SUPPLEMENTAL MATERIAL

Contents:

Methods

Cardiac magnetic resonance imaging acquisition protocol

All examinations were performed with an 8–element torso placed phased–array coil. Images were obtained during suspended respiration at end–expiration. Scout images were acquired initially to identify the cardiac location. Subsequently, balanced steady-state free precession cine images were acquired in three left ventricular long-axis views and a set of multiple contiguous short–axis slices from the atrioventricular ring to the apex (slice thickness 8 mm, intersection gap 2 mm). Typical parameters were echo time 1.6 ms, repetition time 3.6 ms, flip angle 55°, temporal resolution <50 ms, matrix size 224x224, field of view 300-450 mm, and receiver bandwidth 558 Hz/pixel. Delayed enhancement imaging was performed using a segmental inversion recovery sequence at least 10 minutes after intravenous injection of 0.2 mmol/kg gadobutrol (Gadovist, Bayer Schering Pharma, Berlin, Germany). Three standard long-axis and multiple contiguous short-axis views of the left ventricle (LV) were acquired (slice thickness 8 mm, intersection gap 2 mm). Inversion time was continuously adjusted to null normal myocardium. Typical parameters were echo time 3.2 ms, repetition time 6.8 ms, time of inversion 160-320 ms, flip angle 20°, matrix size 224x160, field of view 300-450 mm, and receiver bandwidth 140 Hz/pixel.

Cardiac magnetic resonance imaging data analysis

Maximal ratio of non-compacted to compacted myocardium (NC/C) was measured in end-diastole in the LV long-axis views. The true LV apex (segment 17)[1] was excluded from this

calculation because of expected artificially high values.[2] Measurements of the thickness of the non-compacted myocardium were obtained from base to the peak of the most prominent trabeculae. If the LV cavity at the level of interest was entirely filled with trabeculae, the thickness of non-compacted myocardium was measured to the LV central axis. Meticulous care was taken to exclude the papillary muscles, their heads and the site of attachment of their bases to the trabeculae carneae in the measurements.[3] Measurements of the thickness of the compacted myocardium included fifty percent of the chemical shift artifact on the epicardial surface.[4]

LV volumes and ejection fraction were calculated. Papillary muscles and trabeculations were considered to be part of the LV cavity and included in calculations of LV volumes. LV volumes were indexed to the body surface area to account for the variations in body weight between subjects. Dilated cardiomyopathy phenotype was defined by the presence of LV dilatation (LVEDV>2SD from normal corrected by body surface area and gender) and systolic dysfunction (LVEF<45%) [PMID: 26792875].

Library Preparation

Libraries were constructed into Illumina paired-end pre-capture libraries according to the manufacturer's protocol (*Illumina Multiplexing_SamplePrep_Guide_1005361_D*) with modifications as described in the BCM-HGSC protocol (https://www.hgsc.bcm.edu/content/protocols-sequencing-library-construction). Libraries were prepared using Beckman robotic workstations (Biomek FX and FXp models). Briefly, 0.5 ug of DNA was sheared into fragments of approximately 200-300 base pairs with the Covaris E210 system followed by end-repair (NEBNext End-Repair Module, Cat. No.

E6050L), A-tailing (NEBNext dA-Tailing Module, Cat. No. E6053L), and ligation of the Illumina multiplexing PE adaptors with barcode sequences using the ExpressLink T4 DNA Ligase (a custom product from Life Technologies). Pre-capture ligation-mediated PCR (LM-PCR) was performed for 6-8 cycles using the Library Amplification Readymix containing KAPA HiFi DNA Polymerase (Kapa Biosystems, Inc., Cat. No. KK2612). Universal primer IMUX-P1.0 and IMUX-P3.0 were used in the PCR amplification. Purification was performed with Agencourt AMPure XP beads after enzymatic reactions. Size distribution of the pre-capture LM-PCR product was determined using the LabChip GX electrophoresis system (PerkinElmer), and quantification was performed by gel analysis using AlphaView SA Version 3.4 software.

Capture Enrichment

The pre-capture libraries were pooled together in equimolar amounts (6 samples/pool, totaling 1.5 ug/pool) and then hybridized in solution to the 42Mb HGSC VCRome 2.1 design[5] (NimbleGen, Cat. No. 06266380001) according to the manufacturer's protocol *NimbleGen SeqCap EZ Exome Library SR User's Guide* with minor revisions. Post-capture LM-PCR amplification was performed using the Library Amplification Readymix containing KAPA HiFi DNA Polymerase with 12 cycles of amplification. After the final AMPure XP bead purification, quantity and size of the capture library was analyzed using the LabChip GX electrophoresis system. The efficiency of the capture was evaluated by performing a qPCR-based quality check on the enrichment level of four standard NimbleGen control loci.

Successful enrichment of the capture libraries was estimated to range from a 6 to 9 of ΔCt value over the non-enriched samples.

Sequencing

Library templates were prepared for sequencing using Illumina's cBot cluster generation system with TruSeq PE Cluster Generation Kits (Cat. No. PE-401-3001) according to the manufacturer's protocol. Briefly, these libraries were denatured with sodium hydroxide and diluted to 6-9 pM in hybridization buffer in order to achieve a load density of ~800K clusters/mm. Each library pool was loaded in a single lane of a HiSeq flow cell, and each lane was spiked with 2% phiX control library for run quality control. The sample libraries then underwent bridge amplification to form clonal clusters, followed by hybridization with the sequencing primer. Sequencing runs were performed in paired-end mode using the Illumina HiSeq 2000 platform. Using the TruSeq SBS Kits (Cat. No. FC-401-3001), sequencing-by-synthesis reactions were extended for 101 cycles from each end, with an additional 7 cycles for the index read. With sequencing yields averaging 7 Gb per sample, samples achieved an average of 93% of the targeted exome bases covered to a depth of 20X or greater.

Primary Data Analysis

Initial sequence analysis was performed using the HGSC Mercury analysis pipeline[6] (https://www.hgsc.bcm.edu/content/mercury). In summary, the .bcl files produced on-instrument were first transferred into the HGSC analysis infrastructure by the HiSeq Real-time Analysis module. Mercury then ran the vendor's primary analysis software (CASAVA)

to de-multiplex pooled samples and generate sequence reads and base-call confidence values (qualities), followed by the mapping of reads to the GRCh37 Human reference genome (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/) using the Burrows-Wheeler aligner, http://bio-bwa.sourceforge.net/). The resulting BAM (binary alignment/map) file underwent quality recalibration using GATK (http://www.broadinstitute.org/gatk/), and where necessary the merging of separate sequence-event BAMs into a single sample-level BAM. BAM sorting, duplicate read marking, and realignment to improve in/del discovery all occur at this step.

Variants are subsequently annotated with the Cassandra suite which evalues the variant effect on RefSeq and USCS gene models using AnnoVar. MAFs are reported from multiple sources including 1000 genomes, ExAc and EVS. Variant deleteriousness is predicted from ~10 models from dbNSFP. Gene function, association to disease and associated phenotypes are collected from OMIM, SwissProt, and Entrez. Additionally, variants were filtered if they had a minor allele frequency >0.5% (averaged from Thousand Genomes, ExAc and ESP), >1% if they had been previously associated with disease (HGMD) or >2% if the variant was homozygous or there was a second variant in the gene. Variants were filtered if they were marked as benign or likely benign in ClinVar from multiple ("mult") institutions. Variants were prioritized if they occurred in a known LVNC gene, known cardiomyopathy gene (non-LVNC), or a muscular dystrophy gene (Table S1 and Table S2). Alleles were considered interesting based upon zygosity, minor allele frequency, association to disease and predicted deleteriousness (polyphen HVAR). An exception to this approach was made for *TTN*, in

which only truncating mutations (nonsense, splicing, and frameshift) were considered interesting.

Variant enrichment analyses

Variant of interest (VOIs) enrichment analysis was conducted using an in-house developed script. Variants were considered interesting if they met the following criteria: minor allele frequency (MAF) < 0.2%, variant present in at least 25% of all reads covering variant position, nonsynonymous variants must be considered deleterious by Polyphen2 HVAR, and variant is present in a known LVNC or cardiomyopathy gene (Table S1 and Table S2). To evaluate the significance of this result we obtained a cohort of 425 phenotypically unselected ("normal"), Americans of European descent, controls which had been sequenced, and data processed in an identical manner to our LVNC cohort.

Figures

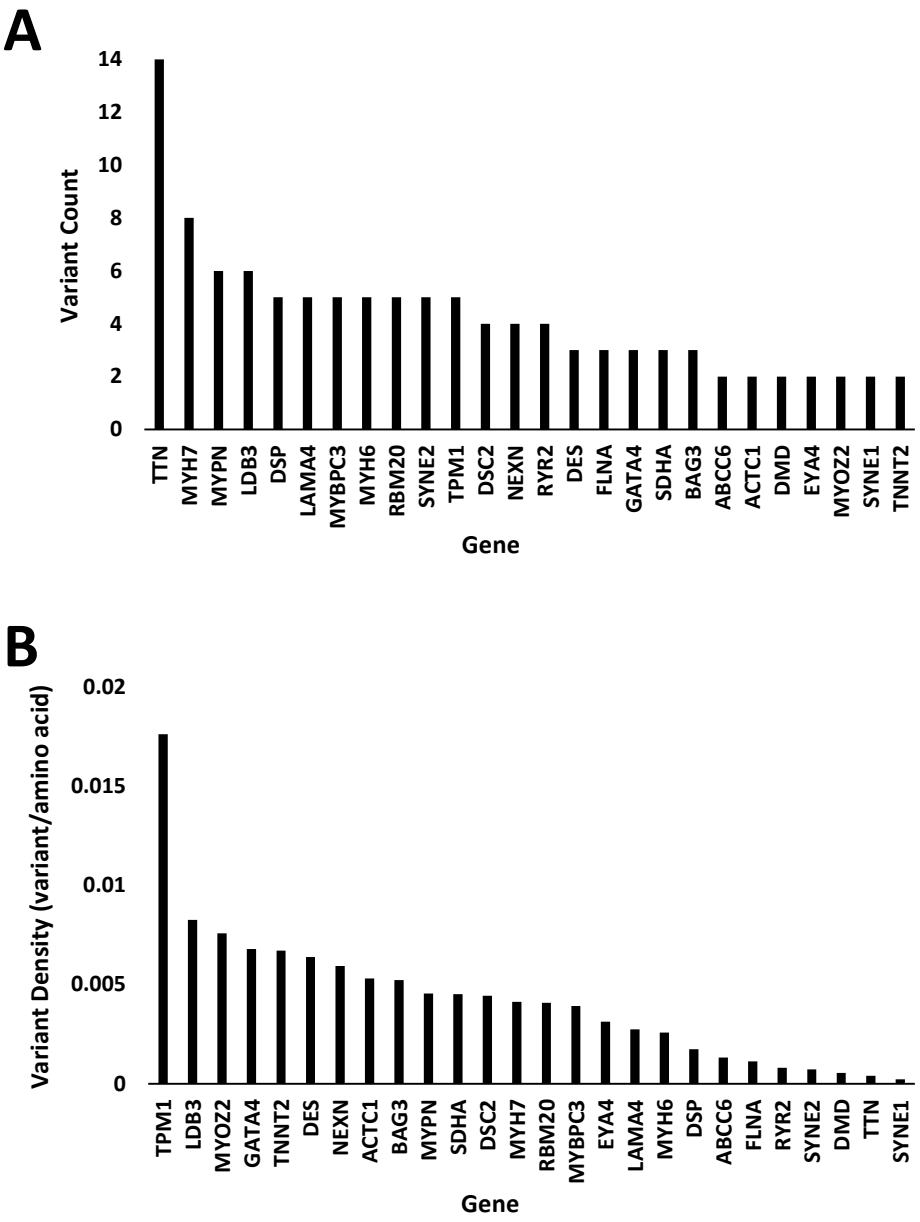

Figure S1. Number of VOIs discovered per gene **(Panel A)**. Number of VOIs discovered per gene normalized by transcript length **(Panel B)**.

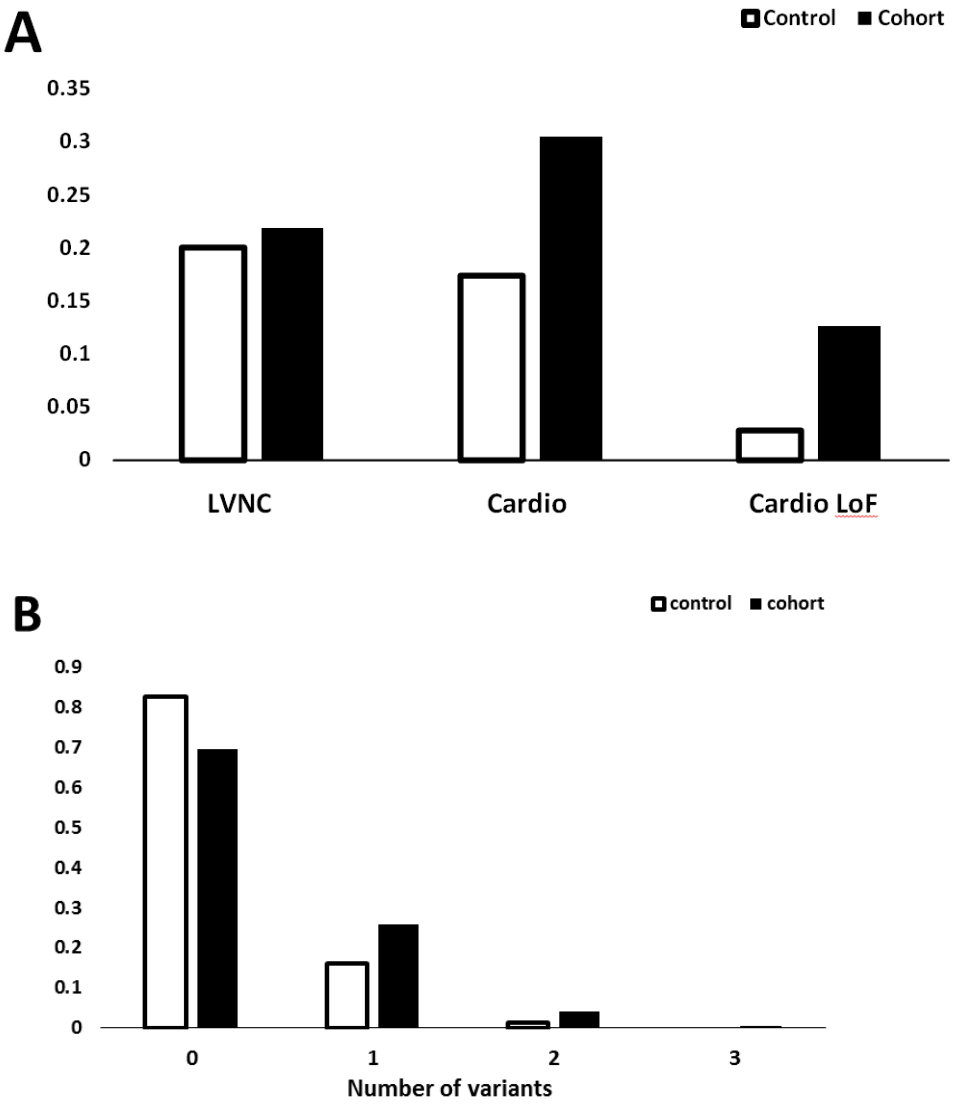

Figure S2. Proportion of patients with at least one variant in the well charctersized LVNC gene set[7] (LVNC), cardiomyopathy gene set (Cardio) or a loss of function mutation in the cardiomyopathy gene set (Cardio LoF) **(Panel A)**. Proportion of subjects with 0, 1, 2 or 3+ variants in cardiomyopathy gene set **(Panel B)**.

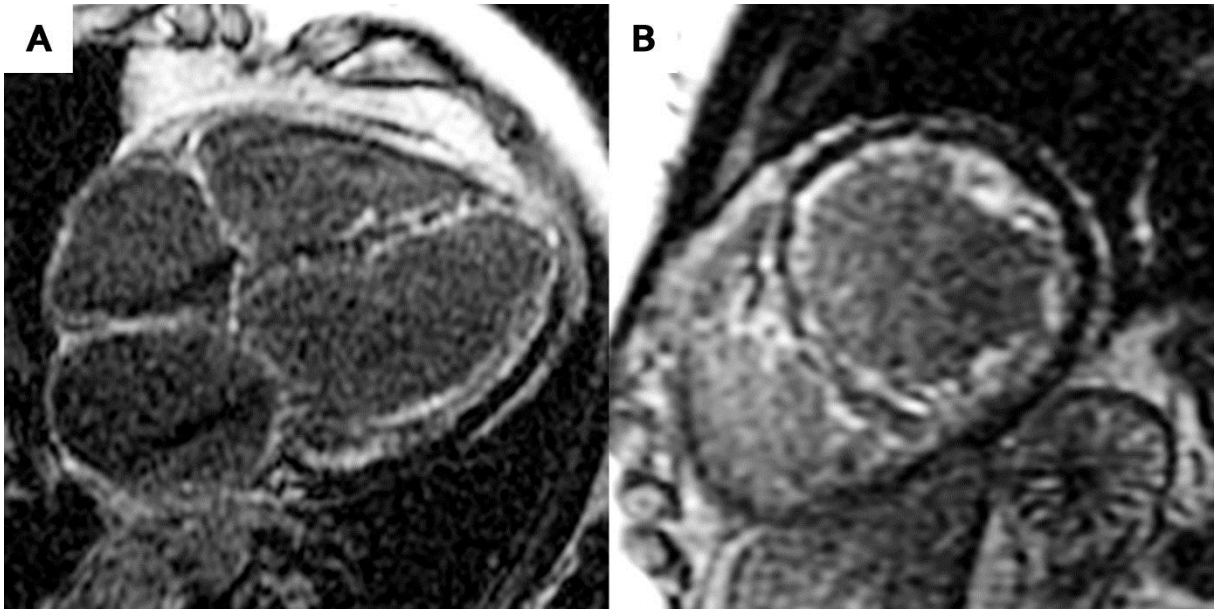

Figure S3. CMR imaging of 12-year old patient with SYNE2 mutation successfully resuscitated after cardiac arrest.

Four-chamber view **(Panel A)** and short axis view **(Panel B)** demonstrating extensive late gadolinium enhancement in subendocardial and non-compacted myocardial layer of left ventricle. Presence of late gadolinium enhancement was not confined only to non-compacted segments indicating LVNC is a diffuse pathology. Five months later patient underwent orthotopic heart transplantation due to end-stage heart failure.

Tables

Table S1. Well characterized LVNC gene list.[7]

ABCCP
ACTC1
ACTN2
CASQ2
DMPK
DSP
DTNA
HCN4
LDB3
LMNA
MIB1
MYBPC3
MYH7
NNT
PKP2
PLEKHM2
PRDM16
RYR2
SCN5A
TAZ
TNNT2
TPM1

Table S2. Cardiomyopathy gene list. Genes commonly associated with cardiomyopathy and with available clinical tests.

ABCC6	*FKTN*	*MTMR14*	*MYLK2*	*SOS1*
ABCC9	*FLNA*	*MTND1*	*MYOZ2*	*SYNE1*
ACTC1	*FLNC*	*MTND5*	*MYPN*	*SYNE2*
ACTN2	*FLT1*	*MTND6*	*NEBL*	*TAZ*
ANG	*GATA4*	*MTTD*	*NEXN*	*TBX1*
ANKRD1	*GATAD1*	*MTTG*	*NRAS*	*TBX20*
BAG3	*GLA*	*MTTH*	*PDLIM3*	*TCAP*
BRAF	*HRAS*	*MTTI*	*PLEC*	*TGFB3*
CAV3	*ILK*	*MTTK*	*PLN*	*TMEM43*
CITED2	*JPH2*	*MTTL1*	*PRDM16*	*TMPO*
CRELD1	*JUP*	*MTTL2*	*PRKAG2*	*TNNC1*
CRYAB	*KCNE1*	*MTTM*	*PRKAR1A*	*TNNI3*
CSRP3	*KRAS*	*MTTQ*	*PSEN1*	*TNNT2*
DES	*LAMA4*	*MTTS1*	*PTPN11*	*TPM1*
DMD	*LAMP2*	*MTTS2*	*RAF1*	*TTN*
DSC2	*LDB3*	*MYBPC3*	*RBM20*	*TTR*
DSP	*LMNA*	*MYH6*	*RYR2*	*VCL*
DTNA	*MAP2K1*	*MYH7*	*SCN5A*	
EMD	*MAP2K2*	*MYL2*	*SDHA*	
EYA4	*MEF2A*	*MYL3*	*SGCD*	

Table S3. Discovered variants in cardiomyopathy genes (N=190).

ID*	Family	Pheno	Solved case	Number of VOIs	Gene symbol	VOI	CHR	POS	REF	VAR	MAF	Protein effect	Deleterious-ness prediction	Disease causing in HGMD	ClinVar	ClinVar source
93	H	LVNC	Yes	4	*ACTC1*	Yes	15	35085571	G	A	0	p.A110V	D	No		
					BAG3	Yes	10	121432151	G	A	0.001034	p.V298M	B	No	US	Single
					TGFB3	Yes	14	76446886	G	A	0.000116	p.H117H	n/a	No		
					TTN	Yes	2	179397443	TCTCA	T	0	p.32991_32992del	n/a	Prox, DCM		
176*	H	LVNC	Yes	4	*ACTC1*	Yes	15	35085571	G	A	0	p.A110V	D	No		
					BAG3	Yes	10	121432151	G	A	0.001034	p.V298M	B	No	US	Single
					TGFB3	Yes	14	76446886	G	A	0.000116	p.H117H	n/a	No		
					TTN	Yes	2	179397443	TCTCA	T	0	p.32991_32992del	n/a	Prox, DCM		
4		LVNC	Yes	3	*CRYAB*	Yes	11	111782333	G	A	0.001244	p.P39L	D	No	US	Single
					LMNA	Yes	1	156105901	C	T	0	p.G382G	n/a	Yes, MDLG	LP|P	Single|Single
					MYLK2	Yes	20	30419883	C	T	0	p.R552X	n/a	No		
90		LVNC	Yes	3	*MYH7*	Yes	14	23900678	G	A	0.001022	p.R249X	n/a	Prox		
					TPM1	Yes	15	63363291	A	G	0.000883	p.K259E	B	No	LB|US|US	Single|No_criteria|Single
					PRKAG2	Yes	7	151257534	T	A	0	p.K133I	D	No		
94		LVNC	Yes	3	*ACTC1*	Yes	15	35084476	C	T	0.000233	p.R208H	B	No		
					DSC2	Yes	18	28648964	G	T	0	p.H802N	P	No		
					TPM1	Yes	15	63340850	G	C	0	p.E26Q	D	No		
					TGFB3	No	14	76446944	G	A	0.001558	p.S98L	B	No	LB	Single

105	LVNC	Yes	3	*MYBPC3*	Yes	11	47372841	CT	C	0	p.A80fs	n/a	Prox, DCM		
				MYH6	Yes	14	23868200	T	C	0.000918	p.K543R	P	No		
				MYH6	Yes	14	23862647	C	G	0	p.E1003D	P	Prox, HCM		
124	LVHT	Yes	3	*DMD*	Yes	X	32305736	G	A	0	p.T2063M	P	Prox, DMD		
				MYPN	Yes	10	69955255	C	T	0.000686	p.R1042C	D	No	US	No_criteria
				VCL	Yes	10	75871701	T	G	0	p.V927G	B	No		
140	LVNC	Yes	3	*MYH7*	Yes	14	23890217	C	A	0.001069	p.D1096Y	D	No	US|US	Single|Single
				SDHA	Yes	5	231050	C	T	0.000836	p.T277M	P	No		
				TTN	Yes	2	179499117	TCTTCTTGCCCTCCACCTCAG	T	0	p.32991_32992del	n/a	No		
187	LVNC	Yes	2	*SCN5A*	Yes	3	38592390	G	A	0	p.L1807F	D	Prox, LQTS		
				SCN5A	No	3	38591847	G	C	0.002172	p.P1988A	B	Yes, LQTS	UNK|US|US	Conf|Single|No_criteria
				TPM1	Yes	15	63363291	A	G	0.000883	p.K259E	B	No	LB|US|US	Single|No_criteria|Single
5	LVNC	Yes	2	*MYBPC3*	Yes	11	47354175	C	T	0	p.R1190H	D	Prox, HCM		
				MYBPC3	No	11	47367871	C	T	0.004326	p.R326Q	B	Yes, HCM	UNK|B|LB|LB|B	Conf|Single|No_criteria|No_criteria|Mult
				TMPO	Yes	12	98931409	T	G	0	p.I241S	P	No		
7	LVNC	Yes	2	*MYH7*	Yes	14	23893148	C	G	0.001326	p.V964L	D	Yes, DCM	US|LP|LP|US	Single|Single|Single|Single
				TTN	Yes	2	179449558	G	A	0	p.R12731X	n/a	No		

31		LVNC	Yes	2	*DSC2*	Yes	18	28672114	C	T	0.001294	p.E102K	B	No	US|LB|US|LP	Single|No_criteria|No_criteria|Single
					LDB3	Yes	10	88452322	G	A	0.001813	p.R365K	B	No		
40		LVNC	Yes	2	*TPM1*	Yes	15	63354797	C	T	0	p.A242V	D	Prox, DCM	US	Single
					TTN	Yes	2	179588756	C	T	0	p.W5833X	n/a	No		
41		LVNC	Yes	2	*RBM20*	Yes	10	112572068	C	T	0	p.P638L	D	Yes, DCM	P,P|P	Single|Single|Single
					TTR	Yes	18	29178580	C	T	0	p.A129V	B	Yes, EH	UNK	No_criteria
43		LVNC	Yes	2	*LAMA4*	Yes	6	112441643	G	A	0	p.S1503F	D	No		
					TBX1	Yes	22	19750765	C	T	0	p.R138W	D	No		
45		LVNC	Yes	2	*ABCC6*	Yes	16	16248830	C	T	0.001168	p.R1314Q	D	Yes, PEAD		
					RBM20	Yes	10	112595636	C	A	0.002091	p.S1195Y	D	No	US	Single
48		LVNC	Yes	2	*DES*	Yes	2	220286116	G	T	0	p.A360S	D	Yes, DRM		
					LDB3	Yes	10	88492723	T	A	0	p.I615N	D	No		
65*	F	LVHT	Yes	2	*FLNC*	Yes	7	128484198	C	T	0	p.Q1024X	n/a	No		
					RYR2	Yes	1	237955507	G	C	0	p.A4556P	P	Yes, VT		
67		LVNC	Yes	2	*RBM20*	Yes	10	112572217	C	T	0	p.R688X	n/a	No	US	Single
					TTN	Yes	2	179537132	A	G	0	g.179537132A>G	n/a	No		
69		LVNC	Yes	2	*LAMA4*	Yes	6	112443287	C	T	0.000882	p.A1462T	D	No	US	Mult
					MYPN	Yes	10	69970208	T	C	0	p.L1320P	D	No		
					NEXN	No	1	78395131	A	C	0.002386	p.E332A	B	No	LB|US	Single|Single
70		LVHT	Yes	2	*DSP*	Yes	6	7585825	C	A	0.000233	p.P2777H	D	No	US	Single
					GATA4	Yes	8	11615875	C	G	0	p.P407R	D	Yes, CHD		

77		LVNC	Yes	2	*LDB3*	Yes	10	88441535	G	A	0.001325	p.A222T	D	No	US|US	Single|Single
					TTN	Yes	2	179454576	G	A	0.000604	p.R11753X	n/a	No	LP|P	Mult|Single
85		LVHT	Yes	2	*MEF2A*	Yes	15	100173373	C	T	0	p.R17X	n/a	No		
					RYR2	Yes	1	237886543	T	C	0	p.V3557A	B	No		
86		LVNC	Yes	2	*FKTN*	Yes	9	108363422	A	G	0.000815	g.108363422A>G	n/a	No	US|US	Single|Single
					MYH6	Yes	14	23855633	T	G	0.000116	p.K1617T	D	No		
87		LVNC	Yes	2	*DMD*	Yes	X	31893318	G	T	0.000539	p.P1021Q	P	Prox, DMD		
					MYH7	Yes	14	23902306	G	A	0	p.S111F	P	Prox, HCM		
136*	L	LVHT	Yes	2	*ABCC9*	Yes	12	21971098	G	C	0	p.L1253V	B	No		
					SYNE2	Yes	14	64542818	G	C	0	p.K3674N	D	No		
137		LVHT	Yes	2	*DSP*	Yes	6	7585486	C	A	0	p.T2664N	P	Prox, ARVD		
					EYA4	Yes	6	133789765	C	T	0.001059	p.T266M	D	Prox, hearing loss	US|US	Single|Single
139		LVHT	Yes	2	*SDHA*	Yes	5	251554	C	T	0.00144	p.R589W	D	Yes, paraganglioma	P	No_criteria
					TTN	Yes	2	179616770	GAA	G	0	g.179616771delAA	n/a	No	LB	Single
145	F	LVNC	Yes	2	*FLNC*	Yes	7	128484198	C	T	0	p.Q1024X	n/a	No		
					RYR2	Yes	1	237955507	G	C	0	p.A4556P	P	Yes, VT		
146		LVNC	Yes	2	*DSC2*	Yes	18	28672114	C	T	0.001294	p.E102K	B	No	US|LB|US|LP	Single|No_criteria|No_criteria|Single
					DSC2	Yes	18	28669437	G	A	0.000929	p.R199C	P	Prox, ARVD		
150		LVHT	Yes	2	*PLEC*	Yes	8	144998035	G	A	0.000127	p.A2025V	D	No		
					PRDM16	Yes	1	3329327	G	A	0.000125	p.A856T	D	No		

					PLEC	No	8	145016631	C	T	0	p.R18Q	B	No	
1		LVNC	Yes	1	*MYH7*	Yes	14	23890217	C	A	0.001069	p.D1096Y	D	No	US|US	Single|Single
					ACTN2	No	1	236902618	G	A	0.001186	p.R298H	P	No	US|US	Single|Single
134		LVHT	Yes	1	*MYBPC3*	Yes	11	47356691	G	A	0.001815	p.T936M	D	No		
					MYBPC3	No	11	47357479	C	T	0.00547	p.V896M	B	No	UNK|UNK	Conf|Conf
6		LVNC	Yes	1	*TNNC1*	Yes	3	52486140	C	T	0	p.D62N	D	Prox, DCM		
8*	A	LVHT	Yes	1	*RYR2*	Yes	1	237801771	A	G	0	p.R2303G	D	Yes, VT		
9		LVNC	Yes	1	*PRKAR1A*	Yes	17	66525105	A	AG	0	p.P288fs	n/a	Yes, Carney complex		
10		LVHT	Yes	1	*GATA4*	Yes	8	11612602	A	T	0	p.K319N	B	Prox, ASD		
12		LVNC	Yes	1	*MYH7*	Yes	14	23882972	G	A	0	p.T1929M	B	Yes, HCM	US	No_criteria
13		LVHT	Yes	1	*MYPN*	Yes	10	69926074	G	A	0	p.G542R	P	No		
15		LVNC	Yes	1	*MYPN*	Yes	10	69925569	G	A	0	p.V532M	D	Yes, RCM	US	Single
17		LVHT	Yes	1	*FLNA*	Yes	X	153588864	A	C	0	p.L1100R	D	No		
20*	B	LVHT	Yes	1	*TTN*	Yes	2	179439257	G	A	0	p.R14995X	n/a	No	P|P	Single|Single
25		LVNC	Yes	1	*SYNE1*	Yes	6	152456241	T	C	0	p.M8596V	B	No	US	Single
27		LVNC	Yes	1	*MYH7*	Yes	14	23896502	T	A	0	p.K635X	n/a	No		
30		LVNC	Yes	1	*LAMA4*	Yes	6	112512888	C	T	0.00085	p.R223H	B	No	LB	Single
					MYBPC3	No	11	47367871	C	T	0.004326	p.R326Q	B	Yes, HCM	UNK|B|LB|LB|B	Conf|Single|No_criteria|No_criteria|Mult
33		LVNC	Yes	1	*NEXN*	Yes	1	78401671	C	G	0	p.A472G	D	No		
34		LVNC	Yes	1	*LAMA4*	Yes	6	112508755	G	C	0.001266	p.A281G	P	No	US	Single

35		LVNC	Yes	1	*MYH7*	Yes	14	23902827	C	T	0.000942	p.V39M	D	No	US|US	Single|No_criteria
36		LVNC	Yes	1	*LAMA4*	Yes	6	112435335	G	A	0	p.P1750L	D	No	US	Single
42		LVNC	Yes	1	*TTN*	Yes	2	179416513	G	A	0	p.Q21499X	n/a	No		
44	A	LVNC	Yes	1	*RYR2*	Yes	1	237801771	A	G	0	p.R2303G	D	Prox, VT		
46		LVNC	Yes	1	*ABCC6*	Yes	16	16244435	C	T	0.000977	p.R1468Q	D	No		
					PRKAG2	No	7	151478279	G	A	0.002132	p.T142I	D	No	US|US	Single|Single
49		LVNC	Yes	1	*TNNT2*	Yes	1	201333484	C	G	0	p.R104P	P	Yes, RCM		
50		LVNC	Yes	1	*LDB3*	Yes	10	88441401	C	T	0	p.A177V	B	No		
					PSEN1	No	14	73673178	A	G	0.017511	p.E318G	B	No	NP|B	No_assertion|Single
53		LVHT	Yes	1	*DSP*	Yes	6	7584061	G	A	0.001119	p.R2189Q	B	No		
54		LVHT	Yes	1	*MYL2*	Yes	12	111352043	G	A	0	p.P74L	D	No		
59		LVHT	Yes	1	*NEXN*	Yes	1	78408504	G	A	0	p.S673N	P	No		
62		LVNC	Yes	1	*LDB3*	Yes	10	88452322	G	A	0.001813	p.R365K	B	No		
63		LVHT	Yes	1	*TTN*	Yes	2	179547454	G	A	0	p.R9778X	n/a	No		
					MYH7	No	14	23889445	T	TG	0	g.23889445insG	n/a	No	B|LB|B|B|LB	Mult|No_criteria|Single|Single|Single
64		LVNC	Yes	1	*TTN*	Yes	2	179407387	AC	A	0	g.179407388delC	n/a	No		Mult|No_criteria|Single|Single|Single
					MYH7	No	14	23889445	T	TG	0	g.23889445insG	n/a	No	B|LB|B|B|LB	Mult|No_criteria|Single|Single|Single
66*	G	LVHT	Yes	1	*TTN*	Yes	2	179460312	G	A	0	p.R10384X	n/a	No	P	Single

					GPD1L	No	3	32200588	C	T	0.000898	p.A280V	B	No	P|P	No_criteria|Single
71*	A	LVHT	Yes	1	*RYR2*	Yes	1	237801771	A	G	0	p.R2303G	D	Yes, VT		
75		LVNC	Yes	1	*TNNI3*	Yes	19	55666174	G	A	0	p.R103C	D	No	LP,US	Single,Single
76		LVHT	Yes	1	*DSP*	Yes	6	7583551	C	T	0	p.A2019V	D	No		
78		LVHT	Yes	1	*FLNA*	Yes	X	153594950	C	T	0.000153	p.E349K	B	Prox, Melnick-Needles syndrome	LP	Single
					DMD	No	X	32509625	A	C	0.010678	p.N797K	P	Yes, DMD	B	Mult
79		LVNC	Yes	1	*SYNE2*	Yes	14	64604613	G	A	0	p.A4919T	B	No		
					RBM20	No	10	112544125	C	T	0.009005	p.S455L	B	No	LB|US|LB	Single|No_criteria|Single
82		LVNC	Yes	1	*ANG*	Yes	14	21162102	G	A	0	p.V127I	B	Yes, ALS		
83		LVHT	Yes	1	*SYNE2*	Yes	14	64599128	G	A	0.000116	p.C4829Y	B	No		
88		LVHT	Yes	1	*GATA4*	Yes	8	11614524	G	C	0.001104	p.E360Q	D	Prox, CHD		
					RBM20	No	10	112540556	C	T	0	g.112540556C>T	n/a	No		
91		LVNC	Yes	1	*MYBPC3*	Yes	11	47364162	C	G	0	p.G531R	D	No		
98		LVNC	Yes	1	*FLT1*	Yes	13	29041266	C	G	0.001156	p.R54S	P	No		
					HADHA	No	2	26418053	C	G	0.001185	p.E510Q	D	Yes, MTPD	P|P|P|P	Single|No_criteria|Single|Single
99	I	LVNC	Yes	1	*MYPN*	Yes	10	69948821	C	T	0.001046	p.R955W	D	Yes, DCM		
100		LVNC	Yes	1	*SYNE1*	Yes	6	152697692	G	C	0.003431	p.L3057V	D	No	US	Single
102		LVNC	Yes	1	*FLNA*	Yes	X	153594950	C	T	0.000153	p.E349K	B	Prox, Melnick-Needles syndrome	LP	Single

104		LVNC	Yes	1	*LDB3*	Yes	10	88485922	C	CCA	0	p.C559fs	n/a	No	
					MYBPC3	No	11	47367871	C	T	0.004326	p.R326Q	B	Yes, HCM	UNK|B|LB|LB|B	Conf|Single|No_criteria|No_criteria|Mult
106		LVNC	Yes	1	*DSP*	Yes	6	7580597	C	T	0	p.R1392W	D	No		
107		LVHT	Yes	1	*TTN*	Yes	2	179424674	TG	T	0	p.V19855fs	n/a	No		
109		LVNC	Yes	1	*RBM20*	Yes	10	112572217	C	T	0	p.R688X	n/a	No	US	Single
111		LVHT	Yes	1	*MYH6*	Yes	14	23855633	T	G	0.000116	p.K1617T	P	No		
113		LVHT	Yes	1	*NEXN*	Yes	1	78392569	C	T	0.000317	p.R286W	P	No	US	Single
114	L	LVHT	Yes	1	*SYNE2*	Yes	14	64542818	G	C	0	p.K3674N	D	No		
115		LVHT	Yes	1	*RAF1*	Yes	3	12626121	TAG	T	0	p.398_398del	n/a	No		
116		LVHT	Yes	1	*RYR2*	Yes	1	237863581	C	G	0	p.L3061V	P	No		
122		LVHT	Yes	1	*PSEN1*	Yes	14	73637754	C	T	0	p.L109L	n/a	No		
128		LVHT	Yes	1	*CRELD1*	Yes	3	9985136	C	T	0.001837	p.R329C	D	Yes, CASD	UNK	No_criteria
					RBM20	No	10	112544125	C	T	0.009005	p.S455L	B	No	LB|US|LB	Single|No_criteria|Single
130		LVHT	Yes	1	*BAG3*	Yes	10	121436505	GGA	G	0	p.480_481del	n/a	Yes, DCM		
131		LVHT	Yes	1	*MYBPC3*	Yes	11	47362719	A	C	0	p.C623G	B	Prox, DCM		
142		LVNC	Yes	1	*MYPN*	Yes	10	69933879	T	G	0	p.L677W	D	No		
148		LVNC	Yes	1	*TNNT2*	Yes	1	201333459	A	C	0	p.N112K	B	Prox, DCM		
153		LVHT	Yes	1	*MYOZ2*	Yes	4	120107309	C	T	0	p.T250I	B	Prox, HCM		
155		LVHT	Yes	1	*MYH6*	Yes	14	23871705	C	A	0	p.R370L	B	No		
156		LVNC	Yes	1	*TPM1*	Yes	15	63340632	G	T	0	p.C132X	n/a	No		
157	G	LVNC	Yes	1	*TTN*	Yes	2	179460312	G	A	0	p.R10384X	n/a	No	P	Single

158	K	LVNC	Yes	1	*BAG3*	Yes	10	121411324	C	T	0	p.T46I	P	No		
159*	I	LVHT	Yes	1	*MYPN*	Yes	10	69948821	C	T	0.001046	p.R955W	D	Yes, DCM		
162		LVNC	Yes	1	*RBM20*	Yes	10	112583337	C	T	0	p.P1139L	B	No		
163		LVHT	Yes	1	*CITED2*	Yes	6	139694540	C	G	0	p.S181T	B	Prox, CHD		
166		LVNC	Yes	1	*NEXN*	Yes	1	78383380	G	A	0.000302	p.E53K	D	No		
167		LVNC	Yes	1	*DES*	Yes	2	220283390	T	C	0	p.L69P	B	No		
170		LVNC	Yes	1	*KCNE1*	Yes	21	35821680	C	T	0.012454	p.D85N	P	Yes, LQTS	UNK|P|UNK|US|UNK	No_criteria|No_criteria|Conf|No_criteria|Conf
171		LVNC	Yes	1	*TBX20*	Yes	7	35244184	CCA	C	0	p.300_300del	n/a	No		
172		LVNC	Yes	1	*DES*	Yes	2	220284873	G	A	0.00086	p.R212Q	D	No	US|US|US	Single|No_criteria|Single
173		LVNC	Yes	1	*MTMR14*	Yes	3	9711175	C	T	0	p.R185X	n/a	No		
175		LVHT	Yes	1	*SDHA*	Yes	5	236628	C	T	0.001812	p.A449V	D	Prox, Paraganglioma		
181		LVNC	Yes	1	*SYNE2*	Yes	14	64586160	A	C	0.000929	p.I4286L	P	No		
182		LVHT	Yes	1	*SYNE2*	Yes	14	64655398	T	C	0	p.I5948T	D	No		
183		LVNC	Yes	1	*EYA4*	Yes	6	133827275	G	A	0	p.R408H	B	No		
184		LVNC	Yes	1	*TTN*	Yes	2	179474705	CA	C	0	p.P8275fs	n/a	No		
188		LVHT	Yes	1	*TTN*	Yes	2	179417257	C	A	0	p.E21251X	n/a	No		
190		LVNC	Yes	1	*MYOZ2*	Yes	4	120107234	C	T	0.000139	p.P225L	B	No	US	Single
19		LVHT	No	0	*ABCC9*	No	12	21971087	A	G	0.003481	p.L1256L	n/a	No	B|B	No_criteria|Mult
21		LVHT	No	0	*TNNC1*	No	3	52485426	G	T	0.00158	p.D145E	D	No		
23		LVNC	No	0	*TGFB3*	No	14	76446944	G	A	0.001558	p.S98L	B	No	LB	Single

37*	D	LVNC	No	0	*MYBPC3*	No	11	47357479	C	T	0.00547	p.V896M	B	No	UNK|UNK	Conf|Conf
38		LVNC	No	0	*PRDM16*	No	1	3328948	C	G	0.001186	p.F729L	B	No		
39		LVNC	No	0	*KCNQ1*	No	11	2869088	G	GC	0	p.G629fs	n/a	Yes, LQTS		
51		LVHT	No	0	*RBM20*	No	10	112581039	G	A	0.003724	p.D888N	P	No	US|LB|LB|LB|LB|US	Single|No_criteria|No_criteria|No_criteria|No_criteria|Single
52		LVHT	No	0	*RYR2*	No	1	237617756	G	C	0.000792	p.S453T	P	No		
55*	E	LVHT	No	0	*LAMA4*	No	6	112476110	C	A	0.000883	p.D660Y	D	No	US|US	Mult|No_criteria
56		LVNC	No	0	*MYH7*	No	14	23885449	C	T	0.001766	p.E1573K	P	No		
					TMPO	No	12	98926856	G	A	0.003902	p.R274K	B	No	LB|LB	Single|Single
57		LVHT	No	0	*RBM20*	No	10	112544125	C	T	0.009005	p.S455L	B	No	LB|US|LB	Single|No_criteria|Single
58		LVHT	No	0	*SCO2*	No	22	50962423	C	T	0.001105	p.E140K	D	No	P|P|P	No_criteria|No_criteria|Single
60		LVHT	No	0	*ACTN2*	No	1	236902618	G	A	0.001186	p.R298H	P	No	US|US	Single|Single
72		LVHT	No	0	*DSP*	No	6	7542236	G	A	0.002856	p.V30M	B	No	P|UNK|UNK|US	No_criteria|Conf|Conf|Mult
80		LVHT	No	0	*SCN5A*	No	3	38645516	C	T	0.000803	p.R526H	B	Yes, Brugada syndrome	P	No_criteria
84	D	LVNC	No	0	*MYBPC3*	No	11	47357479	C	T	0.00547	p.V896M	B	No	UNK|UNK	Conf|Conf
89		LVHT	No	0	*MAP2K2*	No	19	4101253	C	T	0	p.R185Q	B	No		
96		LVHT	No	0	*SCO2*	No	22	50962330	G	A	0	p.R171W	D	No	P	No_criteria

101	J	LVNC	No	0	*DSP*	No	6	7556022	G	A	0.001191	p.C81Y	D	No	US|US	Single|Single
110		LVNC	No	0	*TGFB3*	No	14	76446944	G	A	0.001558	p.S98L	B	No	LB	Single
120		LVHT	No	0	*ACTN2*	No	1	236902618	G	A	0.001186	p.R298H	P	No	US|US	Single|Single
121		LVHT	No	0	*NEB*	No	2	152579918	T	C	0	p.Q232R	P	No		
123		LVHT	No	0	*RYR2*	No	1	237778082	G	A	0.022963	p.G1885E	B	No		
126		LVHT	No	0	*NKX2-5*	No	5	172660192	C	A	0.016123	p.A119S	B	Yes, Thyroid dysgenesis	P|LB	No_criteria|Single
127		LVHT	No	0	*JPH2*	No	20	42788571	T	C	0.004088	p.T286A	P	No	US	Single
132*	J	LVHT	No	0	*DSP*	No	6	7556022	G	A	0.001191	p.C81Y	D	No	US|US	Single|Single
					TNNT2	No	1	201330402	C	T	0	g.201330402C>T	n/a	Prox, DCM		
133		LVHT	No	0	*FLNA*	No	X	153594535	G	A	0.016874	p.T429M	D	No	B	Mult
135		LVHT	No	0	*MYH7*	No	14	23887578	C	T	0.00122	p.R1337Q	B	Prox, HCM	US	Single
141		LVNC	No	0	*MYBPC3*	No	11	47367871	C	T	0.004326	p.R326Q	B	Yes, HCM	UNK|B|LB|LB|B	Conf|Single|No_criteria|No_criteria|Mult
143	M	LVNC	No	0	*ACADVL*	No	17	7128342	C	T	0.001093	p.R632C	D	No		
					DSC2	No	18	28671068	C	T	0.000883	p.A133T	B	Yes, ARVD		
144		LVNC	No	0	*SCN5A*	No	3	38645249	C	T	0.001181	p.G615E	D	Yes, LQTS	P|UNK|US|US|LP	No_criteria|Conf|Single|Single|Single
					JUP	No	17	39925231	G	A	0.002117	p.R233C	D	No		
151		LVHT	No	0	*MYBPC3*	No	11	47357479	C	T	0.00547	p.V896M	B	No	UNK|UNK	Conf|Conf
152		LVHT	No	0	*ITGA7*	No	12	56091332	TG	T	0.004381	g.56091333delG	n/a	No	B	Single

154		LVHT	No	0	*LMNA*	No	1	156100408	C	T	0.003796	p.C7C	n/a	Yes, EDMD	B|US|LB	Single|Single|No_criteria
					SCN5A	No	3	38591847	G	C	0.002172	p.P1988A	B	Yes, LQTS	UNK|US|US	Conf|Single|No_criteria
160*	M	LVHT	No	0	*ACADVL*	No	17	7128342	C	T	0.001093	p.R632C	D	No		
165*	E	LVHT	No	0	*LAMA4*	No	6	112476110	C	A	0.000883	p.D660Y	D	No	US|US	Mult|No_criteria
169		LVHT	No	0	*PKP2*	No	12	33021917	C	G	0.001256	p.A372P	P	No		
177		LVNC	No	0	*FKTN*	No	9	108397472	G	GCT	0	p.R438fs	n/a	No		
180		LVHT	No	0	*BAG3*	No	10	121429462	A	T	0.001732	p.I94F	D	Yes, DCM	UNK|UNK	Conf|Conf
185		LVHT	No	0	*TBX5*	No	12	114793602	G	A	0	p.T381I	D	Yes, Holt-Oram syndrome		
186		LVHT	No	0	*FLNA*	No	X	153594535	G	A	0.016874	p.T429M	D	No	B	Mult
2		LVNC	No	0	-											
3		LVNC	No	0	-											
11		LVHT	No	0	-											
14		LVHT	No	0	-											
16		LVHT	No	0	-											
18		LVHT	No	0	-											
22	B	LVHT	No	0	-											
24		LVNC	No	0	-											
26		LVNC	No	0	-											
28	C	LVNC	No	0	-											
29		LVHT	No	0	-											
32		LVNC	No	0	-											

47	E	LVNC	No	0	-
61		LVNC	No	0	-
68*	E	LVHT	No	0	-
73*	C	LVHT	No	0	-
74		LVHT	No	0	-
81		LVHT	No	0	-
92		LVHT	No	0	-
95		LVHT	No	0	-
97		LVNC	No	0	-
103		LVHT	No	0	-
108		LVHT	No	0	-
112*	K	LVHT	No	0	-
117		LVHT	No	0	-
118		LVHT	No	0	-
119		LVHT	No	0	-
125		LVHT	No	0	-
129		LVHT	No	0	-
138		LVHT	No	0	-
147		LVNC	No	0	-
149		LVHT	No	0	-
161		LVHT	No	0	-
164		LVNC	No	0	-
168		LVHT	No	0	-

174	LVHT	No	0	-
178	LVHT	No	0	-
179	LVHT	No	0	-
189	LVHT	No	0	-

* Multiple family members excluded from statistical analysis.

VOI variant of interest, CHR chromosome, LVHT left ventricular hypertrabeculation, LVNC left ventricular non-compaction, MAF minor allele frequency, PHENO phenotype on CMR, POS genomic position, REF reference allele at chromosome and position, VAR variant allele at position. B benign, D deleterious, P probably deleterious.

HGMD: no 'DM' or 'DM?' in HGMD, Prox within 3 amino acids of a disease causing allele in HGMD, LGMD limb girdle muscular dystrophy, EH euthyroid hyperthyroxinaemia, PEAD pseudoxanthoma elasticum, autosomal dominant, DRM mopathy, desmin related, VT ventricular tachycardia, ARVD arrhythmogenic right ventricular dysplasia/cardiomyopathy, MTPD mitochondrial trifunctional protein deficiency, CASD cardiac atrioventricular septal defect, EDMD muscular dystrophy, Emery-Dreifuss, CHD congenital heart disease, DCM dilated cardiomyopathy, HCM hypertrophic cardiomyopathy, DMD Duchenne muscular dystrophy, LQTS long QT syndrome, ASD atrial septal defect, ALS amyotrophic lateral sclerosis, and RCM restrictive cardiomyopathy.

CLINVAR: P pathogenic, LP likely pathogenic, LB likely benign, B benign, UNK unknown, US unspecified, No_assertion no assertion provided, No_criteria no assertion criteria provided, Single citeria provided single submitter, Mult criteria provided multiple submitters, Conf criteria provided conflicting interpretations.

Table S4. Presence of LVNC gene mutation according to Online Mendelian Inheritance in Man (OMIM) database.*

Characteristic	Previously reported in LVNC (N=27)	Not previously reported in LVNC (N=75)	P Value
Age – yr	32±17	25±16	0.08
Male sex — no. (%)	22 (81)	51 (68)	0.22
LV volume parameters			
LVEF <50% – no. (%)	12 (44)	23 (31)	0.20
LVEF – %	43±20	50±14	0.10
LVEDVI – ml/m^2	128±58	111±45	0.12
LVESVI – ml/m^2	82±62	60±45	0.11
Distribution of non-compaction			
Number of affected segments	8.9±3.1	8.1±2.9	0.24
Inter-ventricular septum affected – no.	9 (33)	16 (21)	0.21
Basal segments affected – no. (%)	11 (41)	20 (27)	0.17
NC/C ratio	2.9±1.0	2.5±0.7	0.01
Fulfilled CMR criteria for LVNC – no. (%)	24 (89)	44 (59)	0.004
Presence of LGE – no. (%)	10 (37)	7 (9)	0.001

* LVNC genes/locus according to OMIM database: ACTC1, DTNA, LDB3, MIB1, MYBPC3, MYH7, PRDM16, TAZ, TNNT2, TPM1, and one locus on chromosome 11p15.

Multiple family members removed.

CMR denotes cardiac magnetic resonance, LGE late gadolinium enhancement, LVEDVI left ventricular end-diastolic volume index, LVEF left ventricular ejection fraction, LVESVI left ventricular end-systolic volume index, LVNC left ventricular non-compaction, and NC/C ratio of non-compacted to compacted myocardium.

Table S5. Sample and variant in long-QT syndrome genes with MAF in European populations.

ID	Gene	Variant	Previously identified in HGMD	MAF
65	*FLNC*	p.Q1024X	No	0.000
145	*FLNC*	p.Q1024X	No	0.000
20	*KCNE1*	p.D85N	Yes	0.011
27	*KCNE1*	p.D85N	Yes	0.011
61	*KCNE1*	p.D85N	Yes	0.011
85	*KCNE1*	p.D85N	Yes	0.011
104	*KCNE1*	p.D85N	Yes	0.011
170	*KCNE1*	p.D85N	Yes	0.011
51	*KCNH2*	p.P1018A	Proximal	0.000
105	*KCNH2*	p.T983I	Yes	0.000
144	*KCNH2*	p.A108T	Yes	0.000
174	*KCNH2*	p.R176W	Yes	0.000
13	*KCNJ2*	p.C43Y	Proximal	0.000
64	*KCNJ2*	p.C43Y	Proximal	0.000
73	*KCNJ2*	p.L408V	Proximal	0.000
39	*KCNQ1*	p.G629fs	Yes	0.000
54	*KCNQ1*	p.G649S	No	0.000
111	*KCNQ1*	p.P197L	Yes	0.000
12	*MEF2A*	p.P277L	Yes	0.001
85	*MEF2A*	p.R17X	Yes	0.000
80	*SCN5A*	p.R526H	Yes	0.000
167	*SCN5A*	p.P1988A	Yes	0.002
187	*SCN5A*	p.P1988A	Yes	0.002
187	*SCN5A*	p.L1792F	Proximal	0.000
47	*SCN5A*	p.P1988A	Yes	0.002
55	*SCN5A*	p.P1988A	Yes	0.002
124	*SCN5A*	p.P1988A	Yes	0.002
144	*SCN5A*	p.G615E	Yes	0.000
154	*SCN5A*	p.P1988A	Yes	0.002
155	*SCN5A*	p.P1988A	Yes	0.002

MAF denotes minor allele frequency.

Table S6. Truncating mutations in TTN, band-location and exon usage.

Variant	Band	Constitutive in CCDS
NM_001256850:exon308:c.98972_98975del:p.32991_32992del	M	Yes
NM_001256850:c.70372+1delG	M	Yes
NM_001256850:exon285:c.C86191T:p.Q28731X	A	Yes
NM_001256850:exon285:c.G85447T:p.E28483X	A	Yes
NM_001256850:exon276:c.81261delC:p.V27087fs	A	Yes
NM_001256850:exon276:c.C66679T:p.R22227X	A	Yes
NM_001256850:exon260:c.C59887T:p.R19963X	A	Yes
NM_001256850:exon254:c.C56953T:p.R18985X	A	Yes
NM_001256850:exon245:c.C52846T:p.R17616X	A	Yes
NM_001256850:exon222:c.46521delT:p.P15507fs	A	Yes
NM_001256850:exon180:c.37448_37467del:p.12483_12489del	I	Yes
NM_001256850:c.34930+2A>G	I	No
NM_001256850:exon133:c.C32113T:p.R10705X	I	No
NM_001256850:exon71:c.G20279A:p.W6760X	I	No

CCDS denotes conserved coding DNA sequence.

References

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