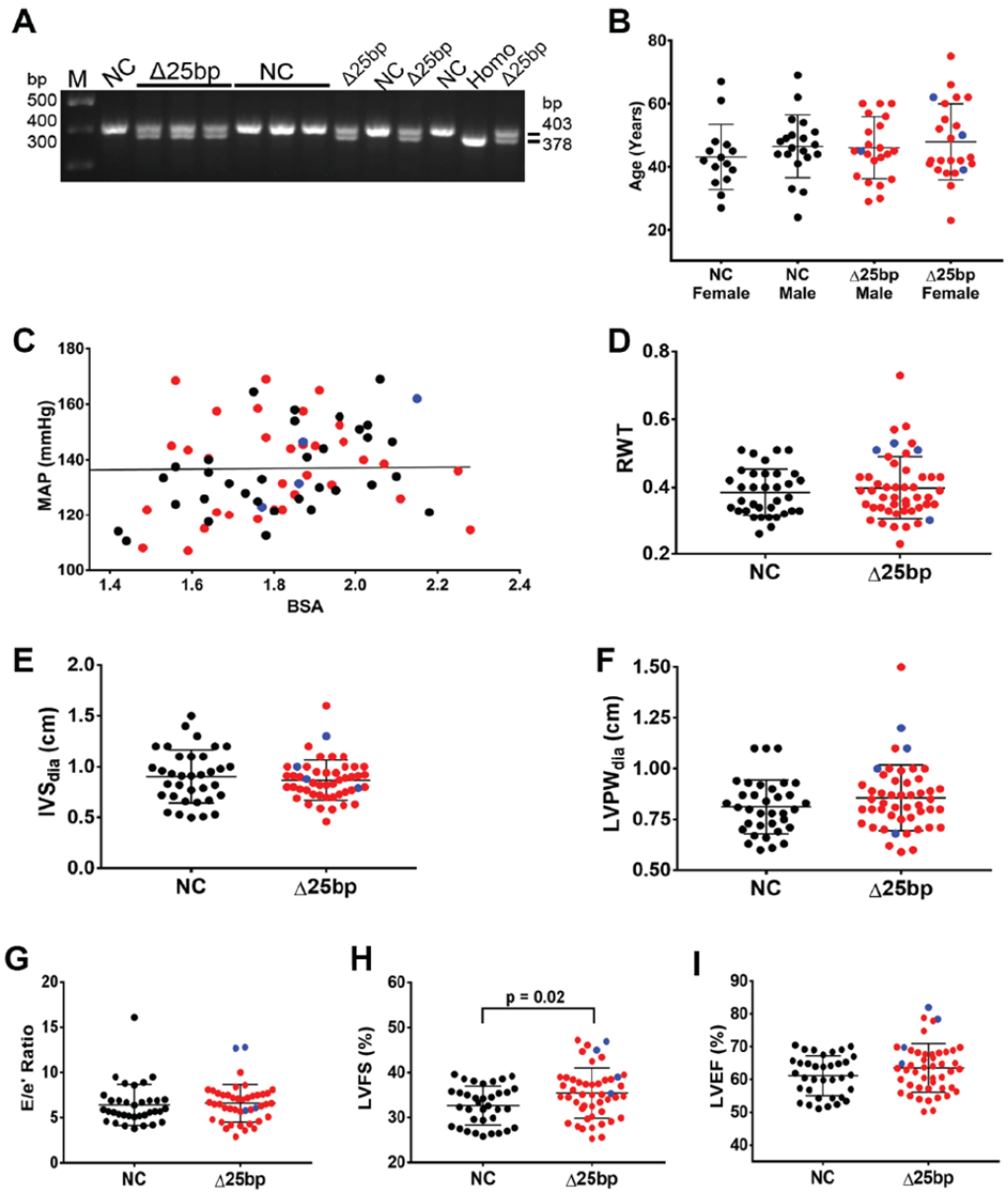
Supplementary Online Content

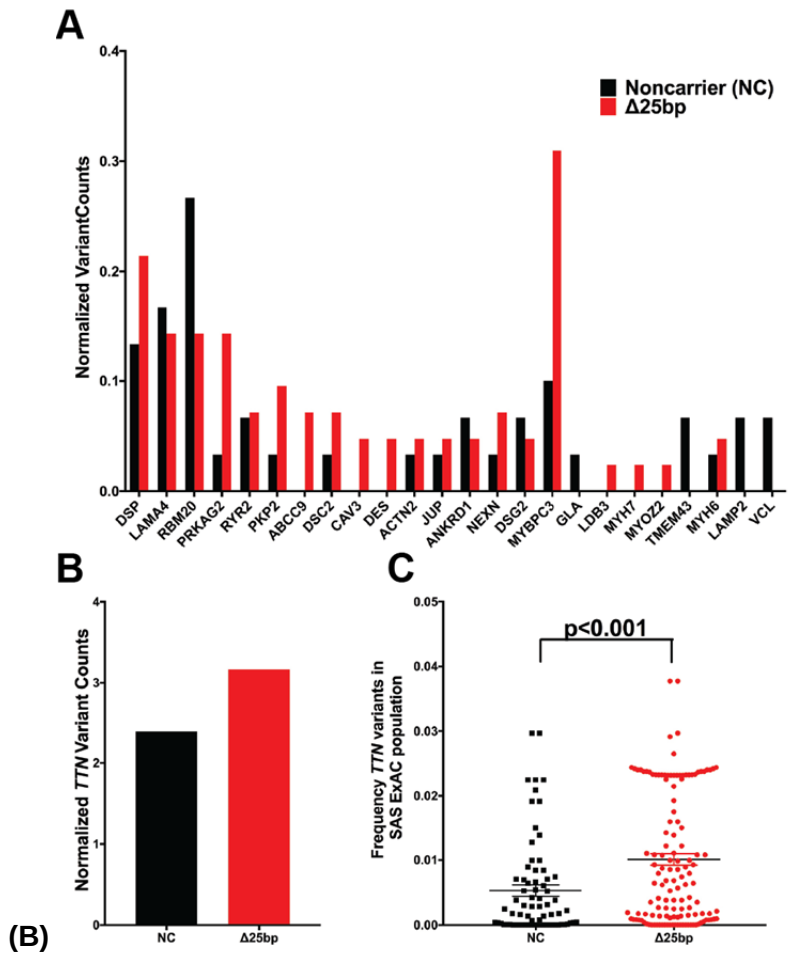
Viswanathan SK, Puckelwartz MJ, Mehta A, et al. Association of Cardiomyopathy With *MYBPC3* D389V and *MYBPC3*$^{\Delta 25bp}$ Intronic Deletion in South Asian Descendants. *JAMA Cardiol*. Published online April 11, 2018. doi:10.1001/jamacardio.2018.0618

This supplementary material has been provided by the authors to give readers additional information about their work.

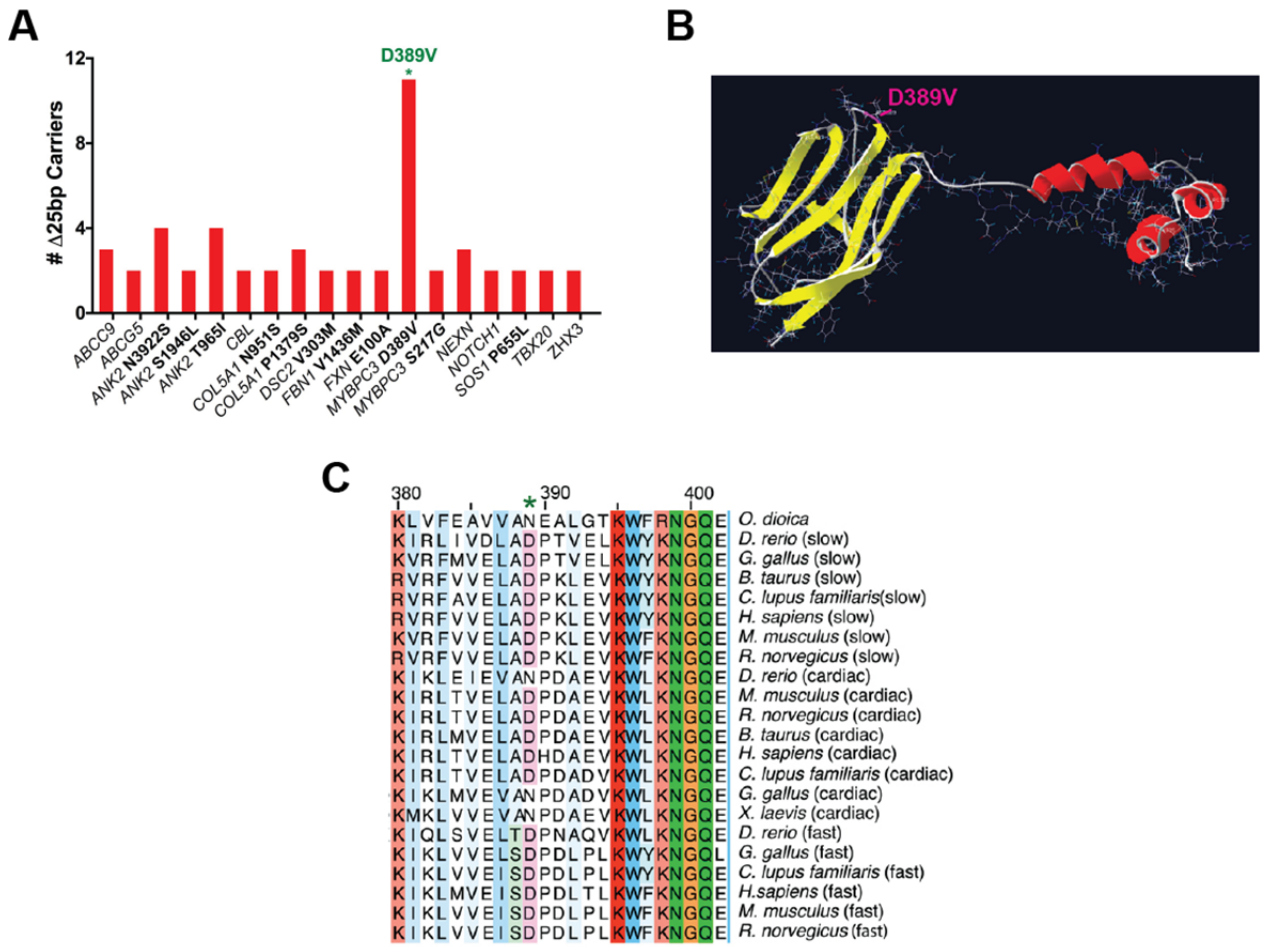


eFigure 1. Genotype-phenotype features of the *MYBPC3*$^{\Delta 25bp}$ variant carriers.

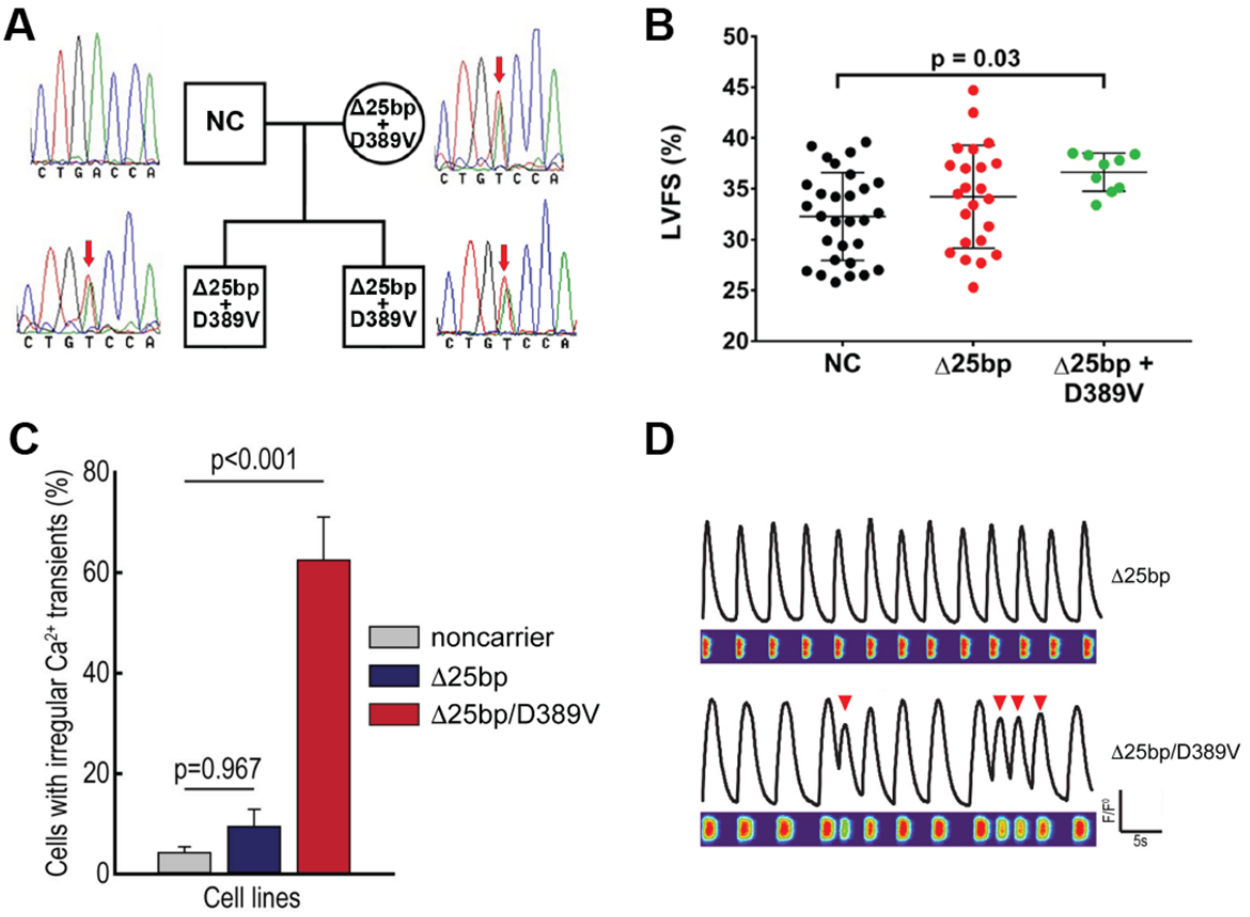
(A) PCR-based genotyping was used to detect noncarriers (NC), heterozygous (*Δ25bp)*, and homozygous (Homo) variant carriers. The symbol Δ indicates the 25bp deletion. **(B)** Age distribution of the subjects who underwent echocardiographic evaluation grouped by genotype and gender (n=82). **(C)** Comparison of mean arterial pressure (MAP) and body surface area (BSA) showing equal distribution of carriers and noncarriers. Comparison of key cardiomyopathy-related echocardiographic parameters namely (**D**) relative wall thickness (RWT), (**E**) ventricular septal thickness during diastole (IVS_{dia}), (**F**) free wall thickness during diastole ($LVPW_{dia}$), (**G**) ratio of early transmitral flow (E) to left ventricular early diastolic velocity (e') (E/e' ratio), (**H**) left ventricular fractional shortening (LVFS %) and (**I**) left ventricular ejection fraction (LVEF %) among *MYBPC3*$^{\Delta 25bp}$ variant carriers (n=47), including homozygous carriers and noncarriers (n=35). Blue markers represent homozygous carriers, while red markers represent heterozygous carriers. Statistical comparison between the groups performed using unpaired *t*-test.



eFigure 2. Evaluation of rare protein-altering variation in 46 cardiomyopathy genes in 72 US-SAs who were carriers or noncarriers of the *MYBPC3*$^{\Delta 25bp}$ variant.
Additional cardiomyopathy genes (n=46) were fully sequenced. Rare protein-altering variants were evaluated, and an excess of rare protein-altering variation was seen in carriers compared to noncarriers. (**A**) The number of variants was normalized to cohort size, and the groups were compared. The genetic signature differed between *MYBPC3*$^{\Delta 25bp}$ carriers (red) and noncarriers (NC, black). (**B**) *MYBPC3*$^{\Delta 25bp}$ carriers had an excess of *TTN* variants with 3.2 rare *TTN* variants, while noncarriers had approximately 2.4 rare *TTN* variants per subject. (**C**) Rare *TTN* variants, as identified in *MYBPC3*$^{\Delta 25bp}$ carriers, had a higher frequency in US-SAs compared to *TTN* variants identified in noncarriers, indicating a distinct genetic background in *MYBPC3*$^{\Delta 25bp}$ carriers compared to noncarriers ($p<0.001$). Using Southeast Asian (SAS) population frequencies from ExAC[18], population frequency differed for *TTN* variants in *MYBPC3*$^{\Delta 25bp}$ carriers compared to the noncarrier group (mean frequency 0.010±0.0009 in *MYBPC3*$^{\Delta 25bp}$ carriers and 0.005±0.001 in noncarriers, $p<0.001$; TTN variants not identified in ExAC were given a population frequency of zero.). This same analysis conducted with European (Non-Finnish) population frequencies showed no difference between *MYBPC3*$^{\Delta 25bp}$ carriers and noncarriers (0.001±0.0002 and 0.001±0.0003, carrier and noncarrier variants, respectively; p=0.9141). This finding was also specific to the *TTN* locus since analysis of non-*TTN* cardiomyopathy genes using SAS population data did not substantially differ between the *MYBPC3*$^{\Delta 25bp}$ carrier and noncarrier groups (0.006±0.001 and 0.004±0.001, carrier and noncarrier variants, respectively; p=0.48).



eFigure 3. *MYBPC3*^{D389V} is conserved and enriched in *MYBPC3*$^{\Delta 25bp}$ *gene carriers.* **(A)** Among *MYBPC3*$^{\Delta 25bp}$ carriers, D389V was overrepresented, compared to other rare variants, and was uniquely found in *MYBPC3*$^{\Delta 25bp}$ carriers (Fisher's test, p=0.0017). **(B)** Ribbon diagram of C2 domain of cMyBP-C indicating the location of Asp 389, which is highly conserved part of a polymorphic protein-binding region. **(C)** ClustalW alignment of cMyBP-C amino acids showing the evolutionary conservation of Asp 389 (green asterisks) across species.



eFigure 4. D389V is carried on the same single allele as *MYBPC3*$^{\Delta 25bp}$ and is associated with increased LVFS and irregular Ca^{2+} transients in iPSC-derived cardiomyocytes.
(A) Family segregation demonstrates that *MYBPC3*$^{\Delta 25bp}$ and D389V (red arrow) are located on the same allele. The parent with both *MYBPC3*$^{\Delta 25bp}$ and D389V has classic LV diastolic dysfunction without LVH while the oldest son has LVH with LA enlargement. PCR-based genotyping was used to detect noncarriers (NC), heterozygous (*Δ25bp)*, and homozygous (Homo) variant carriers. The symbol Δ indicates the 25bp deletion. **(B)** LVFS was significantly elevated (mean ± sem) in subjects that carry *MYBPC3*$^{\Delta 25bp/D389V}$ compared to noncarriers and those with *MYBPC3*$^{\Delta 25bp}$ alone. **(C)** Frequency of cells with Ca^{2+} transient irregularities represented as a percentage. Cells carrying *MYBPC3*$^{\Delta 25bp/D389V}$ (red bar) showed significantly increased frequency (p>0.001) compared to both *MYBPC3*$^{\Delta 25bp}$ alone (blue bar) and noncarrier (grey bar) controls. **(D)** Representative line scan image showing normal spontaneous Ca^{2+} transients in *MYBPC3*$^{\Delta 25bp}$ cardiomyocytes (top) or arrhythmic Ca^{2+} transients in *MYBPC3*$^{\Delta 25bp/D389V}$ cardiomyocytes (bottom). Red arrowheads indicate arrhythmic Ca^{2+} transients. One-way *ANOVA* and Tukey's post hoc test were used.

eTable 1. Illumina TruSight Cardio panel of 174 cardiovascular disease genes

ABCC9	CACNB2	DOLK	GJA5	KCNJ5	MYH11	PRDM16	SGCG	**TNNC1**
ABCG5	CALM1	DPP6	**GLA**	KCNJ8	**MYH6**	**PRKAG2**	SHOC2	**TNNI3**
ABCG8	CALR3	**DSC2**	GPD1L	KCNQ1	**MYH7**	PRKAR1A	SLC25A4	**TNNT2**
ACTA1	**CASQ2**	**DSG2**	GPIHBP1	KLF10	**MYL2**	PTPN11	SLC2A10	**TPM1**
ACTA2	**CAV3**	**DSP**	HADHA	KRAS	**MYL3**	RAF1	SMAD3	TRDN
ACTC1	CBL	**DTNA**	HCN4	LAMA2	MYLK	RANGRF	SMAD4	TRIM63
ACTN2	CBS	EFEMP2	HFE	**LAMA4**	**MYLK2**	**RBM20**	SNTA1	TRPM4
AKAP9	CETP	ELN	HRAS	**LAMP2**	MYO6	RYR1	SOS1	**TTN**
ALMS1	COL3A1	**EMD**	HSPB8	**LDB3**	**MYOZ2**	**RYR2**	SREBF2	**TTR**
ANK2	COL5A1	EYA4	ILK	LDLR	MYPN	SALL4	**TAZ**	TXNRD2
ANKRD1	COL5A2	FBN1	JAG1	LDLRAP1	**NEXN**	SCN1B	TBX20	**VCL**
APOA4	COX15	FBN2	JPH2	LMF1	NKX2-5	SCN2B	TBX3	ZBTB17
APOA5	CREB3L3	FHL1	**JUP**	**LMNA**	NODAL	SCN3B	TBX5	ZHX3
APOB	CRELD1	**FHL2**	KCNA5	LPL	NOTCH1	SCN4B	**TCAP**	ZIC3
APOC2	**CRYAB**	FKRP	KCND3	LTBP2	NPPA	SCN5A	TGFB2	
APOE	**CSRP3**	FKTN	KCNE1	MAP2K1	NRAS	SCO2	TGFB3	
BAG3	**CTF1**	FXN	KCNE2	MAP2K2	PCSK9	SDHA	TGFBR1	
BRAF	**DES**	GAA	KCNE3	MIB1	PDLIM3	SEPN1	TGFBR2	
CACNA1C	DMD	GATAD1	KCNH2	MURC	**PKP2**	SGCB	**TMEM43**	
CACNA2D1	DNAJC19	GCKR	KCNJ2	**MYBPC3**	**PLN**	**SGCD**	TMPO	

This panel sequences all exons of 174 inherited cardiac disease genes. Analysis of results was performed on the entire set and the subset of 46 cardiomyopathy genes, which are in bold.

eTable 2. Rare variants in the 46-gene cardiomyopathy panel found in Southeast Asian cohort, including *TTN* variants

	# of variants including *TTN* variants	# identified in *MYBPC3*$^{\Delta 25bp}$ carriers (n=42)	# identified in noncarriers (n=30)
MODERATE	298	197	101
HIGH	9	7	2

All variants with ExAC ≤ 0.01 MODERATE-effect variants are missense variants and inframe indels, while HIGH-effect variants are protein-disrupting variants, such as frameshifting variants, stop- gain, stop-loss, and splice-site variants.

eTable 3. Rare variants in the 46-gene cardiomyopathy panel found in Southeast Asian cohort, excluding *TTN* variants

	# of variants excluding *TTN* variants	# identified in *MYBPC3*$^{\Delta 25bp}$ carriers (n=42)	# identified in noncarriers (n=30)
MODERATE	103	66	37
HIGH	7	6	1

All variants with ExAC ≤ 0.01, excluding *TTN* variants.

eTable 4. Fisher's Exact test of *MYBPC3*$^{\Delta 25bp}$ carriers versus noncarriers

Gene	Variants	p value
MYBPC3	D389V	0.0019
ABCC9	c.2238G>A	ns
ABCG5	V300I	ns
ANK2	N3922S	ns
ANK2	S1946L	ns
ANK2	T965I	ns
CBL	His36_37insHis	ns
CDC27	I240L	ns
COL5A1	N951S	ns
COL5A1	P1379S	ns
DSC2	V303M	ns
FBN1	V1436M	ns
FXN	E100A	ns
MYBPC3	S217G	ns
NEXN	E318A	ns
NOTCH1	N104S	ns
SOS1	P655L	ns
TBX20	L244F	ns
ZHX3	P258L	ns

Fisher's Exact test comparing the variations identified in *MYBPC3*$^{\Delta 25bp}$ variant carriers and noncarriers

eTable 5. Subjects excluded from analysis and rationale

Subject	Reason for omitting	Possible Genetic Variant
SS44 (Noncarrier)	EF is 2 STD Dev from mean	
SS69 (Noncarrier)	EF = 38.3; LVIDd=5.5; IVSd=1.1; LVPWd=1.1; LA Vol=59.2	DSP, R1509K, PP2=0.956, GERP=5.74, ExAC_SAS=0.001
SS43 (Noncarrier)	EF=62.9; LVIDd=5.7; LVIDs=3.7; IVSd=0.58; LA Vol=63.8	
SS65 (*MYBPC3*$^{\Delta 25bp}$)	EF=78.8; age=75; 1st degree AV block; septal and inferior infarct; LA Vol=54.6	SCN5A, C1004R, PP2=0.999, GERP=5.38, ExAC_SAS=0.002
SS26 (*MYBPC3*$^{\Delta 25bp}$)	EF=74.8; Mitral E to A ratio = 2.4 (highest)	MYH7, D1798N, PP2=1, GERP=5.12, ExAC_SAS=0
SS28 *MYBPC3*$^{\Delta 25bp/D389V}$)	EF=60.4; T wave abn, possible anterolateral ischemia (ECG); LVIDd=5.3; LVIDs=3.6	GAA, G123E, PP2=0.998, GERP=4.85, ExAC_SAS=0.0001; GAA, S448T, PP2=0.8, GERP=4.18, ExAC_SAS=0.008
SS37 *MYBPC3*$^{\Delta 25bp/D389V}$)	EF=57.2; LVIDd=5.1; IVS/PW=0.53; LA Vol=62.4	DSP, FBN1, FBN2, FKTN (all rare, potentially deleterious)

Rationale for exclusion of subjects from analysis: determined to have presence of mutations in genes known to cause cardiomyopathy, determined to have abnormally deviated echocardiographic parameters, or determined to have known prior cardiovascular disease.