titin (*TTN*) variants comprised 34% of these. 7.3% of IHF samples had at least one P/LP variant. *TTN* mutations comprised 35% of these. Among adult nonfailing data, 3 of 64 samples (1.8%) had at least one P/LP variant.
- 5 samples had multiple P/LP variants. 12 P/LP variants were represented by multiple samples. Missense, nonsense, and frameshift variants comprised most P/LP data.
- CVI found frameshift variants missed by FKN, but FKN found splice variants missed by CVI.

Introduction

- >60 million persons are living with heart failure (HF) globally,[4] and 5-year mortality for HF remains 50-75%.[5]
- Causative DCM gene variants can be detected by genetic testing in up to 40% of familial cases.[6] The DCM Gene Curation Expert Panel identified 19 genes with 'high' evidence for their association with developing DCM.[7] Different studies have identified different numbers of target genes for DCM analysis.[8,9,10,11]
- From prior studies, P/LP variants were identified in 38.7% of non-ischemic HF transplant samples[8] or in 44.3% of explanted DCM hearts.[9] Implicated genes included *TTN*, *LMNA*, *BAG3*, *DSP*, and *TNNT2*. Cardiac genes have integrated, overlapping functions including contraction, excitation-contraction coupling, synchronous mechanosensory responses, cell metabolism and turnover, and regulation of gene expression.
- We sought to classify variants in failing hearts alongside variants from nonfailing hearts in a case-control format, as well as search for DCM-related P/LP variants in IHF samples.
- We compared a novel variant identification algorithm with an already established algorithm to ascertain utility.

Methods and Materials

- Explanted hearts were collected via the University of Colorado Heart Tissue Bank.[10] Phenotype and demographic data were collected at the time of transplant.
- Non-failing hearts were defined as physically eligible for transplant but not utilized due to factors such as genetic mismatch or time expiration.
- WGS was done by the Broad Institute, contracted by the NHLBI Trans-Omics for Precision Medicine (TOPMed) program, with variant annotation as previously described.[11]
- 57 genes were studied based on curated genes from the ClinGen program for "DCM" and "arrhythmogenic right ventricular cardiomyopathy." ClinVar divided these into Tier 1 and Tier 2 genes based on evidence. Variant call files were initially analyzed using the Genoox Franklin (FKN) algorithm (https://franklin.genoox.com/clinical-db/home). FKN classifications were scored as P/LP or variants of uncertain significance (VUS), or they were excluded as benign or likely benign (B/LB). Variants found by a proprietary alternative (CVI) algorithm were adjudicated via ClinVar, gnomAD, and medical literature, with decisions of pathogenicity based on American College of Medical Genetics and American College of Pathology guidelines.[12] Variants with minor allele frequencies >0.01 were excluded, and for *FLNC* and *TTN*, only truncation variants were considered.

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Results

Adult Samples	Nonfailing [n = 164]	Failing [n = 520]	DCM [n = 221]	IHF [n = 234]	PPCM [n = 6]
Age [Range]	mean 49.7 y [19, 75]	mean 52.4 y [19, 78]	mean 50.0 y [20, 78]	mean 56.8 y [30, 77]	mean 39.6 y [23, 62]
Sex (M // F // unknown)	77 // 87 // 0	411 // 103 // 6	170 // 50 // 1	201 // 29 // 4	0 // 6 // 0
White	147	381	156	175	3
Black or African American	4	39	26	6	2
Asian	2	11	4	5	0
American Indian/Alaska Native (%)	2	6	4	2	0
More Than One Race	0	4	3	0	0
Race Other, NA, or Unknown	9	79	28	46	1
Not Hispanic or Latino	128	370	159	157	5
Hispanic or Latino	23	47	26	14	1
Ethnicity NA, Unknown or Unreported	13	103	36	63	0
# (%) Positive for P/LP Variant	3 (1.8%)	112 (21.5%)[2]	77 (34.8%)[4]	17 (7.3%)	1 (16.7%)
# (%) Positive for VUS	49 (29.9%)[1]	173 (33.3%)[3]	82 (37.1%)[5]	70 (29.9%)[6]	3 (50.0%)[7]

Table 1. Adult Demographic Data. DCM: Dilated Cardiomyopathy. IHF: Ischemic Heart Failure. PPCM: Peripartum Cardiomyopathy. ([1]Four samples had 2 VUS; [2]Five samples had 2 P/LP; [3]Thirty-five samples had 2 VUS, three samples had 3 VUS, one sample had 4 VUS; [4]Five samples had 2 P/LP; [5]Nineteen samples had 1 VUS, one sample had 3 VUS; [6]Fourteen samples had 2 VUS, one sample had 3 VUS; [7]One sample had 3 VUS)

Adult DCM (Genes with ≥ 3 P/LP Variants)		
Gene	P/LP	VUS
TTN	28 (34%)	8 (8%)
LMNA	14 (17%)	4 (4%)
BAG3	7 (9%)	1 (1%)
FLNC	5 (6%)	0 (0%)
MYH7	5 (6%)	14 (14%)
TNNT2	4 (5%)	3 (3%)
DSP	3 (4%)	9 (9%)
RBM20	3 (4%)	2 (2%)
SCN5A	3 (4%)	6 (6%)

Adult IHF (All 7 Represented P/LP Genes)		
Gene (Adult IHF)	P/LP	VUS
TTN	6 (35%)	4 (5%)
LMNA	3 (18%)	5 (6%)
SCN5A	3 (18%)	13 (15%)
BAG3	2 (12%)	1 (1%)
DSP	1 (6%)	8 (9%)
FLNC	1 (6%)	0 (0%)
MYH7	1 (6%)	5 (6%)

Adult NF (All P/LP Genes and Top VUS Genes)		
Gene	P/LP	VUS
MYBPC3	2 (67%)	4 (8%)
NEXN	1 (33%)	1 (2%)
DSP	0 (0%)	7 (13%)
CDH2	0 (0%)	4 (8%)
MYH6	0 (0%)	4 (8%)
VCL	0 (0%)	4 (8%)
DES	0 (0%)	3 (6%)
MYH7	0 (0%)	3 (6%)
NEBL	0 (0%)	3 (6%)

Table 2. Adult Gene Data for DCM, IHF, and NF (% = percent of each column total, may not add to 100% due to truncation)

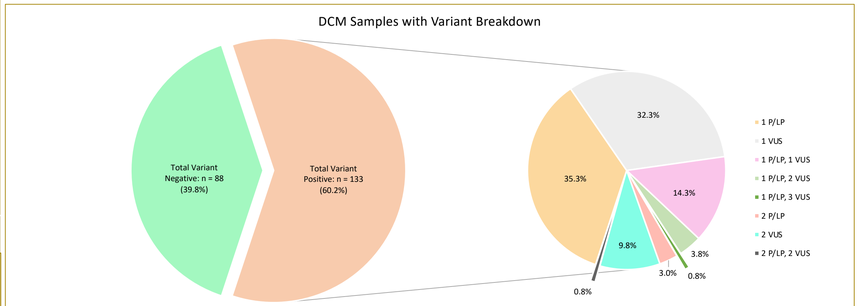

Chart 1. Adult DCM Data with Variant Breakdown. **Legend**: Breakdown of Samples with Various Numbers of P/LP Variants and VUS

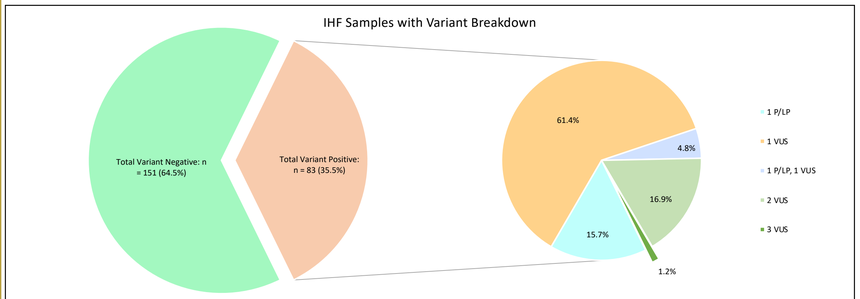

Chart 2. Adult IHF Data with Variant Breakdown. **Legend**: Breakdown of Samples with Various Numbers of P/LP Variants and VUS

Results (continued)

Mutation Type	Missense	Nonsense	Frameshift	Insertion/Deletion	Splice	Stop_Gain
# of Variants	41	39	29	5	4	2

Table 3. Adult P/LP Variant Data by Variant Type

Gene	TNNI3K	LMNA	LMNA	LMNA	TNNT2	TNNT2
Repeated Variant	p.Phe241fs	p.Arg166Pro	p.Arg190Trp	p.Gln353*	p.Glu173del	p.Arg144Trp
# of Repeats	2	2	2	3	2	2
Gene	**BAG3**	**MYBPC3**	**TCAP**	**TTN**	**SCN5A**	**FLNC**
Repeated Variant	p.Arg123*	p.Glu542Gln	p.Arg158Ser	c.86821+2T>A	p.Thr1303Met	p.Gly1891fs
# of Repeats	3	3	2	2	4	2

Table 4. Repeated P/LP Variants

- End-stage samples with *TTN* variants had a mean transplant age of 47.0 years versus 53.5 years for variant-negative samples (p = 0.0011). Age comparisons for other top P/LP genes (*LMNA*, *BAG3*, *FLNC*) did not yield statistically significant results. Similar comparisons of gender and race for these genes were not statistically significant.
- 34.8% of DCM samples had at least one P/LP variant versus 1.8% for NF ($p = 2.86 \times 10^{-15}$). 7.3% of IHF samples had at least one P/LP variant versus 1.8% for NF (p = 0.015). Comparing the DCM and IHF percentages directly yielded ($p = 3.82 \times 10^{-13}$).
- Table 1 was repeated for rarer HF subtypes: hypertrophic cardiomyopathy (HCM), valvular cardiomyopathy (VCM), restrictive cardiomyopathy (RCM), congenital heart disease (CHD), myocarditis, and retransplant. 9 of 11 (81.8%) HCM samples and 3 of 6 (50.0%) myocarditis samples had a P/LP variant; 18 of 20 (90.0%) of retransplants were in male patients.
- There were 3 P/LP nonsense variants identified in the nonfailing population: p.Arg484* in NEXN, p.Arg1271Ter in MYBPC3, and p.Trp1214Ter in MYBPC3.
- 4 P/LP variants (2 splice donor, 1 splice acceptor, 1 missense) were identified by FKN and missed by CVI. 29 P/LP variants (24 frameshift, 3 in-frame deletion, 2 nonsense) were identified by CVI and missed by FKN.

Discussion

- Precision medicine is emerging to individualize treatment of HF. The percentage of P/LP DCM variants was lower but comparable to those of prior studies, suggesting that ~30-40% of end-stage DCM hearts have an ascertainable P/LP cause. As functional studies emerge for variants in target genes, this percentage will likely increase. Genes heavy in VUS should guide further functional studies to pinpoint pathogenicity, as should MYBPC3 given its presence in the nonfailing population. The distribution of variants in DCM and IHF overlapped, suggesting a continuum of HF subtypes, whereas IHF has been historically understood as environmental.
- The presence of P/LP variants in NF samples suggests value in pre-screening donor hearts to mitigate re-transplant. However, this is often impossible due to preservation difficulties.
- Some samples with multiple variants had no P/LP variant among them, raising questions about the additive effect of multiple intermediate variants.
- Some samples had >1 P/LP variants, and some P/LP variants were shared by >1 sample. Both these findings can shape future studies of population, function, and diagnosis.
- Limitations include lack of family history data, as well as possible misdiagnosis of IHF.
- Future efforts include more study of rarer HF subtypes (HCM, VCM, RCM, CHD, myocarditis, and re-transplant), as these were sparsely represented, and investigation of pediatric data.

Conclusions

Our data shows consistency with prior studies and some heterogeneity. The genetic basis of DCM continues to emerge, as does that of IHF. Adjudicating pathogenicity is crucial, as variant structure sometimes does not match clinical effect. Here, learning why function does not follow form helps understand cardiac function holistically. Genetic screening of donor hearts may be useful if feasible. Two different sifting algorithms (FKN and CVI) suggest utility of both. Rarer HF subtypes and pediatric data deserve future attention. Results can drive precision medicine approaches such as gene replacement and signaling pathway modifiers.[13,14,15,16,17]

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