

SUPPLEMENTAL MATERIAL

Phenotypic expression, natural history and risk stratification of cardiomyopathy caused by *FLNC* truncating variants

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Supplemental Table I. List and classification of FLNctv included in the study

	Enrollment Center	ID	Nucleotide Change	Protein Change	ACMG Variant Classification ¹²	GnomAD MAF [‡]	FLNC Domain	Additional Variants Gene	Nucleotide Change	Protein Change	Reference of previous publication
1	FCR/U. Trieste Hospital	TSFDC-MDF-MoMa	c.451G>T	p.Glu151*	P [§]	Absent	ABD [#]				
2	U. Amsterdam Hospital	A03	c.697C>T	p.Gln233*	P/LP**	Absent	ABD	PPA2	c.514G>A;p	Glu172Lys	18
3	U. Amsterdam Hospital	A04	c.697C>T	p.Gln233*	P/LP	Absent	ABD				18
4	U. Amsterdam Hospital	A05	c.697C>T	p.Gln233*	P/LP	Absent	ABD				18
5	U. Amsterdam Hospital	A06	c.697C>T	p.Gln233*	P/LP	Absent	ABD				18
6	FCR/U. Trieste Hospital	TSSDC130-02-2302	c.805C>T	p.Arg269*	P	Absent	ROD1				7
7	FCR/U. Colorado Hospital	DNFDC249-0001-MRGT1	c.964G>T	p.Glu322*	LP	Absent	ROD1				
8	Victor Chang Institute	VCCRI PF-IV-1	c.970-4A>G	Intronic splice	LP ^{††}	0.000143	ROD1				
9	Harvard U. Hospital	WSUS219309	c.1444C>T	p.Arg482*	P	Absent	ROD1				
10	Harvard U. Hospital	OYIM213618	c.1444C>T	p.Arg482*	P	Absent	ROD1				
11	Harvard U. Hospital	OYIR222900	c.1444C>T	p.Arg482*	P	Absent	ROD1				
12	Amsterdam U. Hospital	A01	c.1466_1473delinsA	p.Val489Glyfs*33	LP	Absent	ROD1				18
13	Amsterdam U. Hospital	A02	c.1466_1473delinsA	p.Val489Glyfs*33	LP	Absent	ROD1				18
14	Stanford U. Hospital	STE1	c.1605C>A	p.Cys535*	LP	Absent	ROD1				
15	FCR/U. Colorado Hospital	DNFDC079 02-1517	c.2119C>T	p.Gln707*	LP	Absent	ROD1				7
16	U. Trieste Hospital	TSFDC-MDF2-WBAT	c.2190AC>A	p.Tyr730*	LP	Absent	ROD1				
17	Fuwai Hospital	Fuwai-1	c.2618delA	p.Glu873fs	LP	Absent	ROD1				
18	FCR/U. Colorado Hospital	DNFDC195- 10-003CVI	c.2930-1G>T	splice	P	Absent	ROD1				7
19	FCR/U. Trieste Hospital	TSFDC038-02-0225	c.3791-1G>A	splice	P/LP	Absent	ROD1				7

20	FCR/U. Trieste Hospital	TSFDC038- MDF3-VGP	c.3791-1G>A	splice	P/LP	Absent	ROD1					7
21	FCR/U. Trieste Hospital	TSFDC038- MDF4-SC	c.3791-1G>A	splice	P/LP	Absent	ROD1					
22	FCR/U. Trieste Hospital	TSFDC038-01-0748	c.3791-1G>A	splice	P/LP	Absent	ROD1					
23	FCR/U. Trieste Hospital	TSFDC038-MDF5-SR	c.3791-1G>A	splice	P/LP	Absent	ROD1					
24	FCR/U. Trieste Hospital	TSFDC-MDF6-SMV	c.3791-1G>A	splice	P/LP	Absent	ROD1					
25	FCR/U. Trieste Hospital	TSFDC7-MDF7-BrC	c.3791-1G>A	splice	P/LP	Absent	ROD1					
26	FCR/U. Trieste Hospital	TSFDC043- 02-0215	c.3791-1G>A	splice	P/LP	Absent	ROD1					7
27	Harvard U. Hospital	AMVE221656	c.3791-1G>C	splice	LP	0.00001396	ROD1					
28	Harvard U. Hospital	CSER222507	c.3791-1G>C	splice	LP	0.00001396	ROD1					
29	Harvard U. Hospital	ELYL222127	c.3791-1G>C	splice	LP	0.00001396	ROD1					
30	Harvard U. Hospital	ELLA222701	c.3791-1G>C	splice	LP	0.00001396	ROD1					
31	Victor Chang Institute	VCCRI R-IV-1	c.3791-8G>A	Intronic splice	LP‡‡	0.0001217	ROD1	TTN	c.76115_76116insA	p.Asn25372Lysfs*5		30
32	Victor Chang Institute	VCCRI R-III-10	c.3791-8G>A	Intronic splice	LP‡‡	0.0001217	ROD1					30
33	Utrecht U. Hospital	U01	c.3838dup	p.Leu1280fs	LP	Absent	ROD1					
34	U. Trieste Hospital	TSFDC-MDF8-SaA	c.4108C>T	p.Arg1370*	P	Absent	ROD1					
35	FCR/U. Colorado Hospital	DNFDC-MRGT2	c.4275dup	p.Arg1426Alafs*85	LP	Absent	ROD1					
36	Stanford U. Hospital	STB1	c.4716delG	p.Leu1573Cysfs*34	LP	Absent	ROD1					
37	Stanford U. Hospital	STB2	c.4716delG	p.Leu1573Cysfs*34	LP	Absent	ROD1					
38	Johns Hopkins Hospital	JHH2	c.4737+5G>A	Intronic splice	LP	Absent	ROD1					
39	Johns Hopkins Hospital	JHH3	c.4737+5G>A	Intronic splice	LP	Absent	ROD1					
40	Victor Chang Institute	VCCRI G*-III-1	c.4926_4927insACGTCA CA	p.Val1643Thrfs*26	P	0.00001397	ROD1					
41	Victor Chang Institute	VCCRI G*-III-2	c.4926_4927insACGTCA CA	p.Val1643Thrfs*26	P	0.00001397	ROD1					
42	Victor Chang Institute	VCCRI G*-III-7	c.4926_4927insACGTCA CA	p.Val1643Thrfs*26	P	0.00001397	ROD1					
43	Victor Chang Institute	VCCRI G*-II-1	c.4926_4927insACGTCA CA	p.Val1643Thrfs*26	P	0.00001397	ROD1					
44	Victor Chang Institute	VCCRI G*-III-3	c.4926_4927insACGTCA CA	p.Val1643Thrfs*26	P	0.00001397	ROD1					

4 5	Harvard U. Hospital	OHAM222367	c.4926_4927insACGTCA CA	p.Val1643Thrfs*26	P	0.00001397	ROD1				
4 6	FCR/U.Colora do Hospital	DNFDC057-04-0536	c.5672_5672delG	p.Gly1891Valfs*62	P	Absent	ROD2				7,8
4 7	FCR/U.Colora do Hospital	DNFDC057-18-023CV1	c.5672_5672delG	p.Gly1891Valfs*62	P	Absent	ROD2				7,8
4 8	FCR/U.Colora do Hospital	DNFDC057-19-004CV1	c.5672_5672delG	p.Gly1891Valfs*62	P	Absent	ROD2				7,8
4 9	FCR/U.Colora do Hospital	DNFDC057-01-0987	c.5672_5672delG	p.Gly1891Valfs*62	P	Absent	ROD2				7,8
5 0	U.Trieste Hospital	TSFDC-MDF8-GaSa	c.5842+2T>A	splice	P	Absent	ROD2				
5 1	U.Trieste Hospital	TSFDC-MDF9-GaSi	c.5842+2T>A	splice	P	Absent	ROD2				
5 2	FCR/U. Trieste Hospital	TSFDC-MDF10-ArGi	c.5866C>T	p.Gln1956*	LP	Absent	ROD2				
5 3	FCR/U. Trieste Hospital	TSFDC-MDF11-PaGi	c.6136_6137insG	p.Val2046Glyfs*34	P	Absent	Z-Line				
5 4	FCR/U. Trieste Hospital	TSFDC-MDF12-PaSa	c.6136_6137insG	p.Val2046Glyfs*34	P	Absent	Z-Line				7
5 5	FCR/U. Trieste Hospital	TSFDC-MDF13-PaSt	c.6136_6137insG	p.Val2046Glyfs*34	P	Absent	Z-Line				
5 6	FCR/U. Trieste Hospital	TSFDC-MDF14-CPF	c.6136_6137insG	p.Val2046Glyfs*34	P	Absent	Z-Line				
5 7	FCR/U. Trieste Hospital	TSFDC-MDF15-BaGi	c.6208G>A	p.Gly2070Ser	LP	Absent	Z-Line				
5 8	FCR/U. Trieste Hospital	TSFDC-MDF16-BaVi	c.6208G>A	p.Gly2070Ser	LP	Absent	Z-Line				
5 9	FCR/U. Trieste Hospital	TSFDC-MDF17-BaDi	c.6208G>A	p.Gly2070Ser	LP	Absent	Z-Line				7
6 0	FCR/U. Trieste Hospital	TSFDC-MDF18-NaSa	c.6240_6259del	p.Pro2081Leufs*2	LP	Absent	Z-Line				
6 1	FCR/U. Trieste Hospital	TSFDC-MDF19-NaLu	c.6240_6259del	p.Pro2081Leufs*2	LP	Absent	Z-Line				
6 2	FCR/U. Trieste Hospital	TSFDC20-MDF20- BeDa	c.6240_6259del	p.Pro2081Leufs*2	LP	Absent	Z-Line				
6 3	Utrecht U. Hospital	U02	c.6255_6256del	p.Asp2085fs	LP	Absent	Z-Line				
6 4	FCR/U. Trieste Hospital	TSFDC-MDF21-CeNi	c.6459_6460del	p.Thr2153fs	LP	Absent	Z-Line				7
6 5	FCR/U. Trieste Hospital	TSRVD029-02-1070	c.6565G>T	p.Glu2189*	LP	Absent	Z-Line				9
6 6	FCR/U. Trieste Hospital	TSRVD029-BaRO	c.6565G>T	p.Glu2189*	LP	Absent	Z-Line				9
6 7	Utrecht U. Hospital	U03	c.6614_6622del	p.Val2205_Val2207 del	LP	Absent	Z-Line				
6 8	Utrecht U. Hospital	U04	c.6614_6622del	p.Val2205_Val2207 del	LP	Absent	Z-Line				
6 9	FCR/U. Trieste Hospital	TSFDC-MDF22-CrGi	c.6891_6901	p.Pro2298fs	LP	Absent	Z-Line				
7 0	FCR/U. Trieste Hospital	TSFDC-MDF23-Bu Da	c.6976C>T	p.Arg2326*	P	Absent	ROD2				7

71	Victor Chang Institute	VCCRI DF-III-1	c.7107C>A	p.Cys2369*	LP	Absent	ROD2				30
72	Victor Chang Institute	VCCRI DF-IV-2	c.7107C>A	p.Cys2369*	LP	Absent	ROD2				30
73	Victor Chang Institute	VCCRI DF-III-4	c.7107C>A	p.Cys2369*	LP	Absent	ROD2				30
74	Victor Chang Institute	VCCRI DF-III-2	c.7107C>A	p.Cys2369*	LP	Absent	ROD2				30
75	Victor Chang Institute	VCCRI DF-III-5	c.7107C>A	p.Cys2369*	LP	Absent	ROD2				30
76	FCR/U. Trieste Hospital	TSFDC031-438	c.7251+1A>G	p.Tyr2381Glyfs	P	Absent	ROD2				7,8
77	FCR/U. Trieste Hospital	TSFDC031-322	c.7251+1A>G	p.Tyr2381Glyfs	P	Absent	ROD2				7,8
78	FCR/U. Udine Hospital	TSFDC029-414	c.7251+1A>G	p.Tyr2381Glyfs	P	Absent	ROD2				7,8
79	FCR/U. Udine Hospital	TSFDC029-408	c.7251+1A>G	p.Tyr2381Glyfs	P	Absent	ROD2				7,8
80	FCR/U. Udine Hospital	TSFDC029-409	c.7251+1A>G	p.Tyr2381Glyfs	P	Absent	ROD2				7,8
81	FCR/U. Udine Hospital	TSFDC029-410	c.7251+1A>G	p.Tyr2381Glyfs	P	Absent	ROD2				7,8
82	U. Trieste Hospital	TSFDC-MDF24-GaMa	c.7252-2A>G	Splice	LP	Absent	ROD2				
83	FCR/U. Trieste Hospital	TSFDC25-SuGu	c.8107delG	p.Asp2703Thrfs*69	LP	0.00003197	Dimerization				
84	FCR/U. Trieste Hospital	TSFDC-MDF26-SuSa	c.8107delG	p.Asp2703Thrfs*69	LP	0.00003197	Dimerization				7
85	Johns Hopkins Hospital	JHH 1	c.8107delG	p.Asp2703Thrfs*69	LP	0.00003197	Dimerization				9

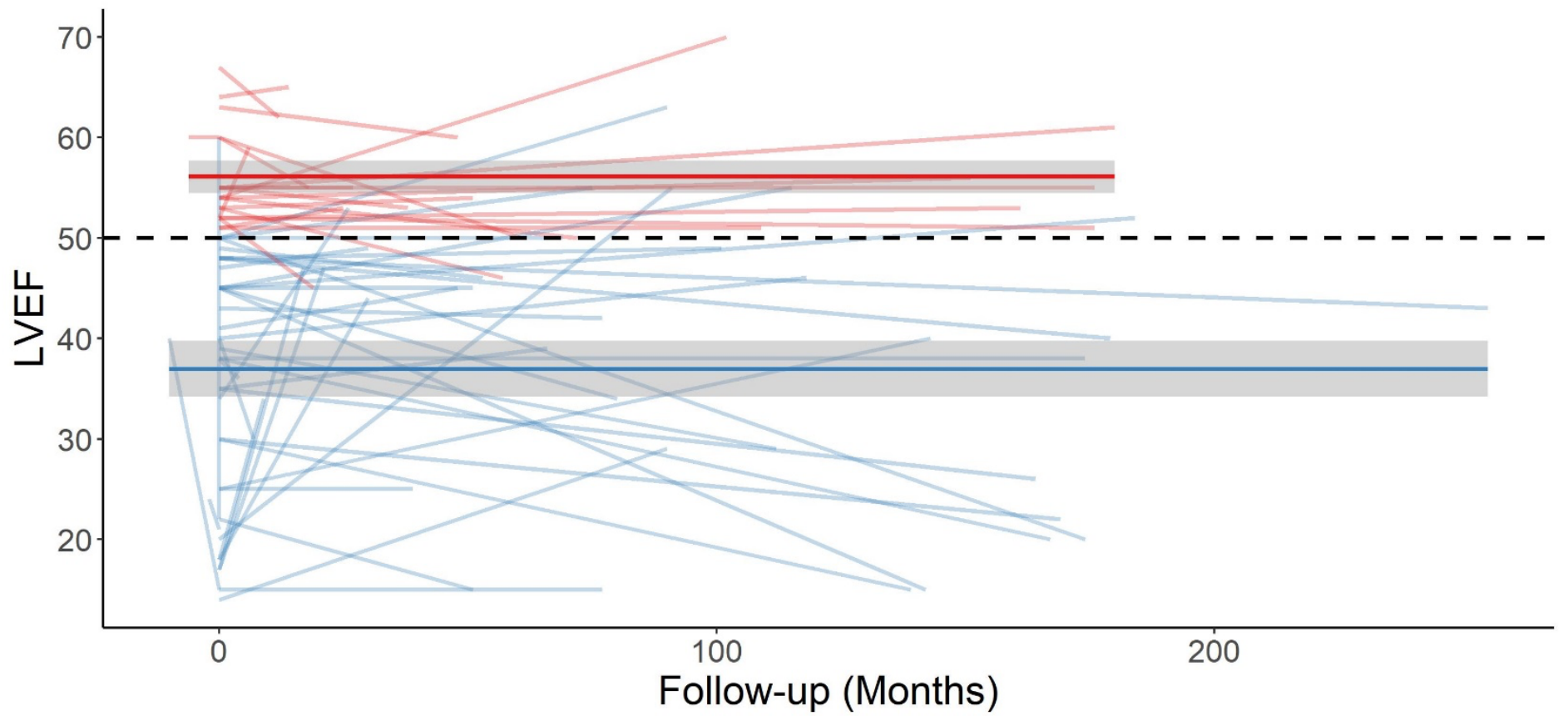
Legend. ‡gnomAD v3.0 (<https://gnomad.broadinstitute.org>). §P: pathogenic variant, LP: likely pathogenic variant. LP^{††}: based on functional assay, cosegregation, bioinformatic algorithm predicting abnormal splicing and truncated protein; LP^{‡*}: based on bioinformatic algorithm predicting abnormal splicing and truncated protein, and cosegregation. Variant location based on UNIPROT (<https://www.uniprot.org/uniprot/Q14315>). For references, see the reference list in the main text.

Supplemental Table II. Phenotype presentation according to variant location (global p-value=0.128)

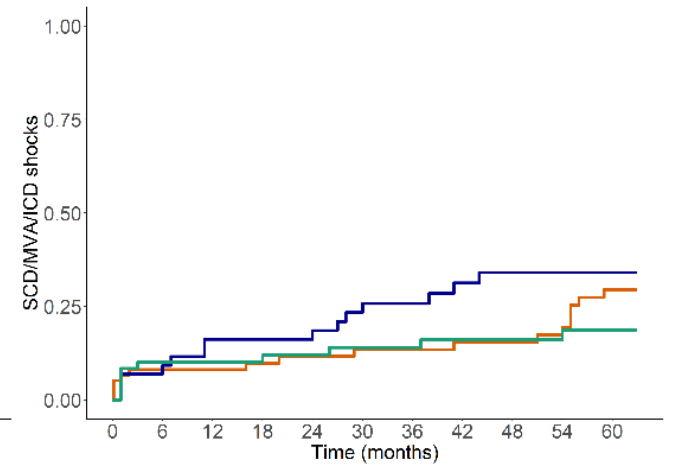
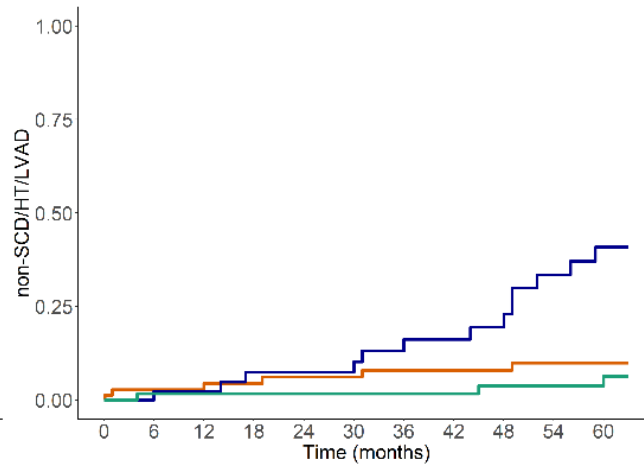
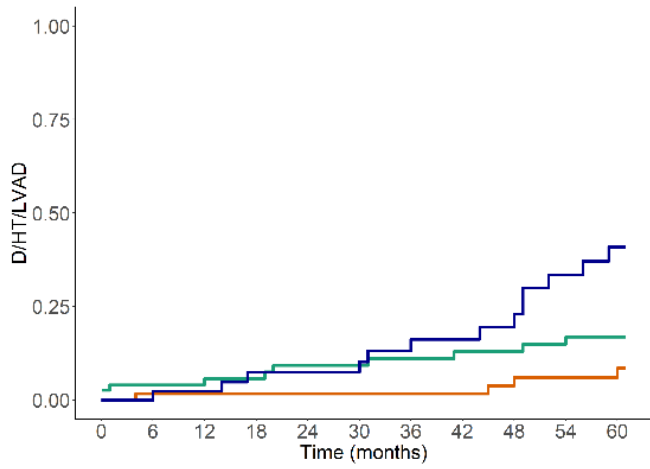
	DCM	ARVC	ALVC	Biventricular ARVC	Minor phenotypic expression	Healthy
ABD (n;%)	6 (100%)	0	0	0	0	0
ROD 1 (n;%)	17 (44%)	0	12 (31%)	0	5 (13%)	5 (13%)
ROD 2 (n;%)	18 (49%)	2 (5%)	8 (22%)	1 (3%)	5 (14%)	3 (8%)
Dimerization (n;%)	1 (33%)	1 (33%)	1 (33%)	0	0	0
Z-line (n;%)	8 (61%)	0	1 (8%)	1 (8%)	2 (15%)	1 (8%)
No Z-line (n;%)	34 (47%)	3 (4%)	20 (28%)	0	8 (11%)	7 (10%)

For abbreviations see table 1 in the main text and table S2.

Supplemental Figure I



Supplemental Figure II



Number at risk

Gene	0	6	12	18	24	30	36	42	48	54	60
LMNA	43	42	39	36	36	33	28	26	23	19	16
DSP	60	58	55	53	52	48	48	45	44	38	38
FLNC	76	62	58	53	51	50	48	46	45	43	40

Cumulative Incidence Function

Gene	0	6	12	18	24	30	36	42	48	54	60
LMNA	0	0.02	0.02	0.07	0.07	0.1	0.16	0.16	0.23	0.33	0.41
DSP	0	0.03	0.04	0.04	0.06	0.06	0.08	0.08	0.08	0.1	0.1
FLNC	0	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.04	0.04	0.06

Cumulative Incidence Function

Gene	0	6	12	18	24	30	36	42	48	54	60
LMNA	0	0.09	0.16	0.16	0.19	0.26	0.26	0.31	0.34	0.34	0.34
DSP	0	0.08	0.08	0.1	0.12	0.13	0.13	0.15	0.15	0.19	0.3
FLNC	0	0.1	0.1	0.12	0.12	0.14	0.14	0.16	0.16	0.19	0.19

Gene — FLNC — DSP — LMNA

Figures Legend

Supplemental Figure I. Trends of LVEF for FLNCtv carriers with available echocardiographic follow-up reassessment. Dashed line indicates the LVEF<50% threshold. Red lines indicate carriers with preserved LVEF at baseline, blue lines indicate carriers with reduced LVEF at baseline. Bold lines represent mean trends during follow-up. Grey shadow is 95% CI.

Supplemental Figure II. Comparison of outcome between the study population of FLNCtv affected carriers (n=76), LMNA affected carriers (n=43) and DSP affected carriers (n=60). Left panel: all-cause mortality/heart transplantation/left ventricular assist device (D/HT/LVAD) in FLNCtv (green lines) vs LMNA mutation (blue lines) vs DSP mutation (red lines). Central panel: Cumulative Incidence Function (CIF) of non-arrhythmic death/HT/LVAD in FLNCtv vs LMNA mutation vs DSP mutation. Right panel: CIF of sudden cardiac death/major ventricular arrhythmias (SCD/MVA) in FLNCtv vs LMNA mutation vs DSP mutation. In FLNCtv patients the risk of D/HT/LVAD was lower compared to LMNA patients (p=0.007) patients and similar to DSP carriers (0.665), the risk of non-SCD/HT/LVAD for FLNCtv patients was lower compared to LMNA patients (p=0.003) patients and similar to DSP patients (0.773) and the risk of SCD/MVA was comparable to LMNA patients (0.258) and DSP patients (p=0.258).