A Gene-Centric Strategy for Identifying Disease-Causing Rare Variants in Dilated Cardiomyopathy

SUPPLEMENTAL MATERIAL

CONTENTS

Supplemental Figure Legends

Figure S1. Principal components analysis (PCA) of study participants. A PCA analysis of Boston and Australian DCM cases (n=203, black dots) and healthy control subjects (n=208, black dots) and UK DCM cases (n=329, red crosses) and control subjects (n=319, green circles) was performed using SmartPCA software (ref) and 127 single nucleotide variants that were common to all groups and had a genotyping rate of >88%.

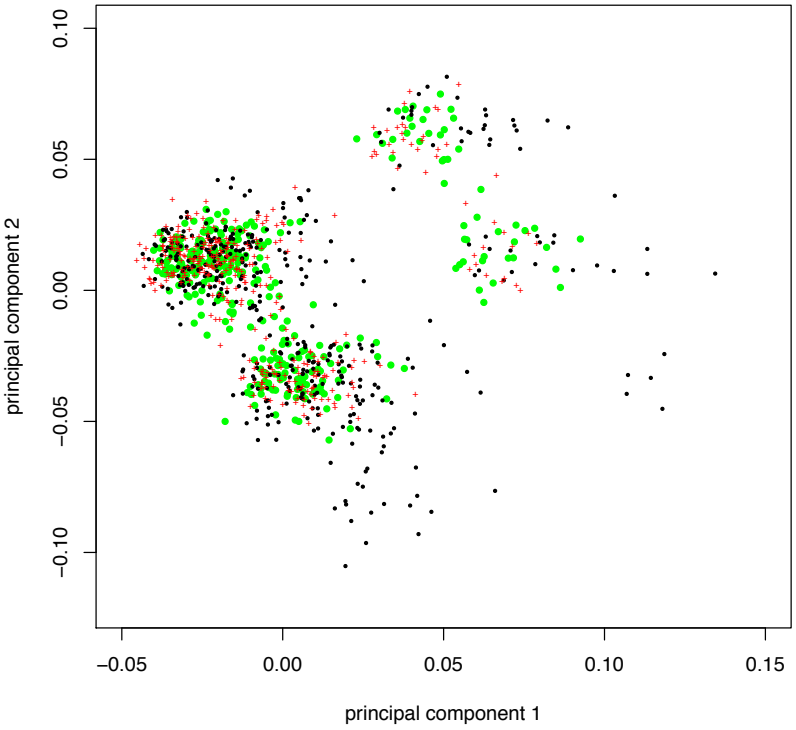
Figure S2. Genotype-phenotype correlations for missense variants. All rare variants (MAF < 0.1%) in the 41-gene data-set that were present in the family proband were evaluated in family members. The presence (+) or absence (-) of variants in relatives is shown. DCM status is denoted as affected (solid symbols), unaffected (open symbols), or unknown (gray symbols); probands are indicated by arrows.

Figure S3. Pedigrees for 5 kindreds in which multiple (5+) rare variants were identified in the family proband. For this analysis, all variants with a MAF < 1.0% in any of the genes in the 69-gene targeted re-sequencing panel were included. The presence (+) or absence (-) of variants in relatives is shown, with co-segregating variants in bold type. DCM status is denoted as affected (solid symbols), unaffected (open symbols), or unknown (gray symbols); probands are indicated by arrows.

Figure S4. Distribution of rare variants in DCM cases and control subjects. Location of rare variants in different protein domains is shown for the group A genes: (**A**) *DES*, (**B**) *SCN5A*, (**C**) *BAG3*, (**D**) *TPM1*, (**E**) *TNNT2*, (**F**) *DSP*, (**G**) *PLN*, (**H**) *DMD*, (**I**) *LDB3*. Variants identified in DCM patients in the discovery cohort (top row) and in the familial DCM (FDCM) replication cohort (second row) are shown: all variants (pink, “DCM all”), damaging variants (red, “DCM

HP"). Variant types are indicated by: circle =missense, square = splice site change, diamond = frameshift, star = stop codon. The third row shows DCM variants identified by two clinical diagnostic laboratories ("DCM Walsh").[1] Missense variants identified in control subjects are shown below the protein schematic: control subjects in this study (Controls), Exome Aggregation Consortium (ExAC) database. See Supplementary Table S9 for protein domain coordinates and statistical analysis.

Figure S1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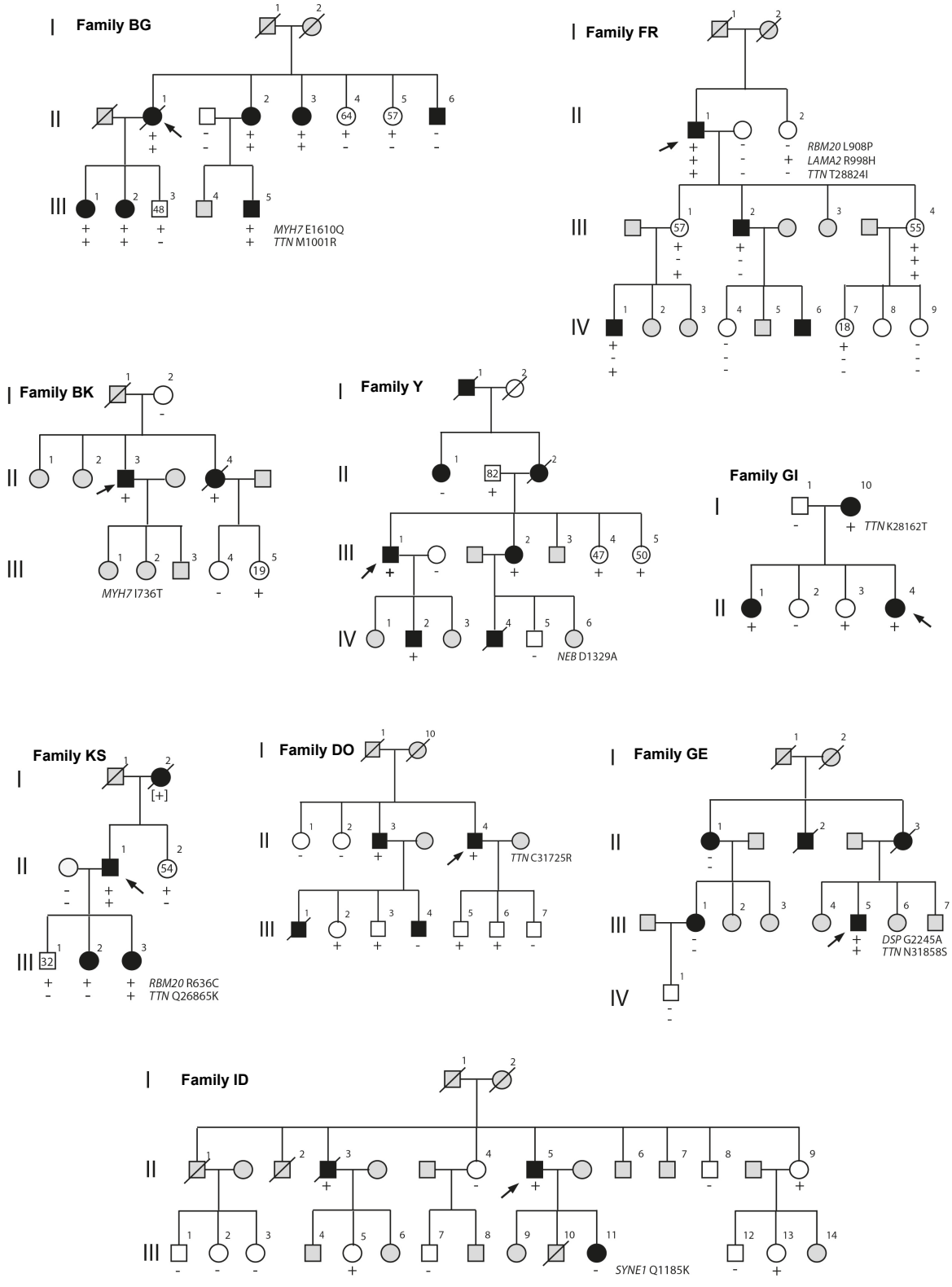
principal component 1

Figure S2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NEB D1329A
Family GI
TTN K28162T
Family KS
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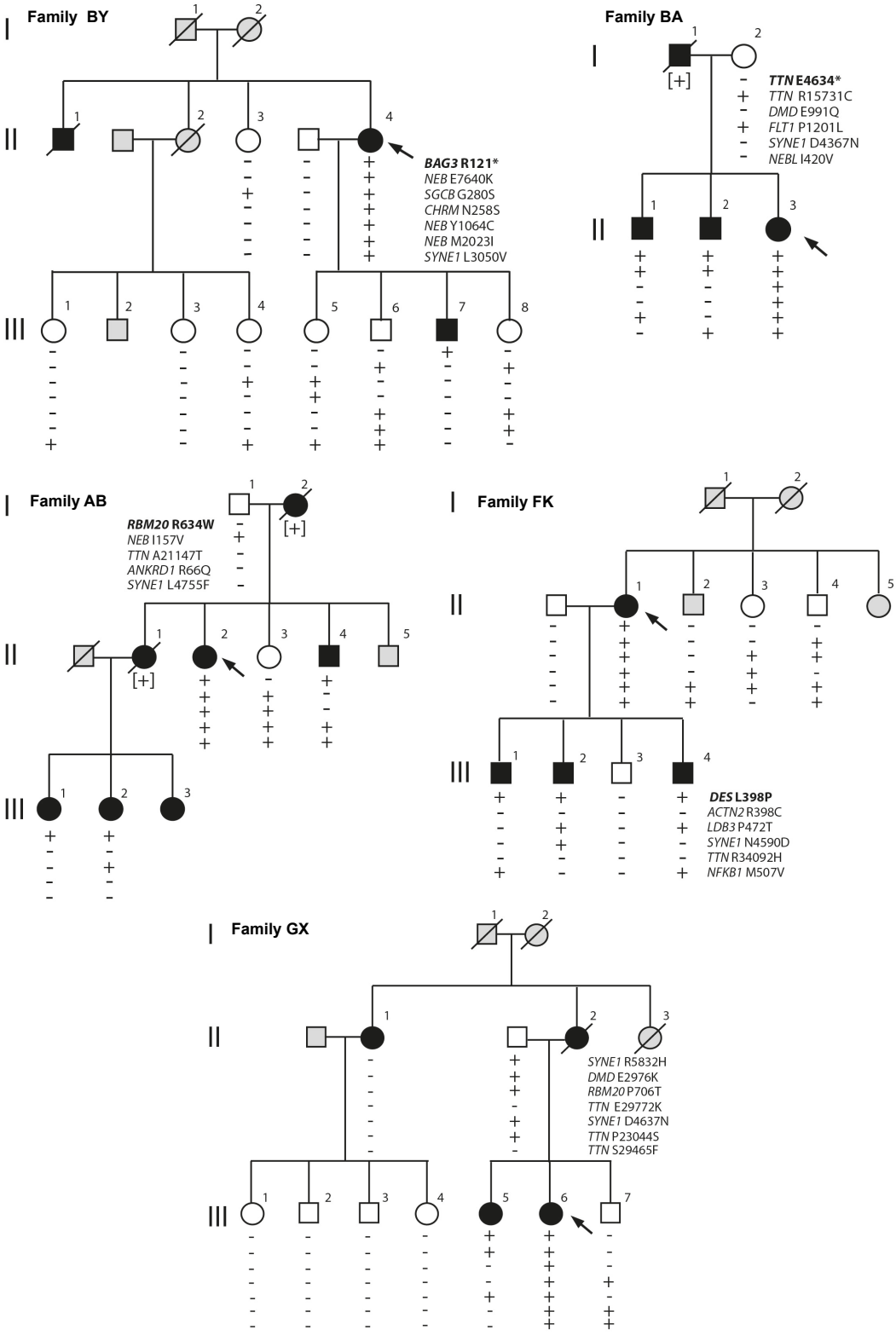
Figure S3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SYNE1 L4755F
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LDB3 P472T
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NFKB1 M507V
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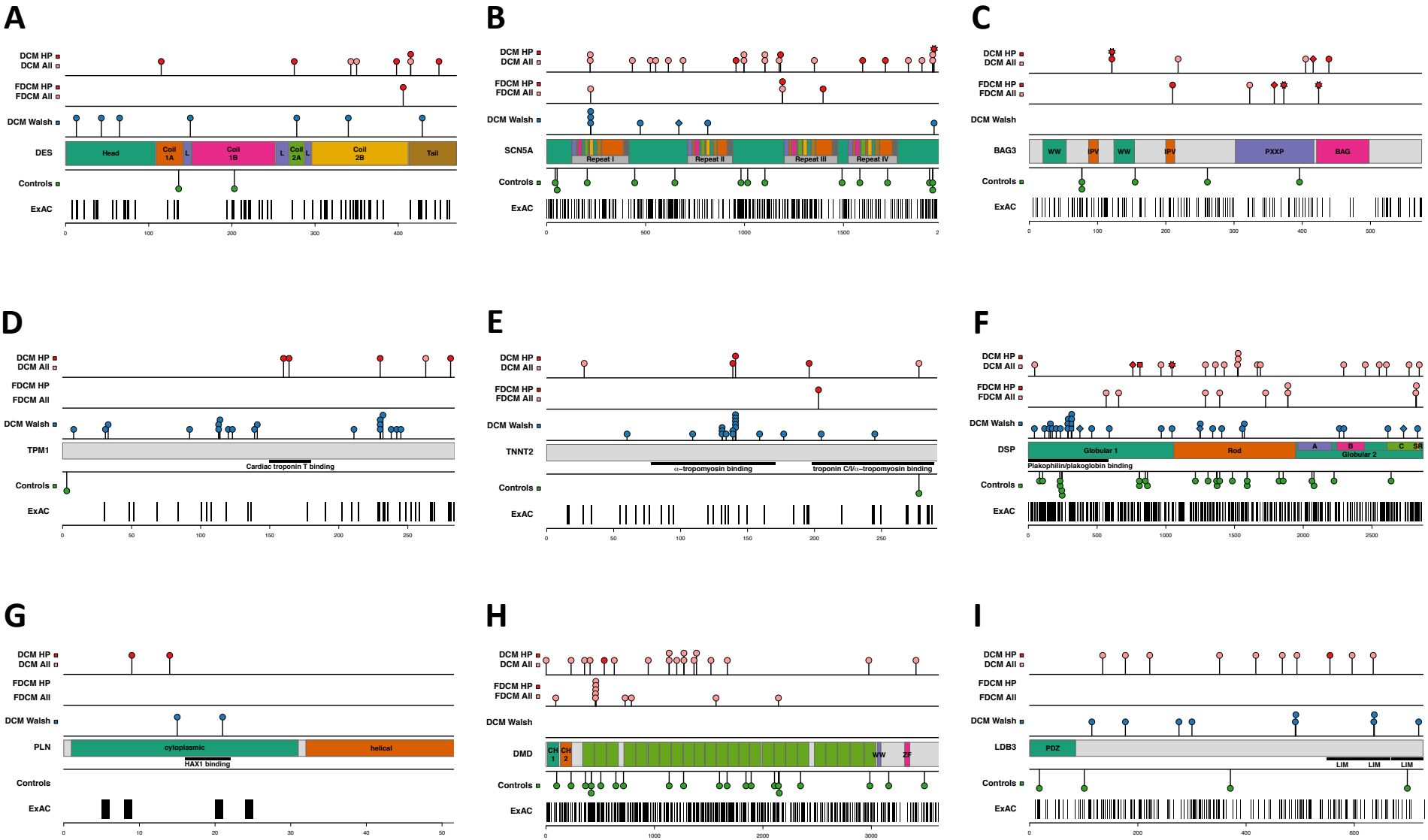
Figure S4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FDCM HP
FDCM All
DCM Walsh
DES
Head
Coil 1A
L
Coil 1B
L
Coil 2A
L
Coil 2B
Tail
Controls
ExAC
B
SCN5A
Repeat I
Repeat II
Repeat III
Repeat IV
C
BAG3
WW
IPV
WW
IPV
PXXP
BAG
D
TPM1
Cardiac troponin T binding
E
TNNT2
α–tropomyosin binding
troponin C/I/α–tropomyosin binding
F
DSP
Globular 1
Rod
A
B
Globular 2
C
SR
Plakophilin/plakoglobin binding
G
PLN
cytoplasmic
helical
HAX1 binding
H
DMD
CH 1
CH 2
WW
ZF
I
LDB3
PDZ
LIM
LIM
LIM

Table S1. Yield of rare (MAF< 0.1%) variants per gene in DCM patients and control subjects.

Gene	Variants (n=770) in DCM cases			Variants (n=589) in control subjects		
	Truncating	Missense	All	Truncating	Missense	All
TTN	83	287	370	6	254	260
SYNE1	0	54	54	3	61	64
LAMA2	2	29	31	4	28	32
RBM20	2	27	29*	0	10	10
MYH7	3	24	27*	0	9	9
DSP	3	16	19	0	23	23
MYBPC3	0	19	19	1	14	15
SYNM	1	18	19	2	18	20
SCN5A	1	17	18	1	14	15
DMD	0	16	16	0	20	20
ACTN2	1	13	14	0	11	11
LMNA	3	11	14*	0	1	1
LAMA4	0	13	13	0	14	14
MYH6	0	12	12	0	16	16
DSG2	0	10	10	0	10	10
LDB3	0	10	10	0	4	4
DES	0	8	8	0	2	2
NEXN	0	8	8	0	4	4
DTNA	1	6	7	0	2	2
VCL	1	6	7	0	9	9
BAG3	2	4	6	0	4	4
ILK	1	5	6	0	6	6
FKTN	0	5	5	1	1	2
TNNT2	0	5	5	0	1	1
TPM1	0	5	5	1	1	2
SDHA	1	3	4	1	5	6
TCAP	0	4	4	0	2	2
ABCC9	1	2	3	0	4	4
ACTC1	0	3	3	0	0	0
ANKRD1	0	3	3	0	6	6
LAMP2	1	2	3	0	1	1
PDLIM3	0	3	3	0	4	4
TNNC1	0	3	3	0	0	0
CRYAB	0	2	2	0	1	1
PLN	0	2	2	0	0	0
SGCD	0	2	2	0	3	3
TNNI3	1	1	2	1	1	2
CAV3	0	1	1	0	2	2
CSRP3	0	1	1	0	0	0
FXN	0	1	1	2	0	2
TAZ	0	1	1	0	0	0

* $P < 0.05$ compared with controls (2x2 chi-squared test; all *TTN* variants excluded).

Table S2. Characteristics of *TTN*tv identified in DCM cases.

Genomic position (Hg19)	Transcript effect	Protein effect	Protein domain	Exon; PSI	Sample ID	Ref.	MAF (ExAC)
179639647	c.6790+1G>T		I-band	29;1.00	10JL01453		Absent
179623709	c.10303+2T>C		I band	44;1.00	10CP01917		0.000008253
179606048	c.11912G>A	p.Trp3971*	I-band, N2B	49;0.95	LW-II-3	R[a]	Absent
179605315	c.12643_12644delCA	p.Gln4215fs	I-band, N2B	49;0.95	10DW00512	R[a]	Absent
179605203	c.12757C>T	p.Gln4253*	I-band, N2B	49;0.95	14MW01583	R[a]	Absent
179604264	c.13696C>T	p.Gln4566*	I-band, N2B	49;0.95	HFA-42	H[a]	0.000008294
179604060	c.13900G>T	p.Glu4634*	I-band, N2B	49;0.95	BA-III-3	R[a] W	Absent
179603994	c.13966C>T	p.Gln 4656*	I-band, N2B	49;0.95	DCM-22	F[a]	Absent
179591957	c.20134delG	p.Ser6712fs	I-band	70;0.69	HFA-9	H[a]	Absent
179571660	c.29062delG	p.Ala9688fs	I-band, N2A	102;0.88	12SK00375	R[a]	Absent
179558736	c.31427-1G>A		I-band	119;0.79	D13KD	H[a]	Absent
179554624	c.31763-1G>A		I-band, PEVK	123;0.88	12PB00376	R[a]	0.00027334
179547630	c.32888-1delG		I-band	136;0.86	10SB00367	R[a]	Absent
179506963	c.40558+1G>A		I-band, PEVK	220;0.96	HFA-26, HFA-41	H[a]	0.0001195
179498590	c.42636del	p.Ala14213fs	I-band	232;0.98	FW-II-1	R[a]	Absent
179498055	c.42947-2A>G		I-band	234;0.97	MIY-11	H[a]	Absent
179497076	c.43544_43545insA	p.Ala14514fs	I-band	237;0.98	MDT-11	H[a]	0.00003414
179495982	c.43792delG	p.Val14598*	I-band	238;0.96	10JM01592	R[a]	Absent
179494967	c.44281+1G>A		I-band	240;0.96	20MC01968	R[a]	0.00000831
179494168	c.44284C>T	p.Arg14762*	I-band	241;0.96	KI-III-2	R[a]	Absent
179487495	c.44816-1G>A		I-band	244;0.96	10BM02061	C	0.00001637
179486244	c.45307C>T	p.Arg15103*	I-band	246;0.97	12RH00086	W	Absent
179485580	c.45756dupA	p.Tyr15253fs	I-band	248;0.97	10BC00144		Absent
179485525	c.45812T>G	p.Leu15271*	I-band	248;0.97	10WS00448	R[a]	Absent
179483495	c.46782C>A	p.Tyr15594*	I-band	252;0.89	20JH01781	R[a] W	Absent
179482120	c.47692C>T	p.Arg15898*	I-band	255;0.86	AP-III-4	R[a]	0.00001504
179480145	c.48527G>A	p.Trp16176*	A-band	260;0.96	10CH00929	R[a]	Absent
179478864	c.49259delA	p.Glu16420fs	A-band	263;0.96	HFA-68	H[a]	Absent
179478665	c.49346-1G>A		A-band	264;0.95	20JB01501	R[a]	Absent

179478552	c.49458G>A	p.Trp16486*	A-band	264;0.95	MO-II-2	R[a]	Absent
179477885	c.49648+2delT		A-band	265;0.96	DM-III-2, MDD-22, MIV-14	H[a]	Absent
179477082	c.50170C>T	p.Arg16724*	A-band	267;0.93	10RN00513	R[a]	Absent
179472127	c.53287+1G>T		A-band	278,0.94	HFA-71	H[a]	Absent
179466192	c.55525_55531delGACAGGA	p.Asp18509fs	A-band	288;0.91	10JF01881	R[a]	Absent
179464422	c.56206delA	p.Thr18736fs	A-band	290;0.96	FQ-III-11	R[a]	Absent
179463603	c.56834delG	p.Gly18945fs	A-band	292;0.94	BM-III-25	R[a]	Absent
179458947	c.58172delA	p.Asp19391fs	A-band	298;0.90	20PD01505	R[a]	Absent
179458293	c.58732+2T>C		A-band	299;0.89	10AL00811	R[a]	Absent
179458075	c.58858delC	p.Glu19619fs	A-band	300;0.90	HFA-63	H[a]	Absent
179457380	c.59352delT	p.Glu19785fs	A-band	302;0.90	AM-III-7	R[a]	Absent
179457005	c.40558G>C		A-band	303;0.91	DCM_UK_B12	H[a]	Absent
179455162	c.61290T>A	p.Cys20430*	A-band	305;0.97	MEH-11	H[a]	Absent
179454957	c.61495C>T	p.Arg20499*	A-band	305;0.97	MDJ-21	H[a] W	Absent
179454576	c.61876C>T	p.Arg20626*	A-band	305;0.97	MEW-11	H[a]	0.00006641
179453427	c.63025C>T	p.Arg21009*	A-band	305;0.97	10TM00933	R[a] W	Absent
179452435	c.63601C>T	p.Arg21201*	A-band	307;0.91	MAO-92, 20EG01127	H[a]	0.000008362
179450018	c.64453G>A	p.Arg21485*	A-band	310;0.91	DCM_UK_B4	W	0.00002508
179441649	c.69412+1G>A		A-band	325;0.93	DCM_UK_B2	H[a]	Absent
179441479	c.69491_69492_delGT	p.Val23164fs	A-band	326;0.94	CS-III-12	R[a]	Absent
179441341	c.69630C>A	p.Tyr23210*	A-band	326;0.94	10CS01472	R[a]	Absent
179438189	c.72668delT	p.Pro24223fs	A-band	327;0.95	MEK-111	H[a]	Absent
179435975	c.74880_74883insACTT	p.Pro24962fs	A-band	327;0.95	DI-II-6	R[a]	Absent
179434743	c.76115insA	p.Asn25372fs	A-band	327;0.95	CT-II-2, R-IV-2, 20SF01123	R[a]	Absent
179433758	c.77101_77102insT	p.Gln25700fs	A-band	327;0.95	HFA-57	H[a]	Absent
179432761	c.78095_78098delAAAG	p.Arg26032fs	A-band	327;0.95	AV-IV-2	R[a] F[a]	Absent
179432675	c.78184G>T	p.Glu26062*	A-band	327;0.95	10BK01803	R[a]	Absent
179432351	c.78507delT	p.Gly26170fs	A-band	327;0.95	10JC00262	R[a]	Absent
179431868	c.78991C>T	p.Arg26331*	A-band	327;0.95	BR-IV-1, EA-II-7	R[a] F[a]	Absent
179429589	c.81262_81269delCAGATGCT	p.Gln27088fs	A-band	327;0.95	10PP00413, 10DM00077	R[a]	Absent
179429538	c.81321C>G	p.Tyr27107*	A-band	327;0.95	12NP00167	R[a]	Absent
179429340	c.81518delC	p.Pro27173fs	A-band	327;0.95	20DH01701	R[a]	Absent

179428345	c.82513delA	p.Ile27505fs	A-band	327;0.95	10CP00605	R[a]	Absent
179426483	c.84376C>T	p.Gln28126*	A-band	327;0.95	LT-II-1	R[a]	Absent
179425091	c.85768C>T	p.Arg28590*	A-band	327;0.95	MGW-11	H[a]	Absent
179424496	c.86363G>A	p.Trp28788*	A-band	327;0.95	MIP-13	H[a]	Absent
179424036	c.86821+2T>A		A-band	327;0.95	MIA-1	H[a] WN	0.000008913
179423219	c.86967G>A	p.Trp28989*	A-band	328;0.96	12JL00046	R[a]	Absent
179422457	c.87624C>A	p.Tyr29208*	A-band	329;0.95	DCM_UK_B6	H[a]	Absent
179413670	c.92683C>T	p.Arg30895*	A-band	340;0.98	10KF00073	R[a]	Absent
179412902	c.93451G>T	p.Glu31151*	A-band	340;0.98	MBG_121	H[a]	Absent
179412874	c.93479G>A	p.Trp31160*	A-band	340;0.98	10AH00506		Absent
179412245	c.94103_94107delTTAAA	p.Thr31366fs	A-band	340;0.98	HFA-46	H[a]	0.000008475
179411592	c.94562dupC	p.Thr31522fs	A-band	342;0.98	20JM01785	R[a]	Absent
179410799	c.95164C>T	p.Gln31722*	A-band	344;0.99	MHQ-12	H[a]	Absent
179410544	c.95415_95416+2delCAGT		A-band	344;0.99	10KW01906	R[a]	Absent
179406990	c.97492+1G>C		A-band	350;1.00	DCM-30, HFA-66, HFA-83	H[a] F[a]	Absent
179404523	c.98265_98268dupAACA	p.His32757fs	A-band	353;1.00	10CS01784	R[a] W	Absent
179403522	c.99034A>T	p.Lys33012*	A-band	355;1.00	MHX-11	H[a]	Absent
179401029	c.100445C>A	p.Ser33482*	A-band	358;0.99	20JR01203	H[a] R[a]	Absent
179400319	c.101021_101022delGA	p.Arg33674fs	A-band	359;1.00	20RD01346	R[a]	Absent
179399346	c.101996G>A	p.Trp33999*	A-band	359;1.00	14CT01557	R[a]	Absent
179393848	c.106629delA	p.Ala35544fs	A-band	361;0.99	12PF00041	R[a]	Absent
179393000	c.107377+1G>A		A-band	362;1.00	20JM01785	R[a]	0.00001661

MAF (ExAC), minor allele frequency in the Exome Aggregation Consortium database; PSI, proportion spliced-in.
[a] This patient reported previously; H = Herman[2], R = Roberts[3], F = Fatkin[4], W = Walsh[1], C = Ceyhan-Birsoy[5].

Table S3. Characteristics of rare missense variants in *TTN* and 40 other cardiomyopathy genes.

Parameter	*TTN* gene			Other cardiomyopathy genes		
	DCM (n=287 variants)	Control (n=254 variants)	*P* value	DCM (n=375 variants)	Control (n=312 variants)	*P* value
Novel (EA-ESP)	163 (57%)	141(56%)	0.79	235 (63%)	193 (62%)	0.87
Novel (ExAC)	76 (27%)	50 (20%)	0.07	121 (32%)	58 (19%)	<0.0001
Deleterious (PolyPhen2/SIFT/PROVEAN)	100 (35%)	95 (37%)	0.59	134 (36%)	80 (26%)	0.005
Deleterious (MetaSVM)	49 (17%)	42 (17%)	0.91	152 (41%)	80 (26%)	<0.0001
Novel (ExAC) + deleterious (PolyPhen2/ SIFT/PROVEAN)	26 (9%)	18 (7%)	0.43	61 (16%)	16 (5%)	<0.0001
Novel (ExAC) + deleterious (MetaSVM)	16 (6%)	8 (3%)	0.21	71 (19%)	15 (5%)	<0.0001

Table S4. Distribution across RVIS percentiles of truncating and missense variants in 40 cardiomyopathy genes (*TTN* excluded).

	RVIS percentile				P value	Residuals				Standardized residuals			
	25th	50th	75th	100th	(2x4)*	25th	50th	75th	100th	25th	50th	75th	100th
All variants													
DCM	205	78	17	81	0.75	0.11	-0.60	0.10	0.39	0.24	-1.01	0.16	0.66
Control	163	73	13	60		-0.12	0.67	-0.11	-0.44	-0.24	1.01	-0.16	-0.66
Truncating variants													
DCM	16	0	4	4	0.01	1.05	-1.36	0.53	-1.06	2.43	-2.28	0.91	-2.03
Control	4	3	1	7		-1.33	1.72	-0.67	1.35	-2.43	2.28	-0.91	2.03
Missense variants													
DCM	189	78	13	77	0.67	-0.15	-0.36	-0.20	0.72	-0.33	-0.61	-0.30	1.19
Control	159	70	12	53		0.17	0.40	0.22	-0.79	0.33	0.61	0.30	-1.19
Missense variants, novel (ExAC)													
DCM	69	22	5	25	0.49	0.16	-0.65	-0.20	0.51	0.43	-1.31	-0.36	1.01
Control	30	15	3	8		-0.24	0.96	0.29	-0.76	-0.43	1.31	0.36	-1.01
Missense variants, deleterious (MetaSVM)													
DCM	94	19	7	28	0.29	-0.16	-0.03	1.11	-0.12	-0.47	-0.05	1.93	-0.23
Control	51	10	0	16		0.23	0.04	-1.54	0.17	0.47	0.05	-1.93	0.23
Missense variants, novel + deleterious													
DCM	47	8	4	12	0.15	0.24	-0.83	0.38	0.13	0.94	-2.17	0.94	0.34
Control	8	5	0	2		-0.51	1.81	-0.84	-0.28	-0.94	2.17	-0.94	-0.34

* A 2 x 4 chi-square test was used to assess differences between cases and controls across different RVIS percentiles. To identify the cells that contribute most to the statistical significant results, raw residuals were obtained by subtracting expected from observed values and standardized residuals were calculated by dividing the raw residuals by the square root of the residual cell variance. The standardized residuals follow a standard normal distribution so any deviations from +2 or –2 corresponds to statistical significance and lack of fit of null hypothesis in that cell.

Table S5. Evidence from animal models implicating different genes in DCM pathogenesis.

Gene	Protein	Gene group	DCM in mouse model		Other species
			Loss-of-function*	Human NS mutation	
MYH7	Myosin heavy chain 7	A	See *MYH6*†; no murine *myh7* KO	Adult KI† mice	Zebrafish embryo morphants Adult fly morphants
DSP	Desmoplakin	A	KO mice: EL (-/-), adult biV ARVC (+/-); cKO mice: adult biV ARVC (-/-)	Adult Tg mice (ARVC mutation)	
SCN5A	Cardiac sodium channel	A	KO mice: EL (-/-); normal FS (+/-); adult Tg mice (80-90% ↓protein): DCM	Adult KI mice	Zebrafish embryo MO: developmental defects
LMNA	Lamin A/C	A	KO mice: NN DCM/death (-/-); adult DCM (+/-)	Adult KI mice	Zebrafish embryo MO
LDB3	LIM domain binding protein 3	A	KO mice: NN death (-/-); adult cKO mice (-/-)	Adult Tg mice	Zebrafish embryo MO: thin ventricular walls
DMD	Dystrophin	A	Adult mdx mice	NA	KO flies
DES	Desmin	A	Adult KO mice: DCM (-/-)	Adult KI mice	Zebrafish embryo MO
TPM1	Tropomyosin α-1 chain	A	KO mice: EL (-/-), normal systolic function (+/-).	Adult Tg mice	
TNNC1	Troponin C	A	KI mice (D73N, Ca^{2+} de-sensitizer): EL (-/-), adult DCM (+/-)	NA	Zebrafish embryo morphant: developmental defects, ↓contraction
TNNT2	Troponin T	A	KO mice: DCM, NN death (-/-), normal FS (+/-).	Adult Tg mice	Zebrafish embryo morphants/MO
BAG3	BCL-2 associated athanogene	A	KO mice: NN death (-/-)	Adult Tg mice	Zebrafish embryo MO: ↓contraction, PE
PLN	Phospholamban	A	Adult Tg mice (over-expression): DCM; KO mice: hypercontractile (-/-)	Adult Tg mice	
RBM20	RNA-binding protein 20	A	NA	NA	Adult KO rats: LV dilation, normal FS

TTN	Titin	A	*TTN*tv Tg mice: EL (-/-), adult stress-induced DCM (+/-)	NA	Zebrafish embryo morphants: ↓contraction, PE (-/-), normal contraction (+/-); Adult *TTN*tv rats: stress-induced DCM
VCL	Vinculin	B	KO mice: EL (-/-); adult stress-induced DCM (+/-); cKO mice: adult DCM (-/-)	NA	Zebrafish embryo MO
LAMA4	Laminin subunit α-4	B	Adult KO mice: LVH, late DCM (-/-)	NA	Zebrafish embryo MO: PE
ILK	Integrin-linked protein kinase	B	Adult KO mice (-/-); adult cKO mice (-/-)	NA	Zebrafish embryo MO: cardiac developmental defects; zebrafish embryo morphant: ↓contraction, PE
MYBPC3	Cardiac myosin binding protein 3	B	KO/KI mice: NN/adult DCM, LVH (-/-), normal adult FS, ± LVH (+/-)	NA	Zebrafish embryo MO: ventricular hypertrophy, normal contraction, PE
CSRP3	Muscle LIM protein	B	KO mice: LVH, NN death or adult DCM (-/-); no early phenotype (+/-)	Adult KI mice: LVH, ↓contractile reserve	Zebrafish embryo MO
CRYAB	αβ-crystallin	B	NA	Adult Tg mice: LVH, late DCM	Zebrafish embryo MO: ↓contraction, PE; NS mutant flies
ACTC1	Cardiac actin	B	KO mice: EL/NN death (-/-)	Adult Tg mice	Zebrafish embryo morphants: cardiac dilation/development defects, ↑contraction, PE
NEXN	Nexilin	B	KO mice: DCM, EFE, NN death (-/-), normal adult FS (+/-)	NA	Zebrafish embryo MO/injected morphants
TAZ	Tafazzin	B	Adult iKO mice (-/-)	NA	Zebrafish embryo MO: developmental defects, PE
LAMP2	Lysosome-associated membrane glycoprotein 2	B	Adult KO mice: ↑ HW/BW, ↓myofibril force (-/-)	NA	

TNNI3	Troponin I	B	KO mice: NN death, ↑ myocyte resting tension, ↓ Ca^{2+} sensitivity (-/-)	NA	Morphant flies
ANKRD1	Cardiac ankyrin repeat protein	B	Adult KO mice: normal FS (-/-)	NA	
DSG2	Desmoglein-2	B	KO mice: EL (-/-), viable, no overt changes (+/-); cKO mice: adult RV dilation, histopathology (-/-)	Adult Tg mice (ARVC mutation)	
MYH6	Myosin heavy chain 6	C	KO mice†: EL (-/-), adult ↓contraction (+/-)	NA	KO xenopus: developmental defects, dilation, ↓contraction
ABCC9	K_{ATP} channel SUR2A subunit	C	KO mice: DCM, NN death (-/-)	NA	KO flies: pacing-induced heart failure
ACTN2	α-actinin	C	NA	NA	Zebrafish embryo MO: ventricular dilation, thin walls
PDLIM3	PDZ and LIM domain protein 3	C	KO mice: adult DCM (RV>LV) (-/-)	NA	
SGCD	δ - sarcoglycan	C	KO mice: late (>50 weeks) DCM (-/-)	Adult KI mice: no DCM	
SYNE1	Nesprin-1	C	KASH-deficient mice: no dilation, late (>52 weeks)↓contraction (-/-)	NA	
FXN	Frataxin	C	KO mice: EL (-/-); cKO mice: adult LVH/DCM (-/-)	NA	KO flies
CAV3	Caveolin-3	C	No DCM in LOF model, Tg overexpression: adult DCM	NA	
TCAP	Telethonin	C	Adult KO mice: normal baseline, post-TAC LVH, DCM (-/-), no change (+/-)	NA	Zebrafish embryo MO
FKTN	Fukutin	C	KO mice: EL (-/-)	NA	
SDHA	Succinate dehydrogenase flavoprotein subunit	D	NA	NA	
DTNA	α-dystrobrevin	D	Adult KO mice: no DCM,	NA	

			isoproterenol-induced ↑mortality (-/-)		
LAMA2	Laminin subunit α-2	D	KO mice: SKM (-/-)	NA	
SYNM	Synemin	D	KO mice: SKM (-/-)	NA	

* Most studies have evaluated homozygous gene loss-of-function models; data from heterozygous mice are shown when available.
† α-MHC is major myosin isoform in murine ventricle: human *MYH7* mutations modelled in murine *myh6* gene.
ARVC, arrhythmogenic right ventricular cardiomyopathy; biV, biventricular; cKO, cardiac-specific knockout; DCM, dilated cardiomyopathy; EFE, endocardial fibroelastosis; EL, embryonic lethal; FS, fractional shortening; HW/BW, heart weight: body weight ratio; iKO, inducible knockout; KASH, Klarsicht Anc-1 Syne Homology domain; KI, knock-in; KO, knockout; LV, left ventricle; LVH, left ventricular hypertrophy; MO, morpholino; NA, not available; NN, neonatal; NS, non-synonymous variant; PE, pericardial effusion; RV, right ventricle; SKM, skeletal myopathy; TAC, transverse aortic constriction; Tg, transgenic; *TTN*tv, truncating variants in the *TTN* (titin) gene.

Table S6. Distribution across gene groups of truncating and missense variants in 40 cardiomyopathy genes (*TTN* excluded).

	Gene group				P value	Residuals				Standardized residuals			
	A	B	C	D	(2x4)*	A	B	C	D	A	B	C	D
All variants													
DCM	162	78	99	61	0.002	1.99	-0.25	-1.48	-0.67	3.67	-0.41	-2.60	-1.09
Control	91	68	110	60		-2.19	0.27	1.63	0.74	-3.67	0.41	2.60	1.09
Truncating variants													
DCM	14	4	2	5	0.01	1.45	0.23	-1.27	-0.80	2.90	0.39	-2.21	-1.49
Control	2	2	6	7		-1.76	-0.28	1.53	0.97	-2.90	-0.39	2.21	1.49
All missense variants													
DCM	148	74	97	56	0.018	1.66	-0.29	-1.23	-0.46	3.05	-0.48	-2.16	-0.75
Control	89	66	104	53		-1.82	0.32	1.35	0.51	-3.05	0.48	2.16	0.75
Missense variants, novel (ExAC)													
DCM	76	14	25	6	0.0009	1.56	-1.13	-0.87	-0.94	3.98	-2.17	-1.76	-1.72
Control	18	14	19	7		-2.26	1.64	1.26	1.36	-3.98	2.17	1.76	1.72
Missense variants, deleterious (MetaSVM)													
DCM	85	24	27	16	0.0005	1.45	0.50	-1.61	-1.09	3.44	0.92	-3.16	-2.01
Control	26	9	29	16		-2.01	-0.69	2.23	1.51	-3.44	-0.92	3.16	2.01
Missense variants, novel + deleterious													
DCM	55	6	8	2	0.001	0.65	0.47	-1.05	-1.05	2.90	1.17	-2.74	-2.58
Control	6	0	6	3		-1.42	-1.02	2.28	2.28	-2.90	-1.17	2.74	2.58

* A 2 x 4 chi-square test was used to assess differences between cases and controls across different gene groups. To identify the cells that contribute most to the statistical significant results, raw residuals were obtained by subtracting expected from observed values and standardized residuals were calculated by dividing the raw residuals by the square root of the residual cell variance. The standardized residuals follow a standard normal distribution so any deviations from +2 or –2 corresponds to statistical significance and lack of fit of null hypothesis in that cell.

Table S7. Damaging* variants in group A genes identified in DCM cases.

Gene	Genomic position(Hg19)	Transcript effect	Protein effect	Protein domain	Variant carriers	Other studies (no.probands)
MYH7	14:23901919	c.431G>T	p.Gly144Val	Motor	10EC00826	DCM (3)
MYH7	14:23900157	c.848A>G	p.Tyr283Cys	Motor	DCM-24	HCM (1)
MYH7	14:23899092	c.1030G>A	p.Glu344Lys	Motor	10EH00627	
MYH7	14:23899016	c.1106G>A	p.Arg369Gln	Motor	CZ-III-4	DCM (4)
MYH7	14:23898294	c.1277C>T	p.Ala426Val	Motor	DCM-2	
MYH7	14:23898175	c.1396G>C	p.Glu466Gln	Motor switch II	DCM-23	
MYH7	14:23897714	c.1573G>A	p.Glu525Lys	Motor	DCM-UK-B5	DCM (1)
MYH7	14:23897708	c.526_+1G>A		Motor	DF-III-1	
MYH7	14:23894983	c.2207T>C	p.Ile736Thr	Motor, converter	BK-III-3	DCM (1), HCM (>10)
MYH7	14:23894584	c.2330G>A	p.Arg777Lys	Motor, converter	20JO01157	
MYH7	14:23894500	c.2414T>C	p.Leu805Pro	Neck, IQ domain	20DH01138	
MYH7	14:23894204	c.2453T>A	p.Ile818Asn	Neck	20RW01823	
MYH7	14:23894037	c.2620G>C	p.Glu874Gln	Rod/hinge	DD-III-4	
MYH7	14:23894036	c.2621A>T	p.Glu874Val	Rod/hinge	10LL01020	
MYH7	14:23892760	c.3094delG	p.Asp1032fs	Rod/hinge	12RC00100	
MYH7	14:23889113	c.3667G>A	p.Glu1223Lys	Hinge	MDU-111	
MYH7	14:23885338	c.4828G>C	p.Glu1610Gln	Rod, LMM	BG-III-1	
MYH7	14:23884251	c.5512A>G	p.Lys1838Glu	Rod, LMM	MDJ_21	
MYH7	14:23884207	c.5556C>G	p.Tyr1852*	Rod, LMM	12DB00017	
MYH7	14:23883041	c.5717C>G	p.Ala1906Gly	Rod, LMM	DCM-26	
RBM20	10:112544606	c.1486C>T	p.Gln496*		12PF00041	
RBM20	10:112572055	c.1900C>T	p.Arg634Trp	RS domain	AB-III-2	DCM (1)
RBM20	10:112572061	c.1906C>T	p.Arg636Cys	RS domain	KS-II-1, MCH-123	DCM (1)
RBM20	10:112572062	c.1907G>A	p.Arg636His	RS domain	MAK-13	DCM (4)
RBM20	10:112572068	c.1913C>T	p.Pro638Leu	RS domain	MJ-11	DCM (4)
RBM20	10:112572419	c.2264G>A	p.Arg755His		10IP00668	
RBM20	10:112572526	c.2371C>T	p.Arg791Trp		HFA-71	
RBM20	10:112581100	c.2723T>C	p.Leu908Pro	Glutamate-rich	FR-I-1	

RBM20	10:112581114	c.2737G>A	p.Glu913Lys	Glutamate-rich	20RJ01217	DCM (2)
RBM20	10:112581427	c.3051_3054delCTCC	p.Ser1018fs		10LS01497	
RBM20	10:112590831	c.3464T>C	p.Val1155Ala		DCM-UK-B8	
RBM20	10:112590924	c.3557A>G	p.His1186Arg	ZF, C2H2-type	10MG01841	
LMNA	1:156084868	c.159A>C	p.Glu53Asp	Coil 1A	20JT01288	
LMNA	1:156104287	c.607G>A	p.Glu203Lys	Coil 1B	12PH00095	DCM (2)
LMNA	1:156104594	c.640-2A>G		Coil 1B	20BB01870	
LMNA	1:156104603	c.647G>A	p.Arg216His	Coil 1B	LZ-III-4	
LMNA	1:156104617	c.661C>T	p.Arg221Cys	Linker 2	10LW02013	
LMNA	1:156104622	c.667_668delGA	p.Glu223fs	Linker 2	20PF01735	
LMNA	1:156104629	c.673C>T	p.Arg225*	Linker 2	MIG111	DCM ± CD (6)
LMNA	1:156108455	c.1875C>A	p.Ser625Arg	Tail	14SD01620	
DES	2:220283527	c.343C>A	p.Leu115Ile	Coil 1A	DCM-UK-C1	
DES	2:220285304	c.823A>G	p.Arg275Gly	Coil 2A	14AM01556	DCM (1)
DES	2:220286231	c.1193T>C	p.Leu398Pro	Coil 2B	FK-II-1	
DES	2:220286282	c.1244G>A	p.Arg415Gln	Tail	12JW00204	
DES	2:220290442	c.1346A>C	p.Lys449Thr	Tail	FG-II-2	MFM (1)
SCN5A	3:38622781	c.2869A>G	p.Asn957Asp	Cytoplasmic	20RJ01812	
SCN5A	3:38616909	c.3545A>G	p.Asp1182Gly	Cytoplasmic	20CP01865	
SCN5A	3:38595793	c.4790T>C	p.Val1597Ala	Transmembrane, S3, repeat IV	14AM01556	
SCN5A	3:38592729	c.5134G>A	p.Gly1712Ser	Extracellular	20AB01158	BS (1)
SCN5A	3:38591991	c.872C>T	p.Arg1958*	Cytoplasmic, C-terminus	C-II-9	DCM (1)
BAG3	10:121429543	c.361C>T	p.Arg121*		BY-III-4	
BAG3	10:121429544	c.362G>A	p.Arg121Gln		20JO01157	
BAG3	10:121436309	c.1243_1244insCT	p.Pro416fs	PXXP domain	20PW01714	
BAG3	10:121436382	c.1316T>A	p.Val439Glu	BAG domain	MBG-121	
TPM1	15:63351866	c.479G>A	p.Arg160His	cTNT binding	DCM-21	
TPM1	15:63351878	c.491A>G	p.Glu164Gly	cTNT binding	MDK-11	
TPM1	15:63354462	c.688G>A	p.Asp230Asn		DCM-20	DCM (3)
TPM1	15:63356332	c.842T>C	p.Met281Thr		SS200	HCM (5)
TNNT2	1:201333470	c.415C>T	p.Arg139Cys	α-TM b.s.	10AB00132	DCM (1)

TNNT2	1:201333464	c.421C>T	p.Arg141Trp	α-TM b.s.	MGY-123	DCM (10), HCM (1)
TNNT2	1:201331144	c.586C>T	p.Arg196Trp		10MP01703	
DSP	6:7574464	c.2276_2277insA	p.Thr760fs	Globular, N-terminal	10AS00411	DCM (1)
DSP	6:7575030	c.2436+2T>C		Globular, N-terminal	20GF01948	
DSP	6:7579556	c.3133C>T	p.Arg1045*	Globular, N-terminal	10KS01707	
PLN	6:118880109	c.25C>T	p.Arg9Cys	Cytoplasmic	20CP01865	DCM (2)
PLN	6:118880125	c.41G>C	p.Arg14Thr	PKA motif	DCM-UK-B11	
DMD	X: 32591954	c.1612G>T	p.Asp538Tyr	Spectrin 2 repeat	10RN00513	
LDB3	10:88476516	c.1664A>G	p.Asn555Ser	LIM zinc-binding 1	10TM00933	

* Damaging variants included truncating (trunc) or novel (ExAC) + deleterious (MetaSVM) missense variants in group A genes (*TTN* excluded).
α-TM, α-tropomyosin; BS, Brugada syndrome; CD, conduction-system abnormalities; cTNT, cardiac troponin T; HCM, hypertrophic cardiomyopathy; LMM, light meromyosin region; MFM, myofibrillar myopathy; PKA, protein kinase A; ZF, zinc finger.

Table S8. Co-segregation analysis of rare (MAF < 0.1%) missense variants in the 41-gene dataset in 14 DCM families.

Family	Gene	Variant	Gene group	MetaSVM	MAF (ExAc)	No. affected/ carriers*	LOD score#
AB	*RBM20*	p.Arg634Trp	A	Deleterious	Absent	6/6	1.46
	TTN	p.Ala21147Thr	A	Deleterious	0.0007573	2/3	-3.82
BG	*MYH7*	p.Glu1610Gln	A	Deleterious	Absent	6/9	-1.47
	TTN	p.Met1001Arg	A	Tolerated	0.0001072	6/6	-3.41
BK	*MYH7*	p.Ile736Thr	A	Deleterious	Absent	2/3	-0.19
CZ	*MYH7*	p.Arg369Gln	A	Deleterious	Absent	4/4	1.11
	TTN	p.Arg18037Trp	A	Tolerated	0.00004172	2/3	0.12
	TTN	p.Val205641Ile	A	Tolerated	0.000008327	4/5	-0.18
DO	*TTN*	p.Cys31725Arg	A	Deleterious	Absent	2/6	0.28
FG	*DES*	p.Lys449Thr	A	Deleterious	Absent	4/4	1.33
	SYNM	p.Ser1089Leu	D	Tolerated	0.0003569	1/4	-0.39
FK	*DES*	p.Leu398Pro	A	Deleterious	Absent	4/4	1.38
	ACTN2	p.Arg398Cys	C	Tolerated	0.00003295	1/2	0.68
	LDB3	p.Pro472Thr	A	Tolerated	Absent	3/5	-3.47
	SYNE1	p.Asn4590Asp	C	Tolerated	0.00009071	2/3	-2.94
	TTN	p.Arg34092His	A	Tolerated	0.00004973	1/3	0.6
FR	*RBM20*	p.Leu908Pro	A	Deleterious	Absent	3/6	0.40
	LAMA2	p.Arg998His	D	Tolerated	0.0001426	1/3	0.58
	TTN	p.Thr28241Ile	A	Tolerated	0.00008418	2/4	-1.11
GE	*DSP*	p.Gly2245Ala	A	Tolerated	0.000008364	1/1	-0.17
	TTN	p.Asn31858Ser	A	Tolerated	0.00003312	1/1	-0.17
GI	*TTN*	p.Lys28162Thr	A	Tolerated	Absent	3/4	-0.08
GX	*DMD*	p.Glu2976Lys	A	Tolerated	0.00002711	2/3	0
	RBM20	p.Pro706Thr	A	Tolerated	Absent	1/2	0
	SYNE1	p.Arg5832His	C	Tolerated	0.00001647	2/3	0
	TTN	p.Glu29772Lys	A	Tolerated	0.0002655	2/2	0
ID	*SYNE1*	p.Gln1185Lys	C	Tolerated	0.00002471	2/5	0.17

KS	*RBM20*	p.Arg636Cys	A	Deleterious	Absent	3/5	-1.11
	TTN	p.Gln28506Lys	A	Tolerated	0.0003342	2/2	-∞
Y	*NEB*	p.Asp1329Ala	D	Deleterious	0.0008056	3/6	-2.64

* Includes all affected individuals and unaffected individuals aged >40 years.
# LOD scores for each variant (theta = 0, penetrance 0.7) were based on all genotyped family members; pedigrees are shown in Figure 2 and Supplementary Figure 2.

Table S9. Distribution of rare variants in DCM cases and controls* across different protein functional domains.

Protein domain	Protein location (aa)	No. variants		Odd ratio	Confidence interval	*P* value
		DCM cases	Controls			
MYH7 (β-myosin heavy chain)						
Myosin motor	85 - 778	42	108	3.35	2.24 - ∞	**2.35E-07**
Neck (IQ)	781 - 810	2	15	0.81	0.13 - ∞	0.72
Tail - Rod/hinge	838 - 1126	15	130	0.65	0.37 - ∞	0.95
Tail - Hinge	1127 - 1279	5	12	2.61	0.87- ∞	0.08
Tail – Light meromyosin (LMM)	1279 - 1935	30	303	0.43	0.29 - ∞	1.00
All domains	1-1935	100	609	NA	NA	NA
RBM20 (RNA-binding protein 20)						
Proline-rich	13 - 86	0	1	0	0 - ∞	1.00
Zinc finger	412 - 436	0	0	0	0 - ∞	1.00
RNA-recognition motif (RRM)	518 - 593	2	4	0.94	0.12 - ∞	0.68
Arg-Ser-rich (RS)	628 - 655	9	4	5.05	1.56 - ∞	**0.008**
Glutamate-rich	843 - 931	6	6	2.02	0.61 - ∞	0.20
Zinc finger	1159 - 1193	1	1	1.89	0.05 - ∞	0.58
All domains	1 - 1227	41	77	NA	NA	NA
LMNA (lamin A/C)						
Head	1 - 33	0	24	0	0 - ∞	1.00
Rod - coil 1A	34 - 70	4	0	∞	4.12 - ∞	**0.001**
Rod - linker 1	71 - 80	0	2	0	0 - ∞	1.00
Rod - coil 1B	81 - 218	9	22	1.98	0.86 - ∞	0.09
Rod- linker 2	219 - 242	6	10	2.86	0.98 - ∞	**0.05**
Rod - coil 2	243 - 383	20	40	3.04	1.64 - ∞	**0.001**
Tail	384 - 646	9	113	0.20	0.09 - ∞	1.00
All domains	1 - 646	48	211	NA	NA	NA
DES (desmin)						

Head	1 - 108	3	30	1.28	0.29 - ∞	0.47
Rod - coil 1A	109 - 141	1	11	1.12	0.05 - ∞	0.62
Rod - linker 1	142 - 151	1	0	∞	0.64 - ∞	0.08
Rod - coil 1B	152 - 252	0	61	0	0 - ∞	1.00
Rod - linker 12	253 - 268	0	0	0	0 - ∞	1.00
Rod - coil 2A	269 - 287	2	2	13.44	1.37 - ∞	**0.03**
Rod - linker 2	288 - 295	0	1	0	0 - ∞	1
Rod - coil 2B	296 - 412	5	57	1.11	0.36 - ∞	0.53
Tail	413 - 470	4	34	1.58	0.45 - ∞	0.32
All domains	1 - 470	16	196	NA	NA	NA
SCN5A (cardiac sodium channel protein 5)						
Cytoplasmic, N-terminus	1 - 126	0	60	0	0 - ∞	1.00
Repeat I	127 - 415	6	110	1.78	0.70 - ∞	0.16
S1, repeat I	127 - 150	0	1	0	0 - ∞	1.00
Extracellular	151 - 158	0	1	0	0 - ∞	1.00
S2, repeat I	159 - 178	0	25	0	0 - ∞	1.00
Cytoplasmic	179 - 191	0	28	0	0 - ∞	1.00
S3, repeat I	192 - 210	0	1	0	0 - ∞	1.00
Extracellular	211- 216	0	17	0	0 - ∞	1.00
S4, repeat I, voltage sensor	217 - 236	6	5	43.35	12.64 - ∞	**2.89E-07**
Cytoplasmic	237 - 252	0	3	0	0 - ∞	1.00
S5, repeat I	253 - 276	0	1	0	0 - ∞	1.00
Extracellular	277 - 389	0	28	0	0 - ∞	1.00
S6, repeat I	390 - 415	0	0	0	0 - ∞	1.00
Cytoplasmic	416 - 711	6	213	0.80	0.32 - ∞	0.75
Repeat II	712 - 939	1	36	0.82	0.04 - ∞	0.71
S1, repeat II	712 - 736	0	2	0	0 - ∞	1.00
Extracellular	737 - 747	0	2	0	0 - ∞	1.00
S2, repeat II	748 - 771	0	4	0	0 - ∞	1.00
Cytoplasmic	772 - 779	0	1	0	0 - ∞	1.00
S3, repeat II	780 - 799	0	9	0	0 - ∞	1.00
Extracellular	800 - 805	0	5	0	0 - ∞	1.00

S4, repeat II, voltage sensor	806 - 825	1	3	10.18	0.38 - ∞	0.12
Cytoplasmic	826 - 841	0	4	0	0 - ∞	1.00
S5, repeat II	842 - 862	0	0	0	0 - ∞	1.00
Extracellular	863 - 913	0	5	0	0 - ∞	1.00
S6, repeat II	914 - 939	0	1	0	0 - ∞	1.00
Cytoplasmic	940 - 1200	9	137	2.35	1.08 - ∞	**0.035**
Repeat III	1201 - 1470	2	148	0.36	0.06 - ∞	0.97
S1, repeat III	1201 - 1224	0	4	0	0 - ∞	1.00
Extracellular	1225 - 1237	0	0	0	0 - ∞	1.00
S2, repeat III	1238 - 1263	0	31	0	0 - ∞	1.00
Cytoplasmic	1264 - 1269	0	1	0	0 - ∞	1.00
S3, repeat III	1270 - 1291	0	18	0	0 - ∞	1.00
Extracellular	1292 - 1295	0	43	0	0 - ∞	1.00
S4, repeat III, voltage sensor	1296 - 1317	0	25	0	0 - ∞	1.00
Cytoplasmic	1318 - 1336	0	3	0	0 - ∞	1.00
S5, repeat III	1337 - 1359	1	11	2.77	0.13 - ∞	0.33
Extracellular	1360 - 1443	1	9	3.39	0.15 - ∞	0.28
S6, repeat III	1444 - 1470	0	3	0	0 - ∞	1.00
Cytoplasmic	1471 - 1523	0	17	0	0 - ∞	1.00
Repeat IV	1524 - 1772	2	55	1.09	0.18 - ∞	0.56
S1, repeat IV	1524 - 1547	0	3	0	0 - ∞	1.00
Extracellular	1548 - 1558	0	1	0	0 - ∞	1.00
S2, repeat IV	1559 - 1582	0	3	0	0 - ∞	1.00
Cytoplasmic	1583 - 1588	0	1	0	0 - ∞	1.00
S3, repeat IV	1589 - 1612	1	16	1.89	0.09 - ∞	0.43
Extracellular	1613 - 1622	0	1	0	0 - ∞	1.00
S4, repeat IV, voltage sensor	1623 - 1644	0	10	0	0 - ∞	1.00
Cytoplasmic	1645 - 1659	0	2	0	0 - ∞	1.00
S5, repeat IV	1660 - 1682	0	9	0	0 - ∞	1.00
Extracellular	1683 - 1747	1	7	4.36	0.19 - ∞	0.23
S6, repeat IV	1748 - 1772	0	2	0	0 - ∞	1.00
Cytoplasmic	1773 - 2017	5	150	0.99	0.36 - ∞	0.58
All domains	1 - 2017	31	926	NA	NA	NA

BAG3 (BAG3)						
WW1	20 - 54	0	9	0	0 - ∞	1.00
IPV	87 -101	0	3	0	0 - ∞	1.00
WW2	124 - 154	0	15	0	0 - ∞	1.00
IPV	200 - 213	1	5	14.57	0.54 - ∞	0.09
PXXP	302 - 417	2	80	1.87	0.25 - ∞	0.38
BAG	421 - 498	1	14	5.17	0.21- ∞	0.21
All domains	1 - 575	6	379	NA	NA	NA
TPM1 (α-tropomyosin)						
Cardiac troponin T binding	150 - 180	2	1	5.42	0.41 - ∞	0.19
All domains	1 - 284	24	62	NA	NA	NA
TNNT2 (cardiac troponin T)						
α-tropomyosin binding	78 - 171	15	33	8.96	3.76 - ∞	**2.85E-06**
Troponin C/I/α-tropomyosin binding	198 - 289	4	93	0.23	0.07 - ∞	1.00
All domains	2 - 298	23	193	NA	NA	NA
DSP (desmoplakin)						
Globular 1	1 - 1056	20	536	1.05	0.62 - ∞	0.48
Plakophilin/plakoglobin binding	1 - 584	15	269	1.78	0.99 - ∞	**0.05**
Rod	1057 - 1945	18	503	0.99	0.57 - ∞	0.57
All Globular 2	1946 - 2871	12	345	0.95	0.50 - ∞	0.61
Globular 2 - domain A	1960 - 2208	0	96	0	0 - ∞	1.00
Globular 2 – domain B	2244 - 2446	3	89	0.93	0.24 - ∞	0.63
Globular 2 – domain C	2609 - 2822	3	73	1.15	0.30 - ∞	0.50
Globular 2 - SR	2824 - 2847	2	14	4.07	0.64 - ∞	0.10
All domains	1 - 2871	50	1384	NA	NA	NA
PLN (phospholamban)						
Cytoplasmic	1 - 31	4	6	0	0 - ∞	1.00

HAX binding	16 - 22	1	3	0.37	0.01 - ∞	0.93
Helical	32 - 52	0	0	0	0 - ∞	1.00
All domains	1 - 52	4	6	NA	NA	NA
DMD (dystrophin)						
Actin binding - CH1	15 - 119	1	16	2.53	0.12 - ∞	0.35
Actin binding - CH2	134 - 237	1	41	0.97	0.05 - ∞	0.65
Spectrin 1	339 - 447	2	88	0.90	0.15 - ∞	0.66
Spectrin 2	448 - 556	6	23	12.82	4.69 - ∞	**4.04E-05**
Spectrin 3	559 - 667	1	58	0.67	0.03 - ∞	0.78
Spectrin 4	719 - 828	2	47	1.74	0.29 - ∞	0.34
Spectrin 5	830 - 934	0	36	0	0 - ∞	1.00
Spectrin 6	943 - 1045	1	45	0.88	0.04 - ∞	0.69
Spectrin 7	1048 - 1154	2	38	2.17	0.36 - ∞	0.26
Spectrin 8	1157 - 1263	1	9	4.53	0.20 - ∞	0.22
Spectrin 9	1266 - 1367	3	42	3.05	0.75 - ∞	0.09
Spectrin 10	1368 - 1463	1	39	1.02	0.05 - ∞	0.64
Spectrin 11	1468 - 1568	2	18	4.66	0.74 - ∞	0.08
Spectrin 12	1571 - 1676	1	60	0.65	0.03 - ∞	0.79
Spectrin 13	1679 - 1778	0	21	0	0 - ∞	1.00
Spectrin 14	1779 - 1874	0	37	0	0 - ∞	1.00
Spectrin 15	1877 - 1979	0	48	0	0 - ∞	1.00
Spectrin 16	1992 - 2101	0	34	0	0 - ∞	1.00
Spectrin 17	2104 - 2208	1	61	0.64	0.03 - ∞	0.80
Spectrin 18	2211 - 2318	0	17	0	0- ∞	1.00
Spectrin 19	2319 - 2423	0	29	0	0 - ∞	1.00
Spectrin 20	2475 - 2577	0	20	0	0 - ∞	1.00
Spectrin 21	2580 - 2686	0	27	0	0 - ∞	1.00
Spectrin 22	2689 - 2802	0	36	0	0 - ∞	1.00
Spectrin 23	2808 - 2930	0	16	0	0 - ∞	1.00
Spectrin 24	2935 - 3040	1	46	0.86	0.04 - ∞	0.70
WW	3055 - 3088	0	1	0	0 - ∞	1.00
Zinc finger	3307 - 3354	0	1	0	0 - ∞	1.00

All domains	1 - 3685	28	1112	NA	NA	NA
LDB3 (LIM domain-binding protein 3)						
PDZ	1-85	0	63	0	0 - ∞	1
LIM zinc-binding 1	549-607	2	40	2.57	0.41 - ∞	0.21
LIM zinc-binding 2	608-667	3	59	2.71	0.66 - ∞	0.13
LIM zinc-binding 3	668-727	1	48	1.00	0.05 - ∞	0.64
All domains	1 - 727	19	914	NA	NA	NA

***** DCM cases include this study (n=532), familial DCM replication cohort (n=101) and patients evaluated in two clinical diagnostic laboratories (reported by Walsh et al.[1]), population control variants were derived from the ExAC database.

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Supplemental References

1. Walsh R, Thomson KL, Ware JS, et al. Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. *Genet Med* 2017;19:192-203.
2. Herman DS, Lam L, Taylor MR, et al. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med* 2012;366:619-628.
3. Roberts AM, Ware JS, Herman DS, et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. *Sci Transl Med* 2015;7:270ra6.
4. Fatkin D, Lam L, Herman DS, et al. Titin truncating mutations: a rare cause of dilated cardiomyopathy in the young. *Prog Ped Cardiol* 2016;40:41-45.
5. Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, et al. Recessive truncating titin gene, *TTN*, mutations presenting as centronuclear myopathy. *Neurology* 2013;81:1205-1214.