SUPPLEMENTARY FILES

Mutations in TRIM63 cause an autosomal recessive form of hypertrophic cardiomyopathy

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Salazar-Mendiguchía J, *et al. Heart* 2020; 106:1342–1348. doi: 10.1136/heartjnl-2020-316913

Additional GENESCOPIC (GENES in Cardiac Outcomes and Prognosis of Inherited Cardiomyopathies) research group collaborators:

Health in Code: Xusto Fernández, Elena Veira, David de Uña, Pablo Iglesias, Noël Brögger, Germán Fernández

Hospital Universitario Puerta de Hierro: Marta Cobo-Marcos, María A. Restrepo-Córdoba, Esther González-López

Saint Bartholomew's Hospital: Luis R. Lopes

Hospital Universitario de Getafe: Jesús Saavedra

Hospital Clínico Universitario de Valladolid: María Sandín-Fuentes, Iria Duro-Aguado

Hospital General Universitario de Alicante: María García Álvarez

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Supplementary Table 1: List of the genes related to inherited cardiovascular disorders and sudden death included in the custom probe library

Gene	Protein name
AARS2	Alanine-tRNA ligase, mitochondrial
ABCC9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
ACAD9	Acyl-CoA dehydrogenase family member 9, mitocondrial
ACADVL	Very long-chain specific acyl-CoA dehydrogenase, mitocondrial
ACTA1	Actin, alfa 1, skeletal muscle
ACTC1	Actin, alpha cardiac muscle 1
ACTN2	Alpha-actinin-2
AGK	Acylglycerol kinase, mitochondrial
AGL	Glycogen debranching enzyme
AGPAT2	1-acyl-sn-glycerol-3-phosphate acyltransferase beta
AKAP9	A-kinase anchor protein 9
ALMS1	Alstrom syndrome protein 1
ANK2	Ankyrin 2
ANK3	Ankyrin-3
ANKRD1	Ankyrin repeat domain-containing protein 1
ANO5	Anoctamin-5
ATP5F1E	ATP synthase subunit epsilon, mitochondrial
ATPAF2	ATP synthase mitochondrial F1 complex assembly factor 2
BAG3	BAG family molecular chaperone regulator 3
BRAF	Serine/threonine-protein kinase B-raf
BSCL2	Seipin
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C
CACNA1D	Voltage-dependent L-type calcium channel subunit alpha-1D
CACNA2D1	Voltage-dependent calcium channel subunit alpha-2/delta-1
CACNB2	Voltage-dependent L-type calcium channel subunit beta-2
CALM1	Calmodulin
CALM2	Calmodulin
CALM3	Calmodulin
CALR3	Calreticulin 3
CAPN3	Calpain-3
CASQ2	Calsequestrin-2
CAV3	Caveolin 3
CAVIN1	Polymerase I and transcript release factor
CAVIN4	Caveolae-associated protein 4
CHRM2	muscarinic acetylcholine receptor M2
COA5	cytochrome c oxidase assembly factor 5

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Gene	Protein name
COA6	cytochrome c oxidase assembly factor 6 homolog
COL7A1	collagen alpha-1(VII) chain
COQ2	4-hydroxybenzoate polyprenyltransferase, mitochondrial
COX15	Cytochrome c oxidase assembly protein COX15 homolog
COX6B1	Cytochrome c oxidase subunit 6B1
CRYAB	Alpha-crystallin B chain
CSRP3	Cysteine and glycine-rich protein 3
CTNNA3	catenin alpha-3
CTNNB1	catenin beta-1
DES	Desmin
DLD	Dihydrolipoyl dehydrogenase, mitochondrial
DMD	Dystrophin
DNAJC19	Mitochondrial import inner membrane translocase subunit TIM14
DNM1L	Dynamin-1-like protein
DOLK	Dolichol kinase
DSC2	Desmocollin 2
DSG2	Desmoglein 2
DSP	Desmoplakin
DTNA	Dystrobrevin alpha
ELAC2	Zinc phosphodiesterase ELAC protein 2
EMD	Emerin
EYA4	Eyes absent homolog 4
FAH	Fumarylacetoacetase
FGF12	Fibroblast growth factor 12
FHL1	Four and a half LIM domains protein 1
FHL2	Four and a half LIM domains 2 (FHL-2)
FHOD3	FH1/FH2 domain-containing protein 3
FKRP	Fukutin-related protein
FKTN	Fukutin
FLNC	Filamin-C
FOXD4	Forkhead box protein D4
FOXRED1	FAD-dependent oxidoreductase domain-containing protein 1
FXN	Frataxin, mitochondrial
GAA	Lysosomal alpha-glucosidase
GATA4	Transcription factor GATA-4
GATA5	Transcription factor GATA-5
GATA6	Transcription factor GATA-6
GATAD1	GATA zinc finger domain-containing protein 1
GFM1	Elongation factor G, mitochondrial
GJA1	Gap junction alpha-1 protein

Gene	Protein name
GJA5	Gap junction alpha-5 protein
GLA	Alpha galactosidase A
GLB1	Beta-galactosidase
GNPTAB	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta
GPD1L	Glycerol-3-phospate dehydrogenase 1-like protein
GREM2	Gremlin-2
GUSB	Beta-glucuronidase
HCN4	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4
HFE	Hereditary hemochromatosis protein
HRAS	GTPase HRas
IDH2	isocitrate dehydrogenase [NADP], mitochondrial
ILK	Integrin-linked protein kinase
IRX3	iroquois-class homeodomain protein IRX-3
JPH2	Junctophilin 2
JUP	Junction plakoglobin
KCNA5	Potassium voltage-gated channel subfamily A member 5
KCND2	Potassium voltage-gated channel subfamily D member 2
KCND3	Potassium voltage-gated channel subfamily D member 3
KCNE1	Potassium voltage-gated channel subfamily E member 1
KCNE2	Potassium voltage-gated channel subfamily E member 2
KCNE3	Potassium voltage-gated channel subfamily E member 3
KCNE5	Potassium voltage-gated channel subfamily E member 1-like protein
KCNH2	Potassium voltage-gated channel subfamily H member 2
KCNJ2	Inward rectifier potassium channel 2
KCNJ5	G protein-activated inward rectifier potassium channel 4
KCNJ8	ATP-sensitive inward rectifier potassium channel 8
KCNK17	Potassium channel subfamily K member 17
KCNK3	potassium channel subfamily K member 3
KCNQ1	Potassium voltage-gated channel subfamily KQT member 1
KLF10	Krueppel-like factor 10
KRAS	GTPase KRas
LAMA2	Laminin subunit alpha-2
LAMA4	Laminin subunit alpha-4
LAMP2	Lysosome-associated membrane glycoprotein 2
LDB3	LIM domain-binding protein 3
LDLR	Low density lipoprotein receptor
LIAS	Lipoyl synthase, mitochondrial
LMNA	Prelamin-A/C
LZTR1	Leucine-zipper-like transcriptional regulator 1

Gene	Protein name
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1
MAP2K2	Dual specificity mitogen-activated protein kinase kinase 2
MIB1	E3 ubiquitin-protein ligase MIB1
MLYCD	Malonyl-CoA decarboxylase, mitochondrial
MRPL3	39S ribosomal protein L3, mitochondrial
MRPL44	39S ribosomal protein L44, mitochondrial
MRPS22	28S ribosomal protein S22, mitochondrial
MTO1	Protein MTO1 homolog, mitochondrial
MYBPC3	Myosin-binding protein C, cardiac-type
MYH11	Myosin-11
MYH6	Myosin-6
MYH7	Myosin-7
MYL2	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform
MYL3	Myosin light chain 3
MYLK2	Myosin light chain kinase 2, skeletal/cardiac muscle
MYOM1	Myomesin-1
MYOT	Myotilin
MYOZ2	Myozenin-2
MYPN	Myopalladin
NEBL	Nebulette
NEXN	Nexilin
NF1	Neurofibromin
NKX2-5	Homeobox protein Nkx-2.5
NKX2-6	Homeobox protein Nkx-2.6
NNT	NAD(P) transhydrogenase, mitochondrial
NOS1AP	Carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase protein
NOTCH1	Neurogenic locus notch homolog protein 1
NPPA	Atrial natriuretic factor
NRAS	GTPase NRas
OBSCN	Obscurin
OBSL1	Obscurin-like protein 1
OPA3	optic atrophy 3 protein
PDHA1	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial
PDLIM3	PDZ and LIM domain protein 3
PERP	p53 apoptosis effector related to PMP-22
PHKA1	Phosphorylase b kinase regulatory subunit alpha, skeletal muscle isoform
PITX2	Pituitary homeobox 2
PKP2	Plakophilin 2
PKP4	Plakophilin 4

Gene	Protein name
PLN	Cardiac phospholamban
PMM2	Phosphomannomutase 2
PPP1R13L	relA-associated inhibitor
PRDM16	PR domain zinc finger protein 16
PRKAG2	5'-AMP-activated protein kinase subunit gamma-2
PSEN1	Presenilin-1
PSEN2	Presenilin-2
PTPN11	Tyrosine-protein phosphatase non-receptor type 11
RAF1	RAF proto-oncogene serine/threonine-protein kinase
RANGRF	Ran guanine nucleotide release factor
RASA2	ras GTPase-activating protein 2
RBM20	Probable RNA-binding protein 20
RIT1	GTP-binding protein Rit1
RRAS	ras-related protein R-Ras
RYR2	Ryanodine receptor 2
SCN10A	sodium channel protein type 10 subunit alpha
SCN1B	Sodium channel subunit beta-1
SCN2B	Sodium channel subunit beta-2
SCN3B	Sodium channel subunit beta-3
SCN4B	Sodium channel subunit beta-4
SCN5A	Sodium channel protein type 5 subunit alpha
SCO2	Protein SCO2 homolog, mitochondrial
SDHA	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
SGCA	Alpha-sarcoglycan
SGCB	Beta-sarcoglycan
SGCD	Delta-sarcoglycan
SHOC2	Leucine-rich repeat protein SHOC-2
SLC22A5	Solute carrier family 22 member 5
SLC25A3	phosphate carrier protein, mitochondrial
SLC25A4	ADP/ATP translocase 1
SLMAP	Sarcolemmal membrane-associated protein
SNTA1	Alpha-1-syntrophin
SOS1	Son of sevenless homolog 1
SOS2	Son of sevenless homolog 2
SPEG	Striated muscle preferentially expressed protein kinase
SPRED1	Sprouty-related, EVH1 domain-containing protein 1
SRY	Sex-determining region Y protein
SURF1	Surfeit locus protein 1
SYNE1	Nesprin-1
SYNE2	Nesprin-2
TAZ	Tafazzin

Gene	Protein name
TBX20	T-box transcription factor TBX20
TBX5	T-box transcription factor TBX5
TCAP	Telethonin
TGFB3	Transforming growth factor, beta 3
TMEM43	Transmembrane protein 43
TMEM70	Transmembrane protein 70, mitochondrial
TMPO	Thymopoietin
TNNC1	Troponin C, slow skeletal and cardiac muscles
TNNI3	Troponin I, cardiac muscle
TNNI3K	Serine/threonine-protein kinase TNNI3K
TNNT2	Troponin T, cardiac muscle
TOR1AIP1	torsin-1A-interacting protein 1
TPM1	Tropomyosin alpha-1 chain
TRDN	Triadin
TRIM63	E3 ubiquitin-protein ligase TRIM63
TRPM4	Transient receptor potential cation channel subfamily M member 4
TSFM	Elongation factor Ts, mitochondria
TTN	Titin
TTR	Transthyretin
TXNRD2	Thioredoxin reductase 2, mitochondrial
VCL	Vinculin
XK	Membrane transport protein XK
ZFHX3	Zinc finger homeobox protein 3
ZFPM2	Zinc finger homeobox protein 3

Supplementary Table 2: Allele frequencies according to gnomAD (v2.1.1), bioinformatic predictors and ACMG classification*.

Protein	cDNA	Allele count gnomAD	Allele number gnomAD	Number of homozygous carriers gnomAD	MAF gnomAD	Polyphen 2	Mutation taster	SIFT	DANN	FATHMM	CADD	ClinVar	Variant classification* (without cosegregation)
p.(Cys23Tyr)	c.68G>A	8	251384	**0**	0.00003	Probably damaging/ 1	Disease-causing / 1	Damaging/ 0	0.997661	0.99477	6.509411	N/A	Likely pathogenic (PS4, PM1,PM2, PP3)
p.(Leu37Val)	c.109T>G	0			0	Probably damaging/ 1	Disease-causing / 0.99	Damaging/ 0	0.997858	0.98309	4.546217	N/A	VUS (PM2, PP3)
p.(Cys75Tyr)	c.224G>A	28	281352	**0**	0.00009	Probably damaging/ 1	Disease-causing / 1	Damaging/ 0	0.997589	0.99484	6.505493	1 VUS	Likely pathogenic (PM1, PM2, PM3, PP3)
p.(Cys145Tyr)	c.434G>A	6	251436	**0**	0.00002	Probably damaging/ 1	Disease-causing / 1	Damaging/ 0	0.997688	0.97016	6.2988	N/A	VUS (PM1,PM2, PP3)
p.(Lys146Thrfs*24)	c.437_442delAGGTGTinsCC	0			0		Disease-causing / 1					N/A	Likely pathogenic (PS4, PM2, PM3, PP3)
p.(Ser161Cysfs*8)	c.481_482delAG	12	282386	**0**	0.00004		Disease-causing / 1				8.107172	N/A	Likely pathogenic (PS4, PM2, PM3, PP3)
p.(Gln247*)	c.739C>T	192	282876	**0**	0.0006		Disease-causing / 1	Tolerated 0.96	0.997504	0.97216	11.872989	1 LB 1 VUS 2 LP	Likely pathogenic (PS3, PM2, PP3)
p.(Leu319Pro)	c.956T>C	24	251442	**0**	0.00009	Probably damaging/ 1	Disease-causing / 1	Damaging/ 0	0.99895	0.99895	5.621389	N/A	VUS (PM2, PP3)

*Note: Care should be taken when applying these rules, as the ACMG guidelines are not intended to be applied in the setting of candidate gene studies.

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Supplementary Table 3. Clinical and genetic characteristics of index cases and evaluated family members.

Family	Subject	Gender	TRIM63 variants	Decade at diagnosis or last FU	Phenotype	LVMWT (mm)	EF (%)	Symptoms at first evaluation	ECG	Events	Additional genetic variants
H8343	**II.2**	F	p.(Cys23Tyr) +/+	20s/40s	HCM	20	68	Asymptomatic	SR, LVHC Holter: NSVT	CVE	
	II.4	M	p.(Cys23Tyr) +/+	20s/40s	HCM	28	70	Asymptomatic	SR, LVHC Holter: NSVT		
	II.6	F	Non-carrier	40s	Normal	8	65	Asymptomatic	SR		
	I.1	M	p.(Cys23Tyr) +/-	60s	Hypertensive heart disease	15	62	Asymptomatic	SR. No LVHC		
	I.2	F	p.(Cys23Tyr) +/-	60s	Normal	12	60	Asymptomatic	SR		
	III.1	F	p.(Cys23Tyr) +/-	10s	Normal	8	65	Asymptomatic	SR		
	III.2	F	?	10s	Normal	7	64	Asymptomatic	SR		
H8758	**II.11**	M	p.(Leu319Pro) +/+	20s/40s	HCM	22	70	Asymptomatic	SR		COQ2 p.(Gln94Hisfs*78) +/-
	II.6	F	p.(Leu319Pro) +/+	40s	Possibly affected	9	68	Asymptomatic	SR, ATWI + LVHC		
	I.1	M	p.(Leu319Pro) +/-	80s	Normal	10	60	Asymptomatic	SR	TIA	
	I.2	F	p.(Leu319Pro) +/-	70s	Normal	10	57	Asymptomatic	SR		
	II.2	M	p.(Leu319Pro) +/-	50s	Normal	11	67	Asymptomatic	SR		
	II.3	F	p.(Leu319Pro) +/-	50s	Normal	8	60	Asymptomatic	SR		
	II.4	F	p.(Leu319Pro) +/-	40s	Normal	8	58	Asymptomatic	SR		
	II.7	F	p.(Leu319Pro) +/-	40s	Normal	9	62	Asymptomatic	SR		
	II.9	M	Non-carrier	40s	Normal	8	64	Asymptomatic	SR		
	II.13	M	p.(Leu319Pro) +/-	30s	Normal	11	60	Asymptomatic	SR		
	II.14	M	p.(Leu319Pro) +/-	30s	Normal	9	65	Asymptomatic	SR		
	II.15	F	p.(Leu319Pro) +/-	30s	Normal	9	65	Asymptomatic	SR		
	III.3	M	?	20s	Normal	9	60	Asymptomatic	SR		
	III.4	M	?	20s	Normal	8	63	Asymptomatic	SR		
	III.6	F	Obligate carrier	10s	Normal	6	60	Asymptomatic	SR		

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H5180	**II.2**	F	p.(Cys23Tyr) +/+	50s/70s	HCM	20	65	Heart Failure (NYHA II)	Second-degree AV block + RBBB		RYR2 p.(His464Gln) +/- TTN p.(Gly16395Glu) +/-
	II.9	F	p.(Cys23Tyr) +/-	60s	Normal	9	60	Asymptomatic	SR		
	III.1	M	p.(Cys23Tyr) +/-	50s	Hypertensive heart disease	13	67	Asymptomatic	SR No LVHC		
	III.2	M	p.(Cys23Tyr) +/-	30s	Normal	11	60	Asymptomatic	SR		
	II.7	M	?	30s/40s	End-stage HCM			Advanced Heart Failure (NYHA IV)		Heart transplant (age 38) Non-CV death (42,hepatocellular carcinoma)	
H10022	**II.2**	M	p.(Gln247*) +/+	20s/30s	HCM	16	56	Palpitations	SR. LVHC Holter: PVC		OBSCN p.(Leu7883Arg) +/-
	II.1	M	p.(Gln247*) +/+	30s	Possibly affected	12	64	Asymptomatic	SR. LVHC		
	II.3	F	Non-carrier	10s	Normal	8	68	Asymptomatic	SR		
	I.1	M	p.(Gln247*) +/-	60s	Normal	11	68	Asymptomatic	SR		
	I.2	F	p.(Gln247*) +/-	50s	Normal	11	65	Asymptomatic	SR		
H2523	**II.2**	M	p.(Lys146Thrfs*24) +/+	40s/70s	End-stage HCM	16	43	Heart Failure (NYHA III)	AF	TIA	MYBPC3 p.(Tyr213*) +/-
	III.1	F	p.(Lys146Thrfs*24) +/-	40s	Normal	10	70	Asymptomatic	SR		MYBPC3 p.(Tyr213*) **-/-**
	III.2	F	p.(Lys146Thrfs*24) +/-	30s	Normal	8	70	Asymptomatic	SR		MYBPC3 p.(Tyr213*) **-/-**
	III.3	F	p.(Lys146Thrfs*24) +/-	30s	Normal	8	65	Asymptomatic	SR		MYBPC3 p.(Tyr213*) **-/-**
H6897	**II.3**	M	p.(Gln247*) +/+	10s/30s	HCM	19	65	Syncope	SR		KCNE1 p.(Arg36His) +/- TCAP p.(Cys38Phe) +/-
	I.1	M	Obligate carrier	50s	Hypertensive heart disease	13	60	Asymptomatic	SR		
H6438	**II.5**	F	p.(Cys23Tyr) +/+	60s/70s	End-stage HCM	12	43	Heart Failure (NYHA II)	SR. LVHC Holter: AF bursts		
	III.1	F	p.(Cys23Tyr) +/-	50s	Normal	8	60	Asymptomatic	SR		

	III.3	M	p.(Cys23Tyr) +/-	40s	Normal	10	69	Asymptomatic	SR		
H997	**II.2**	F	p.(Gln247*) +/- p.(Lys146Thrfs*24) -/+	10s/30s	HCM	24	74	Asthenia	SR. LVHC		DTNA p.(Val174Ile) +/-
	I.1	M	p.(Gln247*) -/- p.(Lys146Thrfs*24) -/+	60s	Normal	12	65	Asymptomatic	SR		
	I.2	F	p.(Gln247*) +/- p.(Lys146Thrfs*24) -/-	50s	Normal	11	62	Asymptomatic	SR		
	II.3	F	p.(Gln247*) +/- p.(Lys146Thrfs*24) -/-	30s	Normal	9	60	Asymptomatic	SR		
H3112	**II.7**	M	p.(Cys75Tyr) +/- p.(Gln247*) -/+	30s/30s	HCM	23	76	Asymptomatic	SR, LVHC		
	II.5	M	p.(Cys75Tyr) +/- p.(Gln247*) -/+	30s	HCM	18	71	Asymptomatic	SR. LVHC		
	II.2	M	?	40s	Normal	11	68	Asymptomatic	SR		
	II.3	M	?	40s	Normal	11	69	Asymptomatic	SR		
	II.4	M	p.(Gln247*) -/+	30s	Normal	9	61	Asymptomatic	SR		
	II.8	F	Non-carrier	20s	Normal	9	65	Asymptomatic	SR		
	I.2	F	p.(Cys75Tyr) +/-	60s	Hypertensive heart disease	13	63	Asymptomatic	SR		
H77085	**II.5**	M	p.(Ser161Cysfs*8) +/+	30s/50s	HCM	17	59	Angina + Heart Failure (NYHA II)	SR. LVHC Holter: NSVT		
	III.2	M	?	60s	Normal	12	67	Asymptomatic	SR		
H8565	**II.1**	M	p.(Cys145Tyr) +/+	50s/50s	RCM	12	55	Palpitations Heart Failure (NYHA II)	SR. LVHC		DSC2 p.(Val129Ile) +/-
	II.2	F	p.(Cys145Tyr) +/-	50s	Normal	6	67	Asymptomatic	SR		
	II.3	F	p.(Cys145Tyr) +/-	50s	Normal	7	72	Asymptomatic	SR		
	II.4	M	p.(Cys145Tyr) +/-	40s	Normal	11	72	Asymptomatic	SR		
FT3104	**II.2**	M	p.(Gln247*) +/+	20s/40s	HCM	21	34	Heart Failure (NYHA II)	SR. LVHC Holter: NSTV	TIA	

	II.1	M	p.(Gln247*) +/-	40s	Normal	11	66	Asymptomatic	SR		
	I.1	M	p.(Gln247*) +/-	60s	Normal	12	64	Asymptomatic	SR		
	I.2	F	p.(Gln247*) +/-	60s	Normal	10	61	Asymptomatic	SR		
H83562	**II.2**	F	p.(Cys75Tyr) +/- p.(Leu37Val) -/+	40s/50s	HCM	17	60	Angina (CCS II) Heart Failure (NYHA II)	SR, LVHC		
	III:1	M	p.(Cys75Tyr) +/-	20s	Normal	11	56	Asymptomatic	SR		
H5850	**II.2**	M	p.(Cys75Tyr) +/+	30s/60s	HCM	21	62	Heart Failure (NYHA II)	Paced	Inappropriate ICD discharge	TCAP p.(Ala118Val) +/-
	III:2	M	?	40s	Normal	11	60	Asymptomatic	SR		
	III:3	F	?	40s	Normal	10	64	Asymptomatic	SR		
	III:4	F	?	30s	Normal	10	59	Asymptomatic	SR		
	II:1	F	?	80s	Normal	12	60	Asymptomatic	SR		
H4094	**II:7**	M	p.(Ser161Cysfs*8) +/+	20s/30s	HCM (ASH)	29	48	Heart Failure (NYHA II)	Paced	Inappropriate ICD discharge	TTN p.(Tyr10050Cys) +/- TPM1 c.375-3C>T +/-
	II:3	F	Non-carrier	50s	Normal	9	60	Asymptomatic	SR		
	II:4	F	p.(Ser161Cysfs*8) +/-	40s	Normal	11	60	Asymptomatic	SR		
	II:5	M	Non-carrier	40s	Normal	10	68	Asymptomatic	SR		
	II:6	M	Non-carrier	40s	Normal	10	65	Asymptomatic	SR		
H12238	**II:1**	M	p.(Gln247*) +/+	40s	HCM	28	60	Asymptomatic	SR		

Note: Subjects in bold correspond to index cases.

ATWI = anterior T-wave inversion; FU = follow-up; f = females; m = males; ? = unknown genotype; HCM = hypertrophic cardiomyopathy; RCM = restrictive cardiomyopathy; SR = sinus rhythm; LVHC = left ventricular hypertrophy criteria; RBBB = right bundle branch block; CVE = cerebrovascular event; TIA = transient ischemic attack; ICD = implantable cardioverter-defibrillator; PVC = premature ventricular contractions; +/- = heterozygous; +/+ = homozygous

Supplementary Table 4: Likelihood of the odds (LOD) score reflecting linkage between *TRIM63* mutations and HCM in individual families.

Family ID	TRIM63 Variant(s)	Family members	Genotyped	Homozygous/ comp. heterozygous	LOD score
H8758	p.Leu319Pro	27	14	2	0.99
H12238	p.Gln247*	7	0	1	0.00
H2768	p.Gln247*	5	2	2	0.73
H83562	p.Cys75Tyr p.Leu37Val	7	1	1	0.00
H4094	p.Ser161Cysfs*8	14	5	1	0.50
H5850	p.Cys75Tyr	10	1	1	0.00
FT3104	p.Gln247*	7	4	1	0.12
H8565	p.Cys145Tyr	6	4	1	0.37
H77085	p.Ser161Cysfs*8	15	1	1	0.00
H3112	p.Cys75Tyr p.Gln247*	17	5	2	0.55
H997	p.Gln247* p.Lys146Thrfs*24	6	4	1	0.12

H3438	p.Cys23Tyr	13	3	1	0.01
H6897	p.Gln247*	8	1	1	0.00
H2523	p.Lys146Thrfs*24 p.Tyr213*	10	4	1	0.01
H5180	p.Cys23Tyr	20	4	1	0.12
H8343	p.Cys23Tyr	12	6	2	0.73
		TOTAL LODs SCORE			**4.25**

LOD score: Logarithms of the odds score calculated for a disease penetrance of 95% (recessive).

Supplementary Table 5. Detailed clinical characteristics of homozygous/compound heterozygous individuals

Family	**Subject**	**Gender**	***TRIM63* variant**	**Decade at diagnosis/ last follow up**	**Diagnosis**	**Reason for diagnosis**	**NYHA functional class**	**LVEDD (mm)**	**LVMWT (mm)**	**LA (mm)**	**EF (%)**	**ECG/ Holter**	**CK level**	**ICD or PM**	**Events**	**Others**
H8343	II.2	F	p.(Cys23Tyr)	20s/40s	HCM (ASH)	Abnormal ECG	I	47	20	40	68	SR, LVHC. NSVT	90		CVE	Pending ICD implantation.
	II.4	M	p.(Cys23Tyr)	20s/40s	HCM (ASH)	Family screening	I	40	28	43	70	SR, LVHC. NSVT		ICD		
H8758	II.11	M	p.(Leu319Pro)	20s/40s	HCM (ASH)	Abnormal ECG	I	52	22		70	SR. LVHC. NSVT	83		TIA	
	II.6	F	p.(Leu319Pro)	40s	Possibly affected (HCM)	Family screening	I	44	9		68	SR, LVHC AnteriorTWI				
H5180	II.2	F	p.(Cys23Tyr)	50s/70s	HCM (ASH)	Dyspnea	II	51	20	43	65	SR. AsVp		PM		Symptomatic second-degree

Family	Subject	Gender	*TRIM63* variant	Decade at diagnosis/ last follow up	Diagnosis	Reason for diagnosis	NYHA functional class	LVEDD (mm)	LVMWT (mm)	LA (mm)	EF (%)	ECG/ Holter	CK level	ICD or PM	Events	Others
																AV block.
H10022	II.2	M	p.(Gln247*)	20s/30s	HCM (concentric)	Palpitations	I	50	16	37	56	SR, LVHC. Isolated VPCs.	490			Normal neurological examination. Normal MRI of limbs.
	II.1	M	p.(Gln247*)	30s	Possibly affected (HCM)	Family screening	I	48	12	35	64	SR, LVHC. Lateral TWI	400			
H2523	II.2	M	p.(Lys146Thrfs*24)	40s/70s	End-stage HCM (concentric)	Dyspnea	III	60	16	51	33	AF Vp		CRT-D	TIA at age 65	AF with uncontrollable ventricular response. AV node

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Family	Subject	Gender	*TRIM63* variant	Decade at diagnosis/ last follow up	Diagnosis	Reason for diagnosis	NYHA functional class	LVEDD (mm)	LVMWT (mm)	LA (mm)	EF (%)	ECG/ Holter	CK level	ICD or PM	Events	Others
																ablation. Carrier or the *MYBPC3* p.(Tyr213*) variant.
H6897	II.3	M	p.(Gln247*)	10s/30s	HCM (concentric)	Syncope	I	47	20	49	65	SR, LVHC. NSVT	205	ICD		Normal CT of limbs. Normal muscle biopsy.
H6438	II.5	F	p.(Cys23Tyr)	60s/70s	End-stage HCM	Dyspnea	II	57	12		43	SR, LVHC. AF bursts				LVMWT 17 mm at first evaluation.
H997	II.2	F	p.(Gln247*) + p.(Lys146Thrfs*24)	10s/30s	HCM (ASH)	Asthenia	I	43	24	40	74	SR, LVHC.	229			

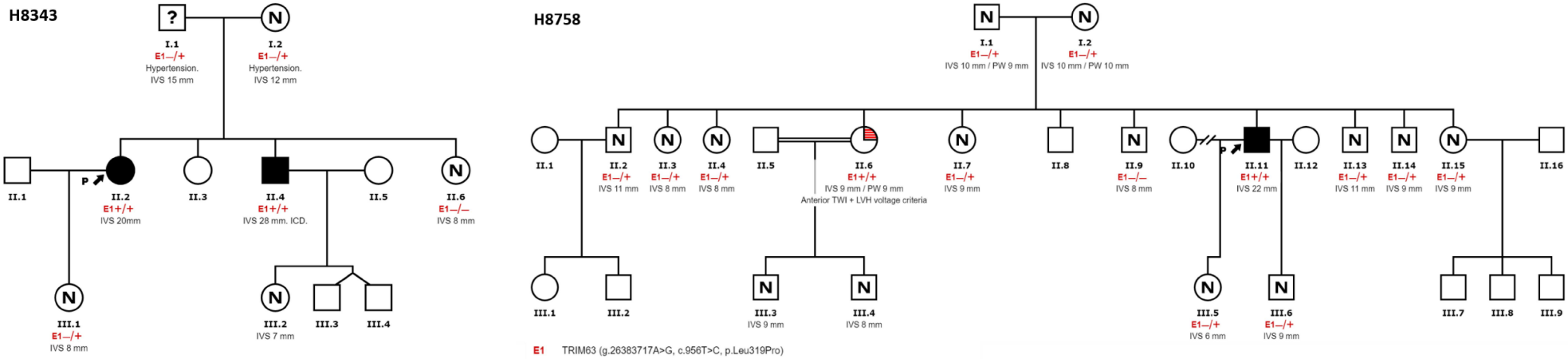
Family	Subject	Gender	*TRIM63* variant	Decade at diagnosis/ last follow up	Diagnosis	Reason for diagnosis	NYHA functional class	LVEDD (mm)	LVMWT (mm)	LA (mm)	EF (%)	ECG/ Holter	CK level	ICD or PM	Events	Others
H3112	II.7	M	p.(Cys75Tyr) + p.(Gln247*)	30s/30s	HCM (concentric)	Abnormal ECG	I	54	23	49	76	SR, LVHC.	70			
	II.5	M	p.(Cys75Tyr) + p.(Gln247*)	30s	HCM (concentric)	Family Screening	I	42	18	37	71	SR, LVHC.				
H77085	II.5	M	p.(Ser161Cysfs*8)	30s/50s	End-stage HCM	Angina	II	35	17	46	59	SR, LVHC. NSVT	100	ICD		LVMWT 33 mm at first evaluation.
H8565	II:1	M	p.(Cys145Tyr)	50s/50s	RCM	Palpitations	II	52	12	55	55	SR, LVHC. NSVT				Elevated filling pressures. PH.
FT3104	II.2	M	p.(Gln247*)	20s/40s	End-stage HCM	Syncope	II	61	21	43	34	SR, LVHC. NSVT	220	ICD	TIA at age 40	Apical aneurysm. Normal CT of limbs. Normal neurological

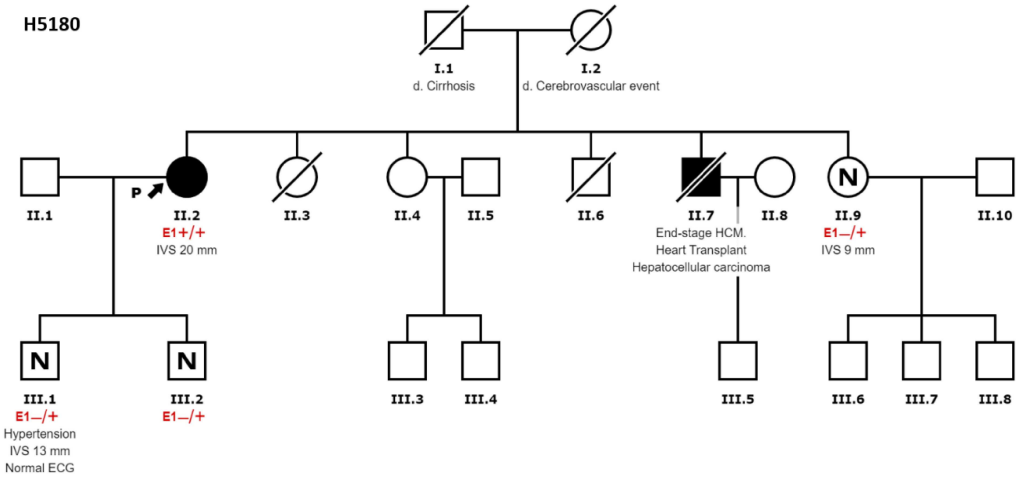
Family	**Subject**	**Gender**	***TRIM63* variant**	**Decade at diagnosis/ last follow up**	**Diagnosis**	**Reason for diagnosis**	**NYHA functional class**	**LVEDD (mm)**	**LVMWT (mm)**	**LA (mm)**	**EF (%)**	**ECG/ Holter**	**CK level**	**ICD or PM**	**Events**	**Others**
																examination.
H83562	II.2	F	p.(Cys75Tyr) + p.(Leu37Val)	40s/50s	HCM (ASH)	Angina	II	47	17	37	60	SR, LVHC.				
H5850	II.2	M	p.(Cys75Tyr)	30s/60s	HCM (ASH)	Palpitations	II	60	21	43	62	AF, NSVT, SVT		ICD	Inappropriate ICD discharge	ICD for secondary prevention (sustained VT).
H4094	II.7	M	p.(Ser161Cysfs*8)	20s/30s	End-stage HCM	Abnormal ECG	II	59	29	51	48	SR, LVHC. NSVT	638	ICD	Inappropriate ICD discharge	
H12238	II:1	M	p. (Gln247*)	40s	HCM	Abnormal ECG	I	43	28	37	60	SR	203			Normal neurological examination.

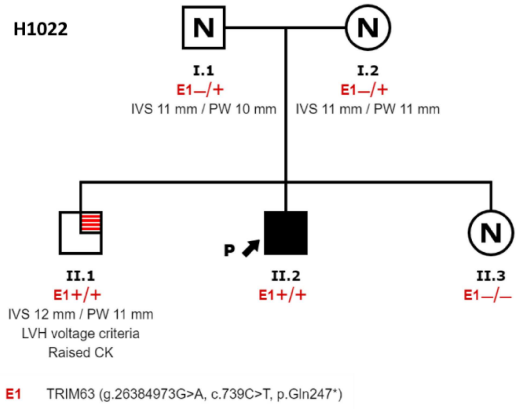
AF = atrial fibrillation; CT = computed tomography; CVE = cerebrovascular event; HCM = hypertrophic cardiomyopathy; ICD = implantable cardioverter-defibrillator; LVHC = left ventricular hypertrophy criteria; PH = pulmonary hypertension; RBBB = right bundle branch block; RCM = restrictive cardiomyopathy; SR = sinus rhythm; TIA = transient ischemic attack; TWI = T-wave inversion; VT = ventricular tachycardia

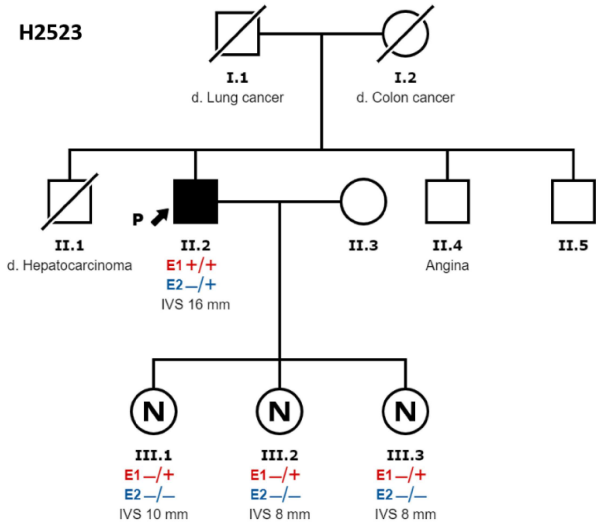
Salazar-Mendiguchía J, *et al. Heart* 2020; 106:1342–1348. doi: 10.1136/heartjnl-2020-316913

Supplementary Figure 1: Pedigrees of the evaluated families



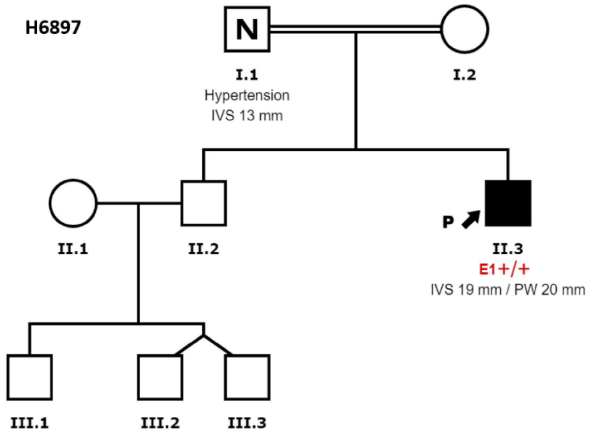

H5180
I.1
d. Cirrhosis
I.2
d. Cerebrovascular event
II.1
P
II.2
E1+/+
IVS 20 mm
II.3
II.4
II.5
II.6
II.7
End-stage HCM.
Heart Transplant
Hepatocellular carcinoma
II.8
N
II.9
E1–/+
IVS 9 mm
II.10
N
III.1
E1–/+
Hypertension
IVS 13 mm
Normal ECG
N
III.2
E1–/+
III.3
III.4
III.5
III.6
III.7
III.8
E1 TRIM63 (g.26393918C>T, c.68G>A, p.Cys23Tyr)


H1022
N
I.1
E1–/+
IVS 11 mm / PW 10 mm
N
I.2
E1–/+
IVS 11 mm / PW 11 mm
II.1
E1+/+
IVS 12 mm / PW 11 mm
LVH voltage criteria
Raised CK
P
II.2
E1+/+
N
II.3
E1–/–
E1 TRIM63 (g.26384973G>A, c.739C>T, p.Gln247*)

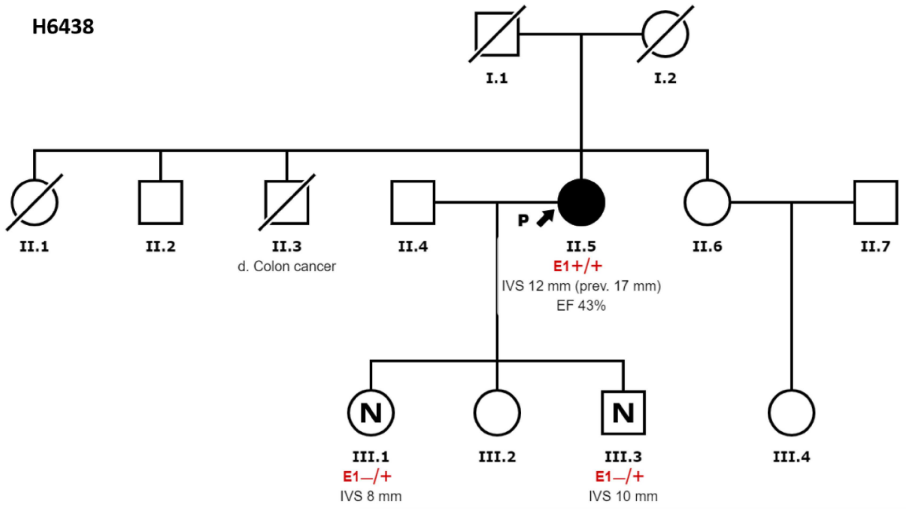


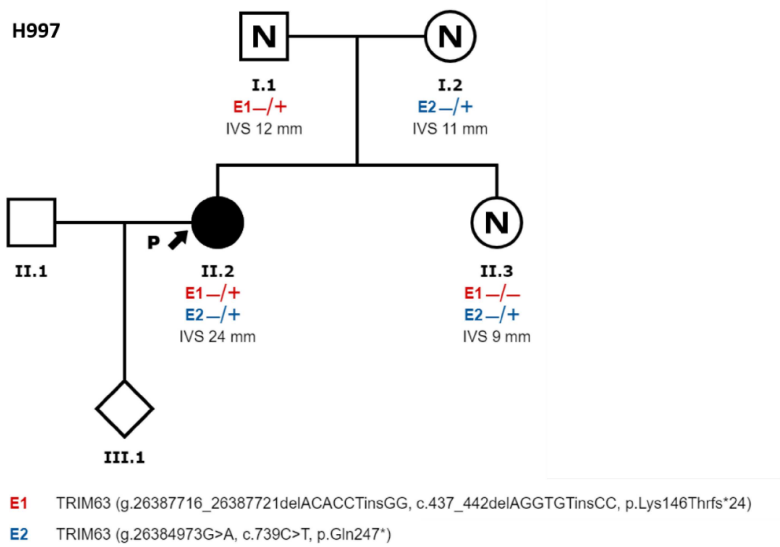
E1 TRIM63 (g.26387716_26387721delACACCTinsGG, c.437_442delAGGTGTinsCC, p.Lys146Thrfs*24)

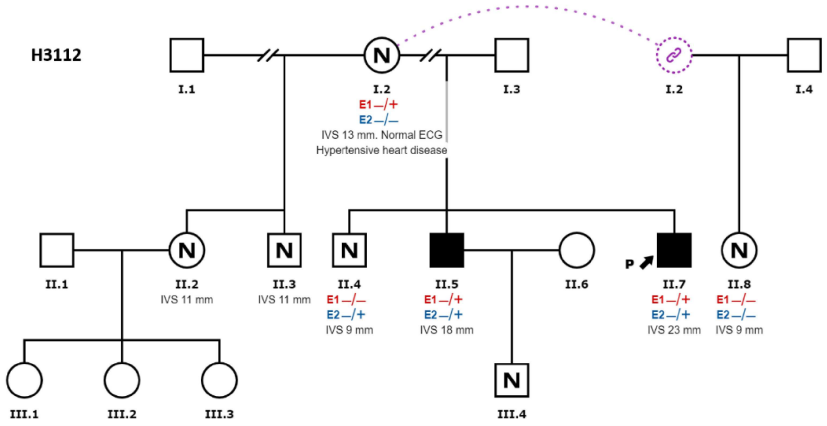
E2 MYBPC3 (g.47371340G>C, c.639C>G, p.Tyr213*)

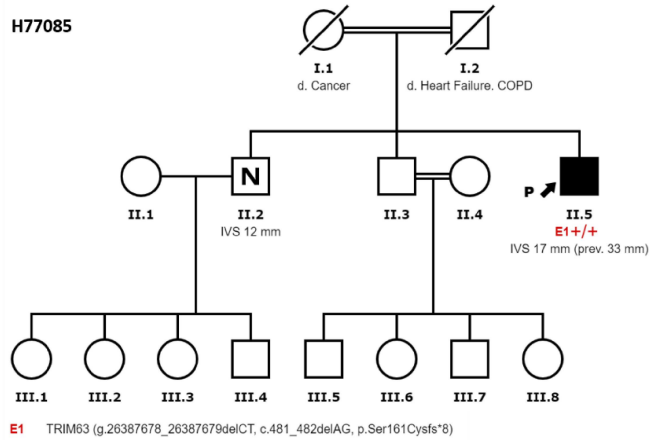


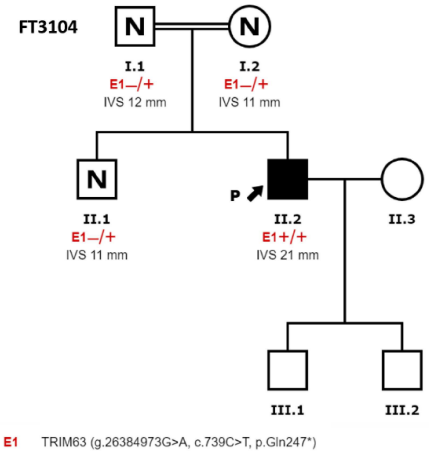
E1 TRIM63 (g.26384973G>A, c.739C>T, p.Gln247*)

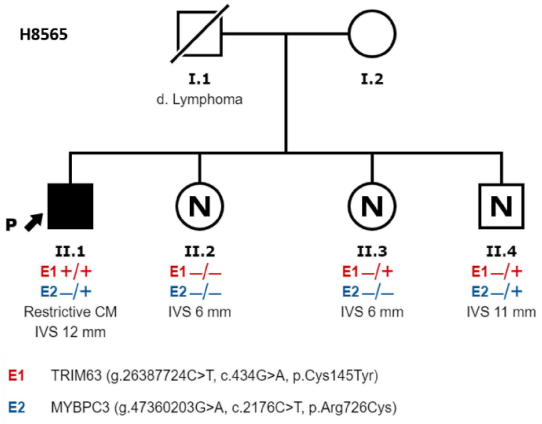

H6438
I.1
I.2
II.1
II.2
II.3
d. Colon cancer
II.4
P
II.5
E1+/+
IVS 12 mm (prev. 17 mm)
EF 43%
II.6
II.7
N
III.1
E1–/+
IVS 8 mm
III.2
N
III.3
E1–/+
IVS 10 mm
III.4
E1 TRIM63 (g.26393918C>T, c.68G>A, p.Cys23Tyr)

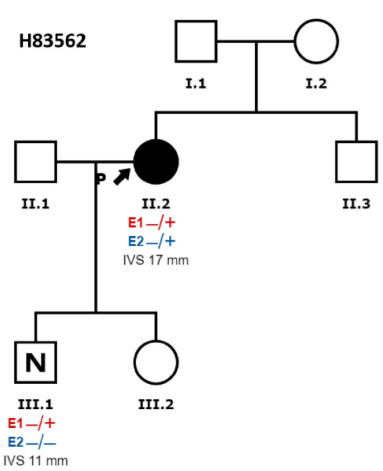

H997
N
I.1
E1–/+
IVS 12 mm
N
I.2
E2–/+
IVS 11 mm
II.1
P
II.2
E1–/+
E2–/+
IVS 24 mm
N
II.3
E1–/–
E2–/+
IVS 9 mm
III.1
E1 TRIM63 (g.26387716_26387721delACACCTinsGG, c.437_442delAGGTGTinsCC, p.Lys146Thrfs*24)
E2 TRIM63 (g.26384973G>A, c.739C>T, p.Gln247*)


H3112
I.1
I.2
E1 –/+
E2 –/–
IVS 13 mm. Normal ECG
Hypertensive heart disease
I.3
I.2
I.4
II.1
II.2
IVS 11 mm
II.3
IVS 11 mm
II.4
E1 –/–
E2 –/+
IVS 9 mm
II.5
E1 –/+
E2 –/+
IVS 18 mm
II.6
II.7
E1 –/+
E2 –/+
IVS 23 mm
II.8
E1 –/–
E2 –/–
IVS 9 mm
III.1
III.2
III.3
III.4
E1 TRIM63 (g.26392867C>T, c.224G>A, p.Cys75Tyr)
E2 TRIM63 (g.26384973G>A, c.739C>T, p.Gln247*)


H77085
I.1
d. Cancer
I.2
d. Heart Failure. COPD
II.1
II.2
IVS 12 mm
II.3
II.4
II.5
E1+/+
IVS 17 mm (prev. 33 mm)
III.1
III.2
III.3
III.4
III.5
III.6
III.7
III.8
E1 TRIM63 (g.26387678_26387679delCT, c.481_482delAG, p.Ser161Cysfs*8)

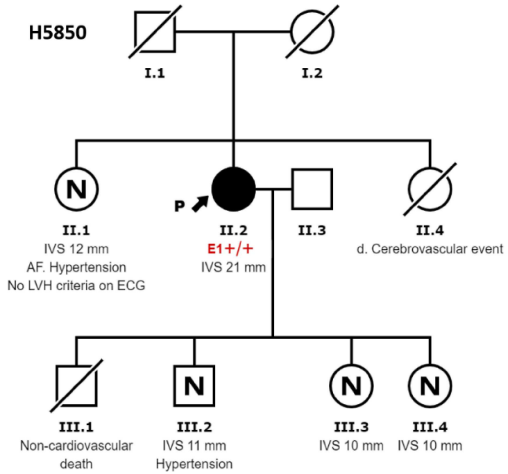

FT3104
I.1
E1–/+
IVS 12 mm
I.2
E1–/+
IVS 11 mm
II.1
E1–/+
IVS 11 mm
II.2
E1+/+
IVS 21 mm
II.3
III.1
III.2
E1 TRIM63 (g.26384973G>A, c.739C>T, p.Gln247*)


H8565
I.1
d. Lymphoma
I.2
II.1
E1+/+
E2 –/+
Restrictive CM
IVS 12 mm
II.2
E1 –/–
E2 –/–
IVS 6 mm
II.3
E1 –/+
E2 –/–
IVS 6 mm
II.4
E1 –/+
E2 –/+
IVS 11 mm
E1 TRIM63 (g.26387724C>T, c.434G>A, p.Cys145Tyr)
E2 MYBPC3 (g.47360203G>A, c.2176C>T, p.Arg726Cys)

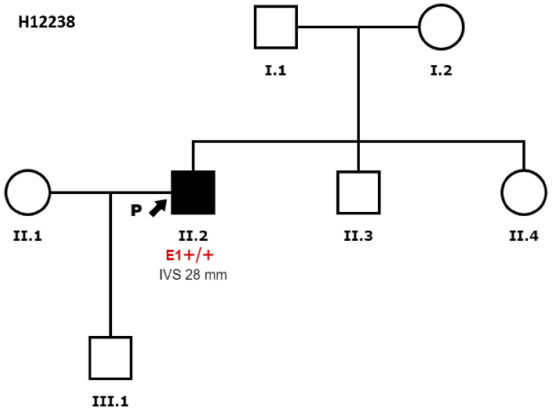


E1 TRIM63 (g.26392867C>T, c.224G>A, p.Cys75Tyr)

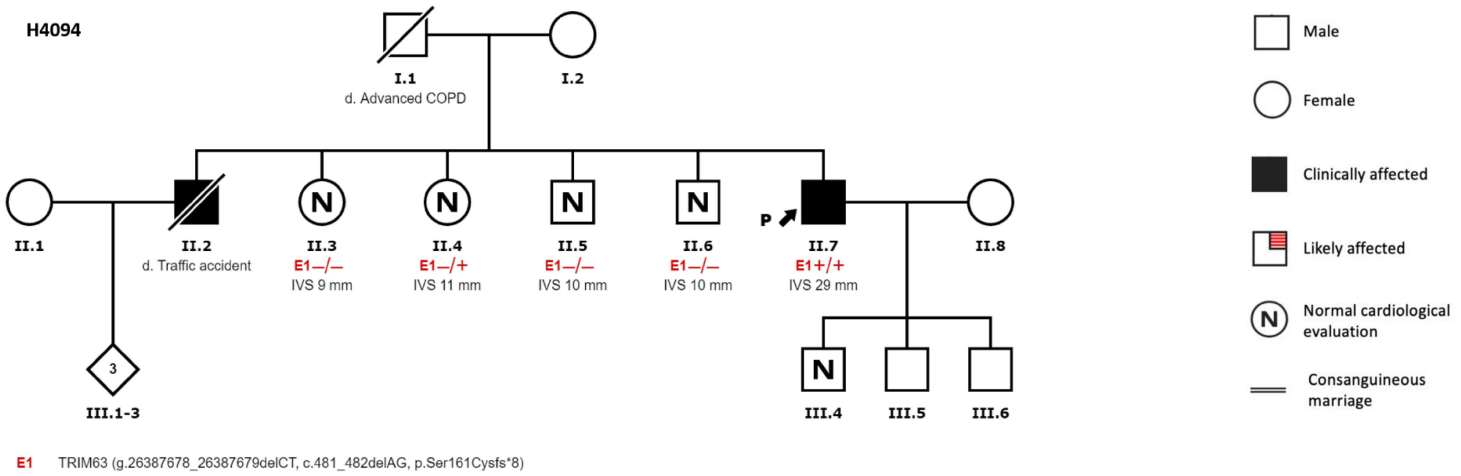
E2 TRIM63 (g.26393877A>C, c.109T>G, p.Leu37Val)



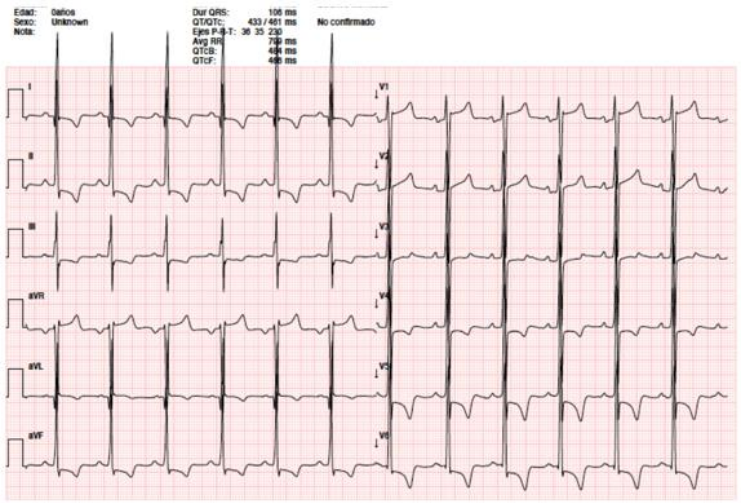
E1 TRIM63 (g.26392867C>T, c.224G>A, p.Cys75Tyr)



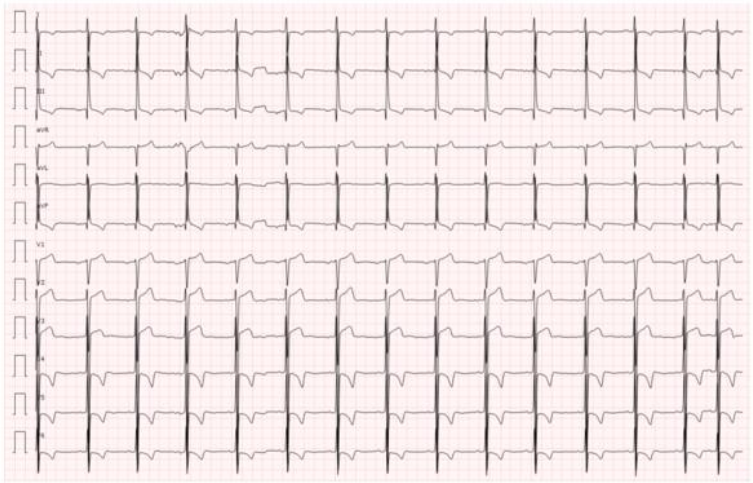
E1 TRIM63 (g.26384973G>A, c.739C>T, p.Gln247*)


H4094
I.1
d. Advanced COPD
I.2
II.1
II.2
d. Traffic accident
II.3
E1–/–
IVS 9 mm
II.4
E1–/+
IVS 11 mm
II.5
E1–/–
IVS 10 mm
II.6
E1–/–
IVS 10 mm
P
II.7
E1+/+
IVS 29 mm
II.8
3
III.1-3
III.4
III.5
III.6
E1 TRIM63 (g.26387678_26387679delCT, c.481_482delAG, p.Ser161Cysfs*8)
Male
Female
Clinically affected
Likely affected
Normal cardiological evaluation
Consanguineous marriage

Supplementary Figure 2. ECG of patients II.6 (family H8758) and II.1 (family H10022) showing LV hypertrophy criteria and repolarization abnormalities.

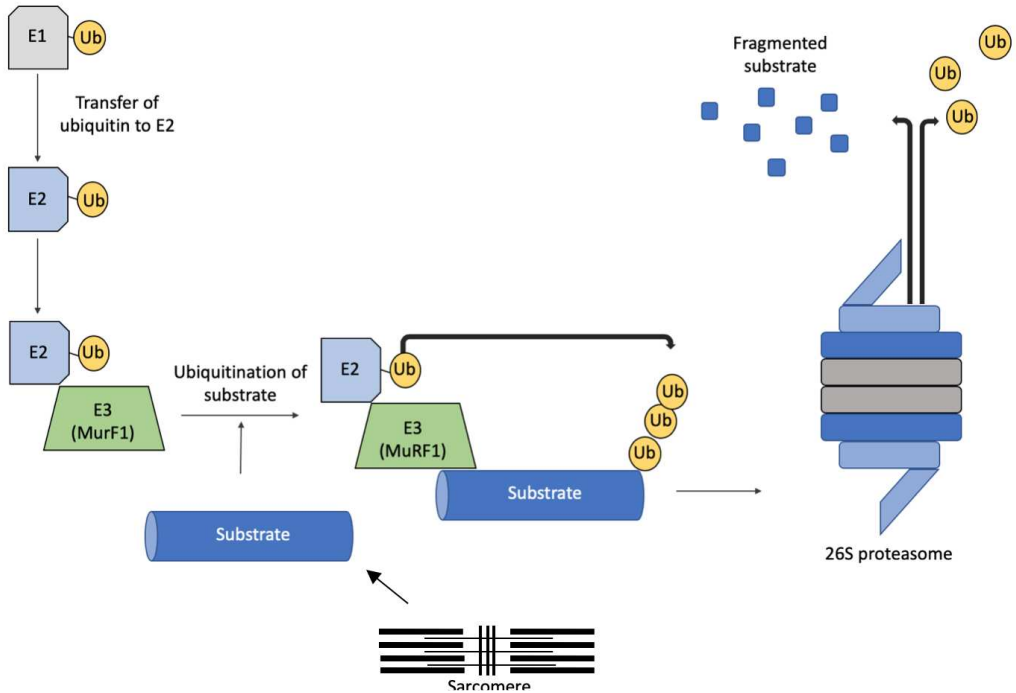


H8758. II.6



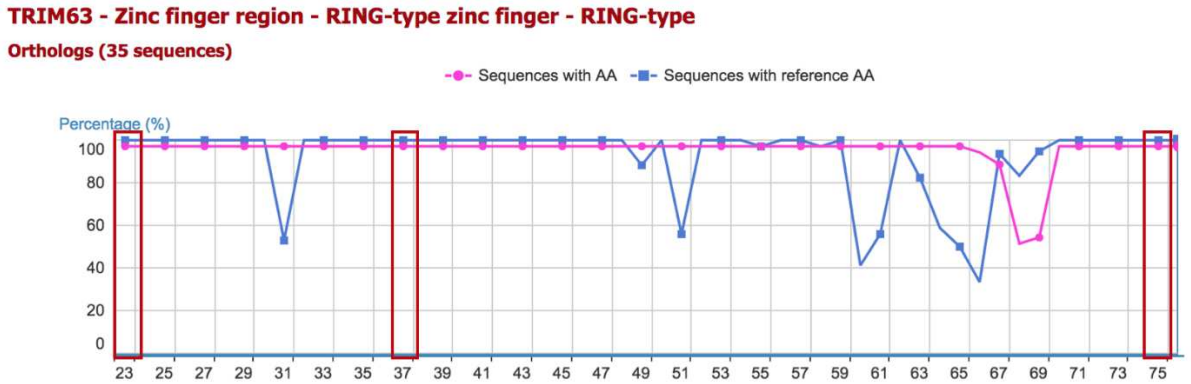
H10022 II.1

Supplementary Figure 3. Schematic representation of the ubiquitin proteasome system



Ubiquitin is activated by the E1 enzyme and is transferred to E2. Afterwards, E2 transfers the activated ubiquitin to a substrate that is bound to an E3 ubiquitin ligase (encoded by *TRIM63*). Ubiquitins serve as a signal for protein degradation by the 26S proteasome.

Supplementary Figure 4. Conservation of the RING domain of TRIM63



Analysis of conservation of the RING domain of TRIM63 in 35 orthologous sequences. The pink line shows the percentage of sequences with amino acids suitable for analysis, whereas the blue line shows the percentage of sequences that contain the reference amino acid (the most-conserved residues are present in 100% of sequences). The red boxes show the amino acids where mutations in the RING domain were found. It can be seen that these mutations are located in extremely conserved regions.