Supplemental Material

Table S1. Gene list.

TIER 1 (N = 74)		TIER 2 (N = 173)			
ABCC9	*MAP2K1*	*NDUFA1*	*AARS2*	*FASTKD2*	*PCCA*
ACTC1	*MAP2K2*	*NDUFA11*	*ACAD8*	*FBXL4*	*PCCB*
ACTN2	*MYBPC3*	*NDUFAF1*	*ACAD9*	*FHL1*	*PDGFRA*
AKAP9	*MYH6*	*NDUFAF2*	*ACADS*	*FHL2*	*PGM1*
ALPK3	*MYH7*	*NDUFAF3*	*ACADVL*	*FIG4*	*PHYH*
ANKRD1	*MYL2*	*NDUFAF4*	*ACTA1*	*FKRP*	*PIGT*
BAG3	*MYL3*	*NDUFAF5*	*ADCY5*	*FOS*	*PMM2*
BRAF	*MYLK2*	*NDUFB3*	*AGK*	*FOXRED1*	*PNPLA2*
CACNA2D1	*MYOZ2*	*NDUFB9*	*AGL*	*FTO*	*POLG*
CALR3	*MYPN*	*NDUFS1*	*AGPAT2*	*GAA*	*POMT1*
CAV3	*NEBL*	*NDUFS2*	*ALG1*	*GBE1*	*PRPS1*
CRYAB	*NEXN*	*NDUFS3*	*ARSB*	*GLB1*	*RAB3GAP2*
CSRP3	*NRAS*	*NDUFS4*	*ATP5E*	*GMPPB*	*RIT1*
DES	*PDLIM3*	*NDUFS6*	*ATPAF2*	*GNAS*	*RMRP*
DMD	*PKP2*	*NDUFV1*	*BCS1L*	*GNPTAB*	*SCO2*
DOLK	*PLN*	*NDUFV2*	*BOLA3*	*GNS*	*SDHAF1*
DTNA	*PRDM16*	*PEX1*	*C10ORF2*	*GPC3*	*SEPN1*
DSC2	*PRKAG2*	*PEX10*	*CAV1*	*GSN*	*SGCA*
DSG2	*PTPN11*	*PEX11B*	*CDKN1C*	*GYS1*	*SGCB*
DSP	*RAF1*	*PEX12*	*CHKB*	*H19*	*SLC22A5*
EMD	*RBM20*	*PEX13*	*CISD2*	*HADH*	*SLC25A20*
EYA4	*RYR2*	*PEX14*	*COA5*	*HADHA*	*SLC25A3*
FKTN	*SCN5A*	*PEX16*	*COG7*	*HADHB*	*SLC2A10*
FLNCA	*SGCD*	*PEX19*	*COL7A1*	*HBB*	*SNAP29*
FXN	*SOS1*	*PEX2*	*COQ2*	*HCCS*	*SYNE2*
GATA4	*TAZ*	*PEX26*	*COX14*	*IDH2*	*TGFB1*
GATAD1	*TCAP*	*PEX3*	*COX6B1*	*IDUA*	*TMEM70*

GLA	*TGFB3*	*PEX5*	*COX7B*	*KCNQ1OT1*	*TPI1*
HRAS	*TMEM43*	*PEX6*	*CPT1A*	*LCRB*	*TPM3*
ILK	*TMPO*	*PEX7*	*CPT2*	*LIAS*	*TSFM*
JPH2	*TNNC1*	*HAMP*	*D2HGDH*	*MGME1*	*TTPA*
JUP	*TNNI3*	*HFE*	*DLD*	*MLYCD*	*UBR1*
KRAS	*TNNT2*	*HFE2*	*DNAJC19*	*MUT*	*VPS13A*
LAMA4	*TPM1*	*MTND1*	*DPM3*	*MYOT*	*WFS1*
LAMP2	*TTN*	*MTND5*	*ELAC2*	*NAGA*	*XK*
LDB3	*TTR*	*MTND6*	*EPG5*	*NEU1*	*YARS2*
LMNA	*VCL*	*MTTD*	*ERBB3*	*NSD1*	
			ERCC4	*NUBPL*	

Table S2. Genes without 100% of coverage of ≥ 20 reads.

Tier 1	**Tier 2**
ACTC1	NEBL
BAG3	ABCC9
DES	SOS1
DMD	HRAS
DSG2	EMD
DSP	SYNE1
LAMP2	RAF1
LMNA	ACTN2
MYBPC3	LAMA2
MYH7	DTNA
RBM20	AKAP9
SCN5A	PDLIM3
TAZ	MYH6
VCL	MYLK2
TTN	MYPN
LDB3	SDHA
	DOLK
	SYNM
	SGCD
	PKP2
	JUP
	NRAS
	CAV3
	RYR2
	TCAP
	GATA4
	JPH2
	PRDM16

	ALPK3
	GATAD1

Table S3. ACMG criteria used to guide classifying likely pathogenic and pathogenic variants.

Subject, gene variant	Classification	Variant information	ACMG criteria
2, NM_001001432.2(*TNNT2*):c.517C>T	P	Heterozygous missense major aa change, very high conservation in silico - consistently pathogenic Absent in population databases Previously described as P/LP in multiple cases with cardiomyopathy Shown to segregate with disease in multiple families Functional studies show abnormal protein function	**PS3** Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product **PM2** Absent from controls (or at extremely low frequency if recessive) (table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) **PP1** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease **PP5** Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation
4, NM_003319.4(*TTN*):c.69855dupA	LP	heterozygous duplication NMD predicted absent in population numerous TV in region associated with DCM classified - LP	**PVS1** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

6, NM_003319.4(TTN):c.21991G>T	LP	Heterozygous nonsense - NMD predicted absent in population databases Segregates with disease within family Classified - LP	**PVS1** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP1** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease
8, NM_003319.4(TTN):c.44430_44449del	LP	heterozygous deletion NMD predicited absent in population databases	**PVS1** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP1** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease
9, NM_133378.4(TTN):c.10361-3042T>A	LP	Heterozygous nonsense - NMD predicted Loss of substantial amount of protein absent in population databases Segregates with disease within family	**PVS1** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP1** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease

1, NM_004415.2(*DSP*):c.2638dupG	LP	Heterozygous frameshift - predicted Absent in population databases Numerous truncating variants downstream reported as pathogenic	**PVS1** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP1** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease
3, NM_001035.2(*RYR2*):c.14570T>A	LP	Heterozygous missense major aa change, very high conservation in silico consistently pathogenic absent from population database confirmed de novo Classified - LP	**PM2** Absent from controls (or at extremely low frequency if recessive) (table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) **PM6** Assumed de novo, but without confirmation of paternity and maternity
5, NM_170707.3(*LMNA*):c.1608+1G>A	LP	heterozygous splice site (cononical) Nucleotide conserved absent from population database previously reported in patient with DCM and LGMD segregation with disease classified - LP	**PVS1** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) **PP1** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease **PP5** Reputable source recently reports variant as pathogenic, but the evidence

			is not available to the laboratory to perform an independent evaluation
7, NM_004281.3(BAG3):c.108G>A	LP	Heterozygous nonsense NMD predicted absent in population databases downstream TV reported pathogenic classified - LP	**PVS1** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
10, NM_005159.4(ACTC1):c.998C>T	LP	ACTC1 Heterozygous missense in silico consistently pathogenic absent in population databases previously desribed as pathogenic in a patient with HCM alternative change classified as pathogenic	**PM2** Absent from controls (or at extremely low frequency if recessive) (table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP1** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) **PP5** Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation **PM5** Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

LP = Likely pathogenic, NMD = nonsense mediated decay, P = pathogenic

Table S4. ACMG criteria used to guide classifying rare variants of unknown significance. *

Variant description	Variant information	ACMG criteria
NM_000257.3(MHY7):c.2923-1G>T	Cononical splice site variant, conserved nucleotide, absent in population, in silico tools suggest aberrant splicing	**PVS1*** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
NM_001134363.1(RBM20):c.3316+1G>A	Cononical splice site variant, conserved nucleotide, absent in population, in silico tools suggest aberrant splicing	**PVS1*** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
NM_020778.4(ALPK3):c.4799G>C	Novel missense variant, very high conservation, major amino acid change (tryptophan to serine), in silico tools suggest deleterious, located in protein kinase domain. Majority of pathogenic variants result in a premature termination codon.	**PM2** Absent from controls (or at extremely low frequency if recessive) (table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

NM_000257.2(MYH7):c.5096G>A	Rare missense variant, very high conservation, minor amino acid change (arginine to glutamine), in silico tools suggest deleterious	**PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
NM_000256.3(MYBPC3):c.3613C>T	Rare missense variant, moderate conservation, major amino acid change (arginine to tryptophan), in silico tools suggest deleterious, located in Ig domain, previously reported in a patient with HCM	**PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) **PP5** Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation
NM_000257.2(MYH7):c.5287G>A	Rare missense variant, very high conservation, minor amino acid change (alanine to threonine), in silico tools suggest deleterious, located myosin tail domain, previously reported in patients with cardiomyopathy	**PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) **PP5** Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation
NM_001035.2(RYR2):c.10046C>T	Novel missense variant, high conservation, major amino acid change (serine to leucine), in silico tools suggest deleterious, conflicting reports of pathogenecity	**PM2** Absent from controls (or at extremely low frequency if recessive) (table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
NM_000257.3(MYH7):c.3035C>A	Rare missense variant, moderate conservation, major amino acid change (alanine to aspartic acid), in silico tools suggest deleterious, previously reported as a VUS in patients with hypertrophic cardiomyopathy, left ventricular noncompaction and myopathy	**PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

NM_001943.4(DSG2):c.3039C>A	Rare nonsense variant, predicted to truncate the protein, previously reported as likely pathogenic, other truncating variants downstream reported in patients with ARVC	**PVS1*** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PP5** Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation
NM_000256.3(MYBPC3):c.604A>C (p.Lys202Gln)	Rare missense variant, very high conservation, minor amino acid change (lysine to glutamine), in silico tools suggest deleterious, located in an Ig domain, previously reported with conflicting interpretations of pathogenicity	**PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
NM_000257.3(MYH7):c.1791C>A (p.Asn597Lys)	Novel missense variant, very high conservation, moderate amino acid change(asparagine to lysine), in silico tools suggest deleterious, previously described in a patient with DCM and another with LVNC	**PM2** Absent from controls (or at extremely low frequency if recessive) (table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium **PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation
NM_000256.3(MYBPC3):c.1334C>T	Rare missense variant, high conservation, moderate amino acid change (threonine to methionine), in silico tools suggest deleterious, situated in an Ig domain	**PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
NM_001458.4(FLNC):c.3125A>G	Rare missense variant, very high conservation, major amino acid change (tyrosine to cysteine),	**PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

	in silico tools suggest deleterious, situated in a Filamin repeat domain	
(NM_00494.3(DSC2):c.2125+1G>T)	Cononical splice site variant, conserved nucleotide, absent in population, in silico tools conflicting	**PVS1*** null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **PM2** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
NM_007078.2(LDB3):c.1675C>T	Rare missense variant, high conservation, major amino acid change (arginine to tryptophan), in silico tools suggest deleterious, situated in a LIM-Zinc domain	**PP3** Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

* Expert team consensus sought to adjust the appropriate strength level of PVS1 by addressing issues specific to each variant type (duplication, deletion, splice site, nonsense/frameshift, initiation codon) as well as recommendations for determining if loss of function is a disease mechanism for the gene of interest.

Table S5. List of genes with pathogenic or likely pathogenic variants and coverage by commercially available gene panels in Victoria, Australia (October 2018).

Gene	Number	Included on dilated cardiomyopathy panel	Included on cardiomyopathy full panel	Included on comprehensive cardiac panel
DSP	1	N	Y	Y
TNNT2	1	Y	Y	Y
TTN	3	Y	Y	Y
LMNA	1	Y	Y	Y
BAG3	1	Y	Y	Y
RYR2	1	N	Y	Y
ACTC1	1	Y	Y	Y

Y = Yes, N = Not covered