SUPPLEMENTARY DATA

Table 1 of the supplementary data

213 genes related to inherited cardiovascular diseases and sudden death included in our custom probe library

AARS2	Alanine-tRNA ligase, mitochondrial
ABCC9	ATP-binding cassette, subfamily C (CFTR/MRP), member 9
ACAD9	Acyl-CoA dehydrogenase family member 9, mitochondrial
ACADM	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial
ACADVL	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial
ACTA1	Actin, alfa 1, skeletal muscle
ACTA2	Actin, aortic smooth muscle
ACTC1	**Actin, alpha cardiac muscle 1 ****
ACTN2	Alpha-actinin-2
ACVRL1	Serine/threonine-protein kinase receptor R3
ADAMTSL4	ADAMTS-like protein 4
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
APOA5	Apolipoprotein A-V
APOB	Apolipoprotein B-100
APOC3	Apolipoprotein C-III
ATPAF2	ATP synthase mitochondrial F1 complex assembly factor 2

BAG3	BAG family molecular chaperone regulator 3
BMPR1B	Bone morphogenetic protein receptor type-1B
BMPR2	Bone morphogenetic protein receptor type II
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
CALR3	Calreticulin 3
CAPN3	Calpain-3
CASQ2	Calsequestrin-2
CAV1	Caveolin-1
CAV3	Caveolin-3
CBL	E3 ubiquitin-protein ligase CBL
CBS	Cystathionine beta-synthase
CETP	Cholesteryl ester transfer protein
COL1A1	Collagen alpha-1(I) chain
COL1A2	Collagen alpha-2(I) chain
COL3A1	Collagen alpha-1(III) chain
COL5A1	Collagen alpha-1(V) chain
COL5A2	Collagen alpha-2(V) chain
COQ2	4-hydroxybenzoate polyprenyltransferase, mitochondrial
COX15	Cytochrome c oxidase assembly protein COX15 homolog
COX6B1	Cytochrome c oxidase subunit 6B1
CRELD1	Cysteine-rich with EGF-like domain protein 1
CRYAB	Alpha-crystallin B chain

CSRP3	Cysteine and glycine-rich protein 3
CTF1	Cardiotrophin 1
CTNNA3	Catenin alpha-3
DES	**Desmin ****
DLD	Dihydrolipoyl dehydrogenase, mitochondrial
DMD	Dystrophin
DNAJC19	Mitochondrial import inner membrane translocase subunit TIM14
DOLK	Dolichol kinase
DSC2	Desmocollin 2
DSG2	Desmoglein 2
DSP	Desmoplakin
DTNA	Dystrobrevin alpha
ELN	Elastin
EMD	Emerin
ENG	Endoglin
EYA4	Eyes absent homolog 4
FAH	Fumarylacetoacetase
FBN1	Fibrillin 1
FBN2	Fibrillin 2
FHL1	**Four and a half LIM domains protein 1 ****
FHL2	Four and a half LIM domains 2
FHOD3	**FH1/FH2 domain-containing protein 3 ****
FKRP	Fukutin-related protein
FKTN	Fukutin
FLNA	Filamin-A
FLNC	Filamin-C
FOXD4	Forkhead box protein D4
GAA	Lysosomal alpha-glucosidase
GATA4	Transcription factor GATA-4

GATA6	Transcription factor GATA-6
GATAD1	GATA zinc finger domain-containing protein 1
GDF2	Growth/differentiation factor 2
GFM1	Elongation factor G, mitochondrial
GJA1	Gap junction alpha-1 protein
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
GUSB	Beta-glucuronidase
HCN4	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4
HFE	Hereditary hemochromatosis protein
HRAS	GTPase HRas
JAG1	Jagged-1
JPH2	Junctophilin 2
JUP	Junction plakoglobin
KCNA5	Potassium voltage-gated channel subfamily A member 5
KCND3	Potassium voltage-gated channel subfamily D member 3
KCNE1	Potassium voltage-gated channel subfamily E member 1
KCNE1L	Potassium voltage-gated channel subfamily E member 1-like protein
KCNE2	Potassium voltage-gated channel subfamily E member 2
KCNE3	Potassium voltage-gated channel subfamily E member 3
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
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
LRP6	Low-density lipoprotein receptor-related protein 6
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
MRPS22	28S ribosomal protein S22, mitochondrial
MTO1	Protein MTO1 homolog, mitochondrial
MURC	Muscle-related coiled coil protein
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 ****
MYLK	Myosin light chain kinase, smooth muscle
MYLK2	Myosin light chain kinase 2, skeletal/cardiac muscle
MYOT	Myotilin
MYOZ2	Myozenin 2
MYPN	Myopalladin

NEBL	Nebulette
NEXN	Nexilin
NKX2-5	Homeobox protein Nkx-2.5
NOTCH1	Neurogenic locus notch homolog protein 1
NOTCH3	Neurogenic locus notch homolog protein 3
NPPA	Atrial natriuretic factor
NRAS	GTPase NRas
OBSL1	Obscurin-like protein 1
PCSK9	Proprotein convertase subtilisin/kexin type 9
PDHA1	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial
PDLIM3	PDZ and LIM domain protein 3
PHKA1	Phosphorylase b kinase regulatory subunit alpha, skeletal muscle isoform
PITX2	Pituitary homeobox 2
PKP2	Plakophilin 2
PLN	Cardiac phospholamban
PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1
PMM2	Phosphomannomutase 2
PRDM16	PR domain zinc finger protein 16
PRKAG2	**5'-AMP-activated protein kinase subunit gamma-2 ****
PRKG1	cGMP-dependent protein kinase 1
PSEN1	Presenilin-1
PSEN2	Presenilin 2
PTPN11	**Tyrosine-protein phosphatase nonreceptor type 11 ****
RAF1	RAF proto-oncogene serine/threonine-protein kinase
RANGRF	Ran guanine nucleotide release factor
RBM20	Probable RNA-binding protein 20
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
SGCA	Alpha-sarcoglycan
SGCB	Beta-sarcoglycan
SGCD	Delta-sarcoglycan
SHOC2	Leucine-rich repeat protein SHOC-2
SKI	Ski oncogene
SLC22A5	Solute carrier family 22 member 5
SLC25A4	ADP/ATP translocase 1
SLC2A10	Solute carrier family 2, facilitated glucose transporter member 10
SLMAP	Sarcolemmal membrane-associated protein
SMAD1	Mothers against decapentaplegic homolog 1
SMAD3	Mothers against decapentaplegic homolog 3
SMAD4	Mothers against decapentaplegic homolog 4
SMAD9	Mothers against decapentaplegic homolog 9
SNTA1	Alpha-1-syntrophin
SOS1	Son of sevenless homolog 1
SPRED1	Sprouty-related, EVH1 domain-containing protein 1
SURF1	Surfeit locus protein 1
TAZ	Tafazzin
TBX1	T-box transcription factor TBX1
TBX20	T-box transcription factor TBX20
TBX5	T-box transcription factor TBX5
TCAP	Telethonin
TGFB2	Transforming growth factor beta-2
TGFB3	Transforming growth factor, beta 3
TGFBR1	TGF-beta receptor type-1

TGFBR2	TGF-beta receptor type-2
TMEM43	Transmembrane protein 43
TMEM70	Transmembrane protein 70, mitochondrial
TMPO	Thymopoietin
TNNC1	**Troponin C, slow skeletal and cardiac muscles ****
TNNI3	**Troponin I, cardiac muscle ****
TNNT2	**Troponin T, cardiac muscle ****
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

****** The genes considered priority for HCM are indicated in bold (n = 18).

Table 2 of the supplementary data

TPM1 p.Arg21Leu study population

Total individuals		n = 226
1. *Carriers*		83
Male	42 (50.6)	
Female	41 (49.4)	
1.a: Carriers with clinical data		67
1.b: Carriers without clinical data		2
1.c: Obligate simple heterozygous carriers without clinical data		14
2. Patients reported as hypertrophic cardiomyopathy without clinical and genetic data		13
3. Noncarriers with clinical data		24
4. First-degree relatives without clinical or genetic evaluation		106
5. Families		
5.a: Pedigrees		27
*5.b: Index cases **		31

5.c: No. carriers/family	2.7
5.d: Pedigrees with affected carriers in ≥ 2 generations	12

The data are presented as absolute numbers or No. (%).

****** Four index cases were reported without pedigree data.

Table 3 of the supplementary data

Clinical features of the *TPM1* p.Arg21Leu carriers diagnosed under the age of 35 years (n = 12)

Pedigree	Id	Sex	Second variant	Index case	Phenotype	Age at Dx	Age	NYHA	AF	FHSD	Max LVH	TV / FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others	SCD risk
#3	IV.1	M	No	No	HCM atypical	18	27	I	-	-	33	-	-	-	-	-	-	Echo: LA 40 mm.	Competitive athlete – football	2.92
#6	IV.1	M		Yes	HCM septal	15	22	II	-	+	39	+	-	-	+(35 exerc)	-	-	Holter: 1 episode NSVT (8 complex) MNR: LGE 3 segments	Competitive athlete - football ICD	6.37
#7	IV.2	M	No	No	HCM septal	26	35	I	-	+	14	-	-	-	-	-	-		Competitive athlete – football	2.48
#11	V.1	M	No	No	HCM septal	17	28	I	-	-	22	-	-	-	-	-	-	Echo: LA 46 mm ECG: negative T waves, high QRS voltages		3.29

#13	II.3	M	No	Yes	HCM septal	30	44	I	-	-	18	-	-	+	+(60)	-	-	Echo: LA 40 mm, diastolic dysfunction I. ECG: pathologic Q wave, abnormal repolarization, high QRS voltages.		2.24
#17	II.1	M	*MYH7* Lys351 Asn (?)	Yes	HCM septal	33	36	I	-	-	16	-	-	-	-	-	-	Echo: LA 42 mm ECG: normal		2.00
#18		M	No	Yes	HCM septal	13	32	III	-	-	51	-	+	+	+(140)	-	-	Echo: LA 47 mm, RA enlarged, severe mitral regurgitation, SAM. Wave S < 8 (tissue Doppler)	Myectomy. ICD	9.94
#19	II.1	M	No	Yes	HCM septal	33	47	I	-	-	23	+	-	-	-	-	-	ECG: first-degree AVB, High QRS Voltage	Primary prevention – ICD implanted	4.59
#20	II.2	M	No	Yes	HCM septal	11	17	I	-	-	17	-	-	-	-	-	-			2.43
#25	II3	M	No	Yes	HCM septal	26	28	I	-	-	32	+	-	-	-	-	-	Echo: LA 35 mm	Treacher-Collins syndrome	5.86
#26	II.1	M	No	Yes	HCM septal	20	23	I	-	-	26	-	-	-	-	-	-	MRI: LGE 2 segments, LA 41 mm	Competitive athlete – football	3.46

#29	II.3	F	No	Yes	HCM septal	15	24	II	-	+	33.5	-	-	-	+(90)	-	-	MRI: LGE in all segments, LA 32 mm	Myectomy	5.35

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; AVB, atrioventricular block; Dx, diagnosis; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; ICD, implanted cardiodefibrillator; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad); left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NSVT, nonsustained ventricular tachycardia; SAM, systolic anterior motion; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation.

Table 4 of the supplementary data

Major and nonmajor adverse cardiovascular events reported in the *TPM1* p.Arg21Leu pedigrees

Individual	Major cardiovascular events (age)	Observations
Carriers		
1. Male	Sudden death (55 y)	No left ventricular hypertrophy (autopsy)
2. Male	Heart transplant (48 y)	
3. Female	Heart failure death (68 y)	Systemic sclerosis (pulmonary fibrosis)
4. Obligate carrier, female	Unspecified cardiac death (79 y)	Unavailable clinical data
First-degree relatives without genetic testing		
5. Male	Sudden death (58 y)	Unknown genetic status
6. Male	Sudden death (40 y)	Unknown genetic status

		Additional familial genetic variant *TPM1* p.Met281Val – pathogenic
7. Female	Sudden death (19 y)	Unknown genetic status Additional familial genetic variant *MYH7* p.Gly741Arg – pathogenic
8. Male	Stroke-related death (64 y)	Unknown genetic status
9. Female	Heart failure death (58 y)	Unknown genetic status
10. Female	Stroke-related death (36 y)	Unknown genetic status
11. Female	Unspecified cardiac death (49 y)	Unknown genetic status Valvular heart disease
12. Male	Unspecified cardiac death (>65 y)	Unknown genetic status
Second-degree relatives without genetic testing		
13. Male	Sudden death (21 y)	Unknown genetic status. Sudden cardiac death during military exercise
14. Male	Sudden death (40 y)	Unknown genetic status
15. Male	Heart failure death (40 y)	Unknown genetic status
16. Male	Unspecified cardiac death (>56 y)	Unknown genetic status
Nonmajor adverse cardiovascular events (age) **		
Carriers		
1. Female	Septal myectomy (23 y)	Echo 33.5 mm
2. Male	Septal myectomy (32 y)	Echo 51 mm
3. Female	Mitral valve replacement (41 y)	Systolic anterior motion of mitral valve
4. Male	Nonfatal stroke (65 y)	
5. Female	Nonfatal stroke (73 y)	

[**] Nonmajor adverse cardiovascular events were not included in the survival curves.

Table 5 of the supplementary data

Clinical features of homozygous *TPM1* p.Arg21Leu carriers (n = 4)

Pedigree	Id	Sex	Age at Dx	Age, y	Additional variant	Max wall thickness	Diastolic dysfunction	Left atrium	LVOTO	Ventricular arrhythmias	LGE	SCD risk
#1	II.3	Male	71	75	No	22 mm, asymmetric septal	II	48	120 mmHg exercise	NSVT	NR	3.85
#10	II.6	Female	67	70	No	17 mm, apical	II	46	118 mmHg at rest	Rare EVB	NR	3.04
#10	II.7	Female	61	68	*MYBPC3* p.Asp75Asn	28 mm, apical. Biventricular LVH	Restrictive	54	No	NSVT	Extensive	4.08
#13	II.2	Male	40	48	No	22 mm, asymmetric septal	II	45	40 mmHg at rest	No	NR	2.56

EVB, ectopic ventricular beats; Dx, diagnosis; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO, left ventricle outflow tract obstruction;

MRI, cardiac magnetic resonance; NSVT, nonsustained ventricular tachycardia; NR, not reported; SCD, sudden cardiac death.

Table 6 of the supplementary data

Clinical features of *TPM1* p.Arg21Leu carriers with an additional genetic variant (n = 12)

Pedigree	Id	Sex	Second variant	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others	SCD risk
#3	III.3	M	*MYH7* p.Thr1019Asn	Not affected		51	I	-	-	11	-	-	-	-	-	-	ECG normal		-
#9	II.1	M	*TNNT2* p.Arg278Cys	HCM Apical	43	45	I	-	-	17	-	-	-	-	-	-	Echo: LA 40mm. MNR: LGE 4 segments		1.67
#9	II.3	F	*TNNT2* p.Arg278Cys	SAM Mitral	41	41	I	-	-	-	-	-	-	-	-	-	ECG normal		0.87
#9	I.2	F	*TNNT2* p.Arg278Cys	HCM apical	76	76	I	-	-	+	-	-	-	-	-	-	MNR: no LGE		0.61
#10	III.6	F	*MYBPC3* p.Asn75Asp	Not affected	-	42	I	-	-	-	-	-	-	-	-	-	-		-

#10	II.7	F	*MYBPC3* p.Asp75Asn	HCM Apical	<61	68	III	-	-	28	+	-	-	-	-		Holter: NSVT MRI: LA 54 mm, restrictive pattern, biventricular hypertrophy and LGE (subepicardical, medium mural, RV and apical). Apical hypokinesis.	Homozygous p.Arg21Leu	4.8
#15	III.5	F	*MYL3* p.Met173Val	HCM septal	43	45	II	-	-	15	-	-	-	+(30)	-	-	MNR: Mild LGE in 2 segments	SAH	1.48
#15	II.3	M	*MYL3* p.Met173Val	HCM reported	?	75	?	?	?	+	?	?	?	?	?	-	?	Patients were evaluated in other center	-
#17	II.1	M	*MYH7* p.Lys351Asn	HCM septal	33	36	I	-	-	16	-	-	-	-	-	-	Echo: LA 42 mm	Mild SAH	2.0
#23	II.2	F	*MYH7* p.Leu1333Val	HCM apical	61	63	I	-	-	15	-	-	-	-	-	-			0.87
#28		M	*MYH7* p.Tyr582Cys	HCM septal	56	57	II-III	-	-	17	-	-	-	+(110)	-	-	MRI: No LGE, LA 45 mm		2.39
#30	II.2	M	*TPM1* p.Met281Val	HCM septal	39	56	I	-	+	23	+	-	-	-	-	-	MRI: LGE >3 segments		4.71

																	Holter: NSVT 3 beats		

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; ICD, implanted cardiodefibrillator; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricular outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); Max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NSVT, nonsustained ventricular tachycardia; SAM, systolic anterior motion; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation.

Comments regarding disease expression of each patient and the pathogenicity of the additional genetic variant are briefly described below:

- **Pedigree #3:** The single p.Arg21Leu carrier (III.3) with the additional variant (*MYH7* p.Thr1019Asn) in this family was unaffected at age 51 years. Both variants have been described as having late/incomplete penetrance.

  We consider that ***MYH7* p.Thr1019Asn** is a likely rare pathogenic variant (present in 4 heterozygous carriers in the gnomAD database). It has been reported in the literature in a French family of African descent, in which 4 carriers had dilated cardiomyopathy and 5 carriers were unaffected. In our center, we identified it in some DCM cases, but mainly in multiple HCM patients. In some cases, the variant was identified in association with another sarcomere variant, as in this family.

- **Pedigree #9:** Individuals II.1, II.3 and I.2 were carriers of a pathogenic *TNNT2* variant, and expressed relatively mild phenotypes

  We consider ***TNNT2* p.Arg278Cys** as a pathogenic HCM-associated variant. It has been identified in more than 220 carriers from >130 families. The variant appears to be associated with late/incomplete penetrance and mild-moderate hypertrophies. This incomplete penetrance explains the presence of this variant in a relevant number of individuals in control populations (98 heterozygous carriers in the gnomAD database). Other 3 missense variants affecting the same amino acid p.Arg278Pro/Leu/His have been also identified in multiple HCM patients with a similar clinical profile. Among the families carrying variants in Arg278, we have reported 9 sudden deaths in carriers and 17 sudden deaths in first/second-degrees relatives without genetic testing.

- **Pedigree #10:** *TPM1* p.Arg21Leu homozygous sisters (II.7 and II.6) showed severe phenotypes, although diagnosed at advanced ages (see: *Pedigree #10*). The homozygous carrier II.7 had a more marked phenotype that may be related to the presence of an additional likely pathogenic variant in *MYBPC3*. On the other hand, his 42-year-old daughter (III.6), clinically unaffected, was a double heterozygous carrier of *TPM1* p.Arg21Leu and *MYBPC3* p.Asp75Asn variants.

  We consider ***MYBPC3* p.Asp75Asn** as a likely rare pathogenic variant (4 carriers in the gnomAD database). This variant has been identified in several HCM index cases, and familial studies showed the presence of both affected and unaffected carriers, suggesting late/incomplete penetrance.

- **Pedigree #15:** There are 2 carriers with complex genotype in this pedigree, but individual II.3 was studied in another center and no clinical details coul d be obtained.

  We consider ***MYL3* p.Met173Val** as a likely pathogenic variant that has been associated with the development of hypertrophic cardiomyopathy (28 carriers from 14 families –half of them either unaffected of with unknown phenotype; so, familial cosegregation has not been clearly documented). It is present in

a single individual from the gnomAD database (control). A functional study suggesting a damaging effect has been published. Data suggest that this variant could be associated with late disease onset and incomplete penetrance.

- **Pedigree #17:** The young age at diagnosis of this carrier (II.1) could be related to the presence of the *MYH7* variant; however, he did not have a severe phenotype.

  We consider ***MYH7* p.Lys351Asn** as a variant of unknown clinical significance. It has been described to date in this single HCM-patient, and also in a single carrier from the gnomAD population (control). Two missense variants located at the same amino acid (p.Lys351Glu/Thr) have been identified in HCM cases (6 unrelated carriers).

- **Pedigree #23:** Patient II.2, with a mild phenotype, carried an additional *MYH7* variant of uncertain significance.

  We consider ***MYH7* p.Leu1333Val** to be a variant of unknown clinical significance. This is the third HCM index case that we have identified with the variant, and we have identified it in another index case with DCM phenotype. It has been reported in 4 heterozygous individuals from the gnomAD database. These data could suggest a late/incomplete penetrance for the variant, but its pathogenicity is still uncertain.

- **Pedigree #28:** Carrier of an additional variant of uncertain significance in *MYH7* with mild phenotype and low SCD risk. No myocardial fibrosis on MRI.

  We consider ***MYH7* p.Tyr582Cys** to be a variant of unknown clinical significance. It has been identified only in this single patient to date. The variant is absent in control populations. It is located within a protein domain in which many other variants have been associated with HCM.

- **Pedigree #30**: Two pathogenic variants in *TPM1* were identified in this family. Only the individual II.2 carried both variants. Compared to his brother (II.1), who carried only *TPM1* p.Met281Val, the clinical expression of the index case was more severe and could suggest a synergic effect of the 2 variants. The paternal uncle (I.1) had a sudden death at 40 years of age; we cannot determine if he had only one of the family's variants or both.

  We consider ***TPM1* p.Met281Val** to be a rare pathogenic variant (8 individuals in gnomAD). This variant has been identified in at least 25 HCM-pedigrees (30 affected carriers and 8 unaffected relatives). Familial cosegregation has been documented in at least 1 large family. Clinical data of the carriers suggest late/incomplete penetrance (only 5 carries have been diagnosed under the age of 45 years). Another rare variant affecting the same amino acid (p.Met281Thr) has been identified in 5 HCM patients (from 4 pedigrees).

Table 7 of the supplementary data

Criteria for classifying *TPM1* p.Arg21Leu pathogenicity

Criteria*	Description	References **
(PS4) The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	• *TPM1* p.Arg21Leu present in 23/4099 (0.56%) hypertrophic cardiomyopathy probands, including 4 homozygous carriers. It was not present in 6462 patients (controls) sequenced with other inherited cardiac disorders in the same period ($P < .0001$) • The variant is listed in simple heterozygosity in 10/62784 (0.015%) individuals from the TOPMed program, and 5/120,158 individuals (0.004%) (age range 55-65 y) from the gnomAD database (non-TOPMed samples)	This study

(PP1) Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	• Combined LOD score = 3.95	This study
(PP2) Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are common mechanisms of disease	• *TPM1* gene Z = 2.87	the gnomAD database, 13
(PP3) Multiple lines of computational evidence supporting a deleterious effect	• Polyphen-2, MutationTaster, FATHMM, DANN	*In silico* predictors
(PM5) Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been previously seen	• p.Arg21His was identified in a single hypertrophic cardiomyopathy index case • *In vitro* functional evidence supporting a damaging effect caused by p.Arg21His	1,2
(PM1) Located in a critical and well-established functional domain (N-terminal amino acids 1-25)	*TPM1* variants: p.Met8Arg, p.Gln9Lys/His/Leu, p.Lys15Asn/Glu/Arg, p.Glu16Gln, p.Asp20Asn, p.Ala22Thr, p.Glu23Gln, and p.Ala25Thr have been reported in multiple affected carriers (16 HCM and 8 DCM-patients) vs only p.Asn17Lys and p.Arg21Leu, p.Ala22Thr in the general population (gnomAD). [see figure 3 of the supplementary data] *In vitro* functional assays: 1) Disruption of the coiled coil structure in residues 15–22 N-terminal. Lys15, Ala18 and Ala22 and those of their coiled coil mates were critical for stability of N:C-terminal junction. Salt bridges and H-bonds, including Asp-20, Arg-21, Gln-24, and Glu-26, reinforce the close interhelix packing. N-terminal region of tropomyosin is necessary for stable binding to actin filaments 2) p.Met8Arg and p.Lys15Asn cause changes to various properties of *TPM1* molecules, disrupting the interaction with F-actin. *In vitro* data obtained	2-12

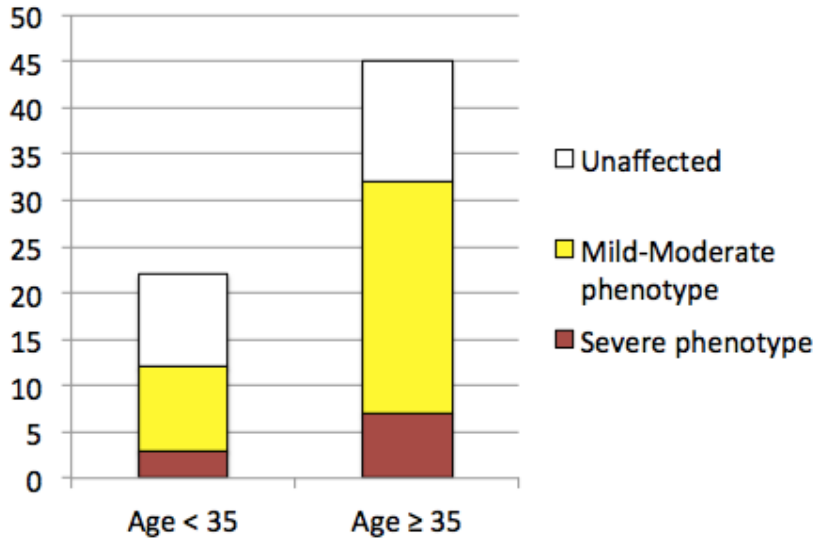
	point out that the N-terminal variants are more crucial for the head-to-tail interaction than those in the C-terminus 3) 1-21 N-terminal residues of tropomyosin are involved in interactions with leiomodin protein (actin-binding capsular protein). p.Lys15Asn reduces binding affinity for both leiomodin and tropomodulin, which are responsible for correct lengths of thin filaments 4) An intact coiled coil at the N-terminus of the *TPM1* is essential for tropomodulin binding	

[*] American College of Medical Genetics Criteria for interpretation of sequenced variants.

[**] See references at the end of the supplementary data.

Figure 1 of the supplementary data.

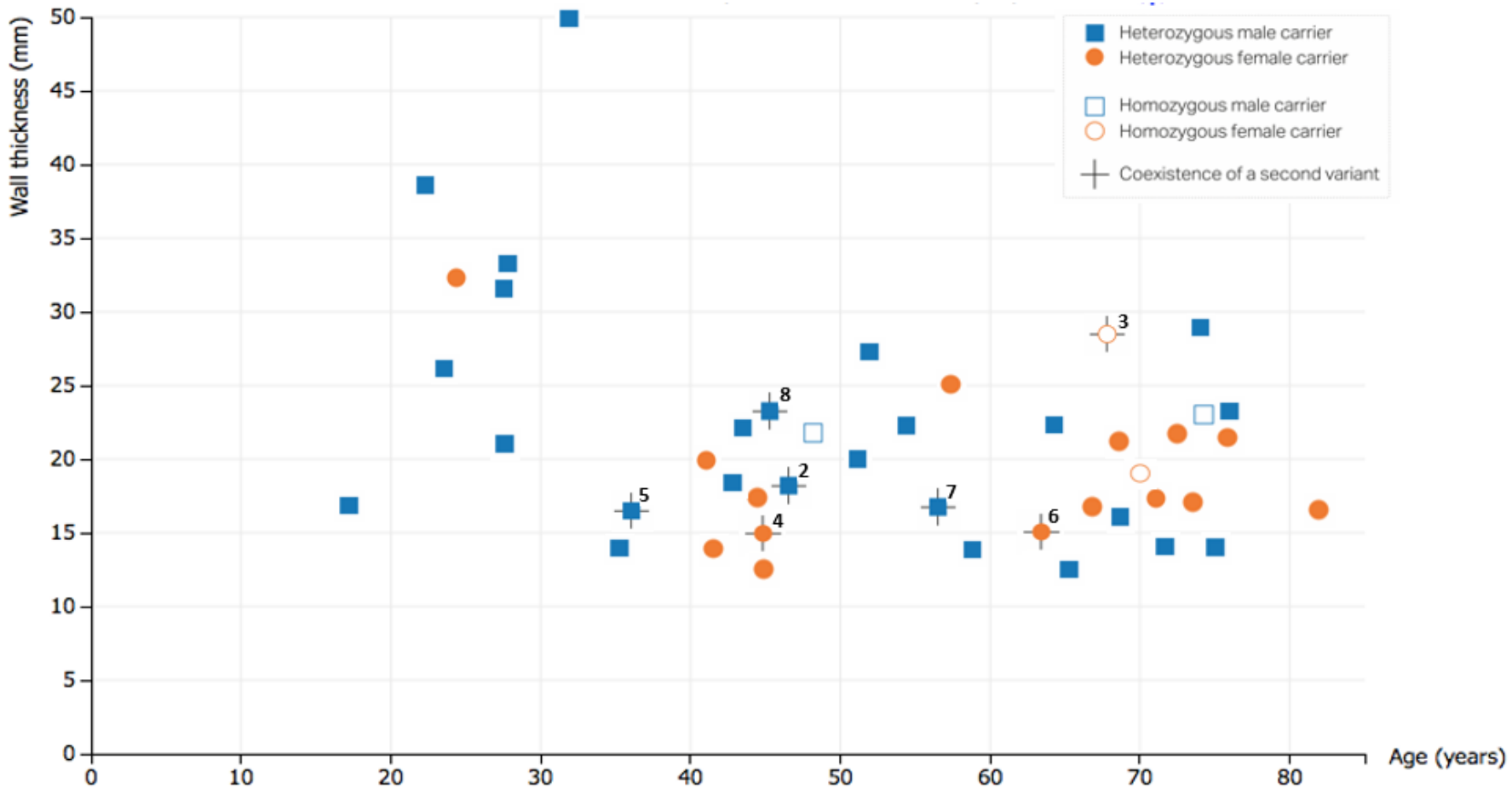
Carriers with clinical data (n = 67) by age and phenotype severity*



(*) Severe phenotype criteria: *TPM1* p.Arg21Leu carriers who experienced a major cardiovascular event or those at high sudden cardiac death risk.

Figure 2 of the supplementary data.

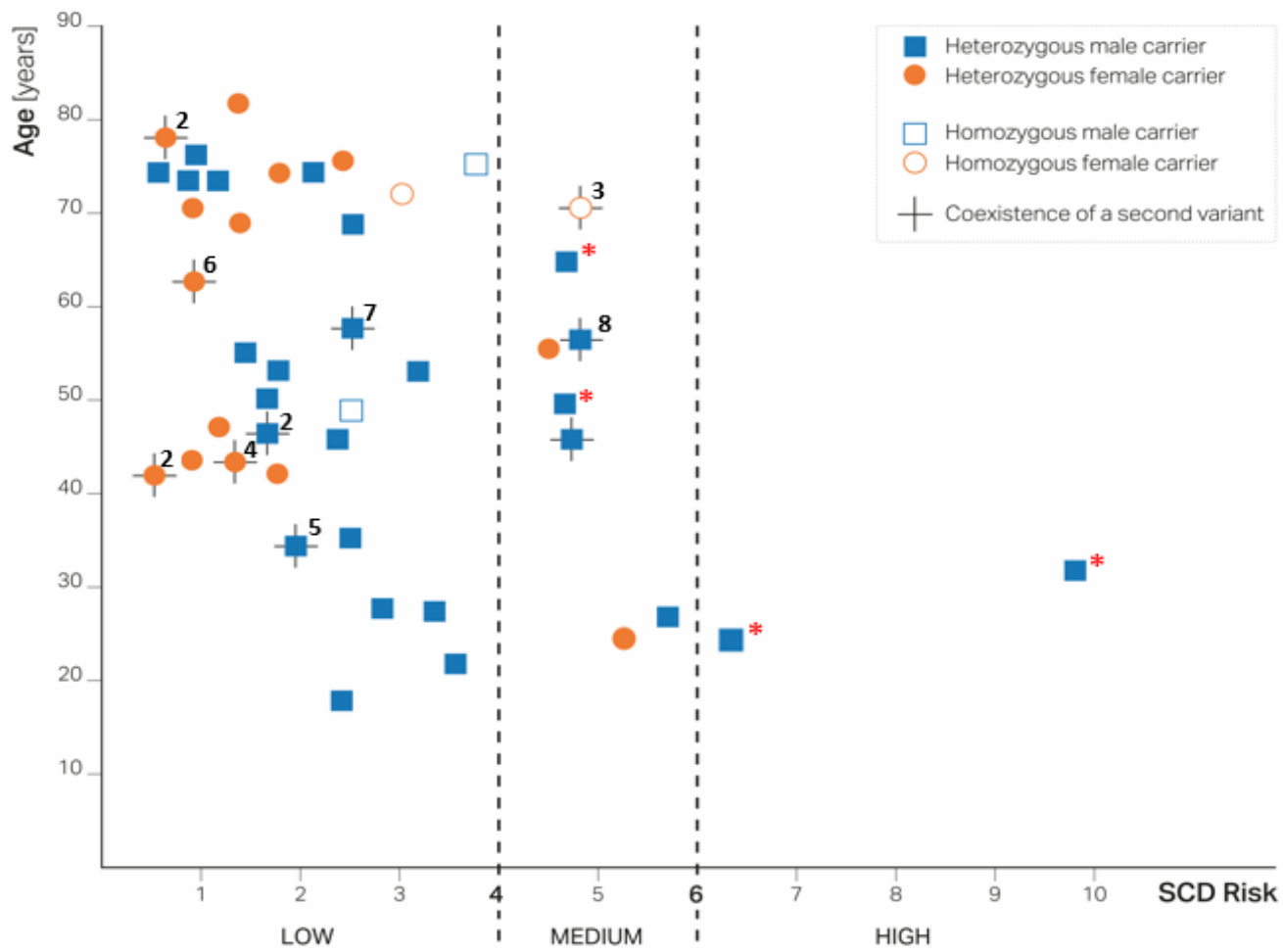
Maximum left ventricular wall thickness (in mm) by sex, genotype and age at the last follow up in *TPM1* p.Arg21Leu affected carriers.



Numbers detail the genotype: 2. *TNNT2* p.Arg278Cys (+++); **3.** *MYBPC3* p.Asp75Asn (+?); **4.** *MYL3* p.Met173Val (+?); **5.** *MYH7* p.Lys351Asn (?); **6.** *MYH7* p.Leu1333Val (?); **7.** *MYH7* p.Tyr582Cys (?); **8.** *TPM1* p.Met281Val (+++). (+++) indicates a pathogenic variant, (+?) likely pathogenic variant, and (?) variant of unknown clinical significance.

Figure 3 of the supplementary data

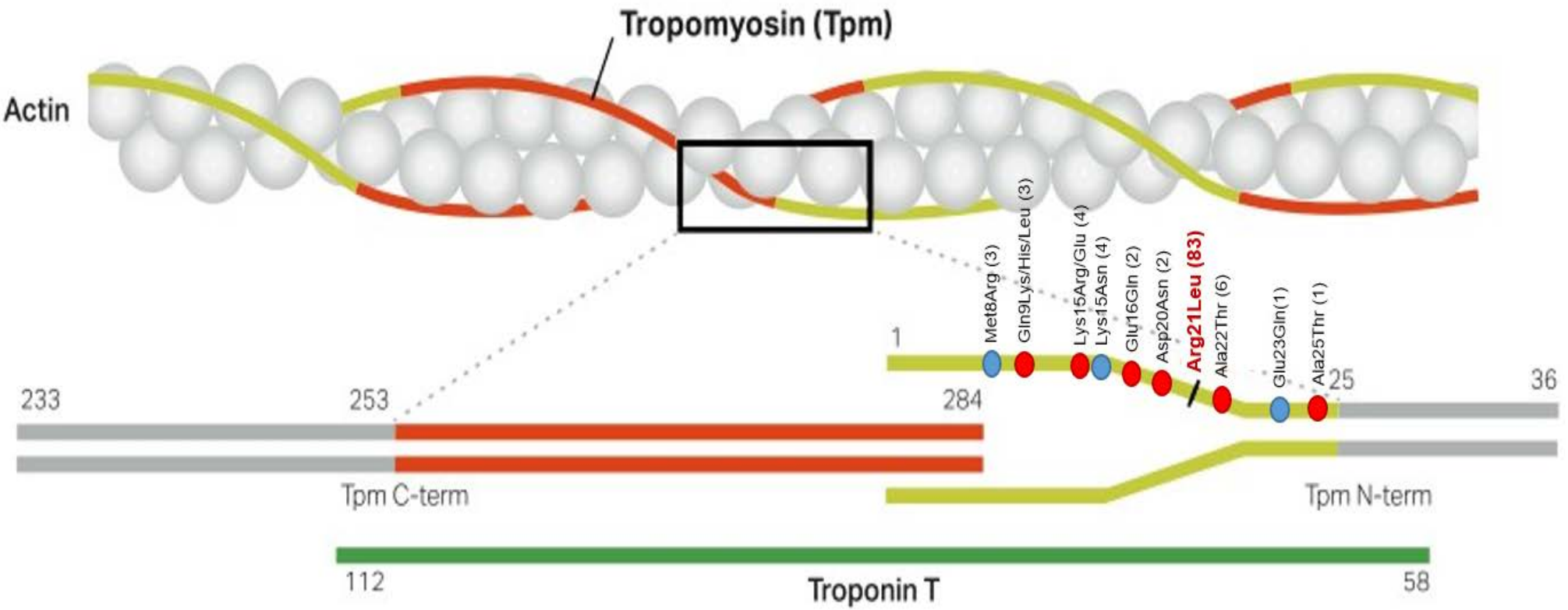
TPM1 p.Arg21Leu sudden cardiac death risk scores (ESC calculator) by sex, genotype and age at the last follow up.



Numbers detail the genotype: see page above. (*) Red asterisks: carriers with ICD.

Figure 4 of the supplementary data.

N-terminal: C-terminal overlapping junction between 2 subsequent αTpm.1.



At the top: αTpm.1 molecules across the actin groove (one tropomyosin is represented in yellow, and the subsequent one in orange), with the N-terminal:C-terminal junction highlighted with a black rectangle**.** Below: The junction has been also reported as tail-head overlapping region; 1-25 amino acids in the N-terminal region participating in the junction, including Arg21Leu. HCM-related variants are shown in red circles, and DCM-variants in blue circles (n = number of carriers).

The junction is one of the 2 αTpm.1 binding-sites with troponin T (in green). Figure drawn by the authors with data from Murakami K, Stewart M, nozawa K et al. Structural basis for tropomyosin overlap in thin (actin) filaments and the generation of a molecular swivel by troponin T. Proc Natl Acad Sci U S A. 2008;105:7200-7205.

***TPM1* p.Arg21Leu pedigrees**

31 index cases:

27 pedigrees

12 pedigrees with LOD score calculated

Legends:

Narrow indicates the **P**roband or index case

Square indicates male/circle indicates female

HCM affected male/female carrier

Unaffected (?) male/female carrier-with only minor ECG/Echo changes

Normal male/female—clinically evaluated

Deceased male/female individual

*** Obligate carrier** is described below of the individual.

NCE No clinical evaluation

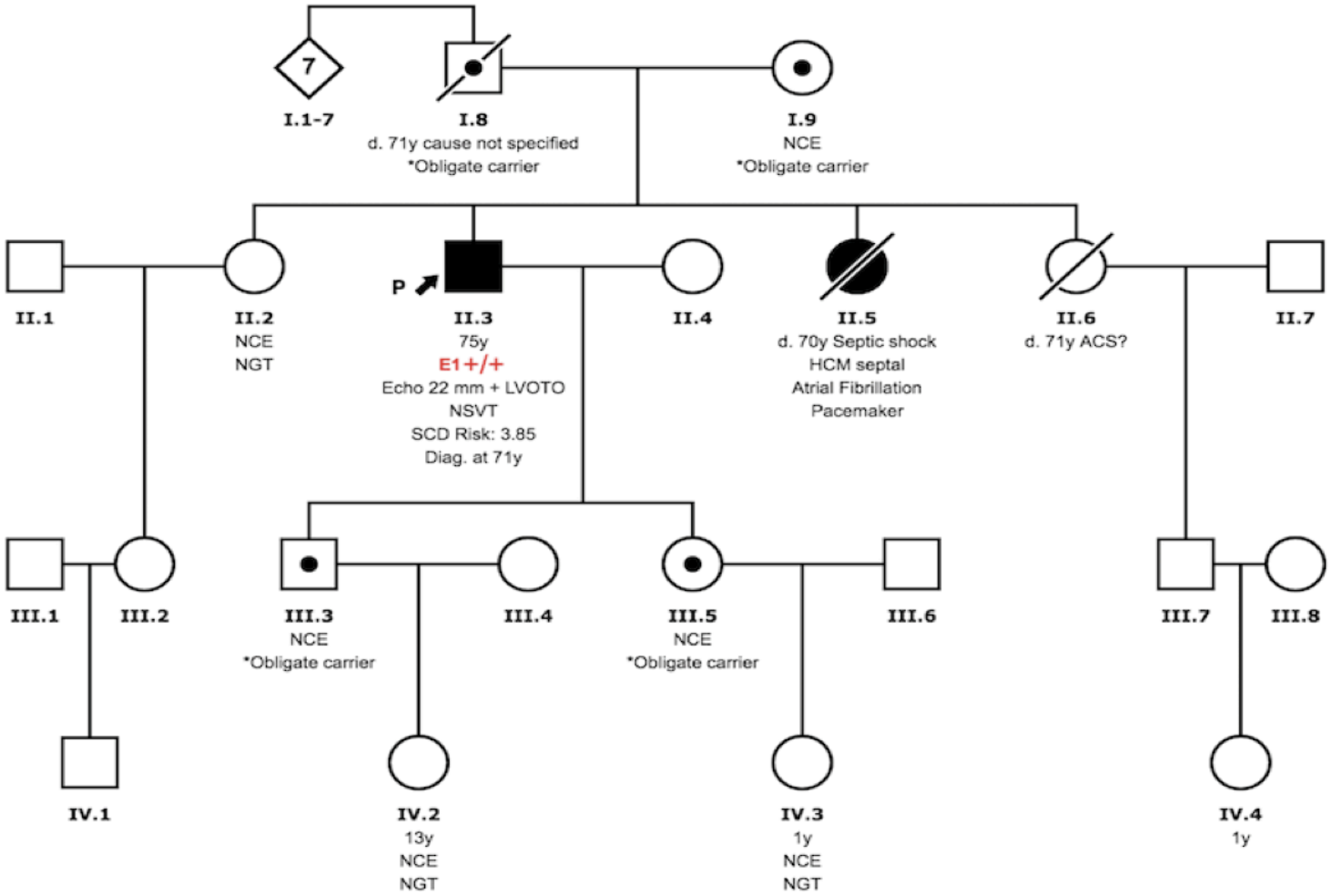
NGT No genetic testing

Unaffected male/female carrier

Male individual with strong suspicious of HCM/female individual or clinical data not reported

Presence of a second genetic disorder in a male/female individual

Pedigree #1



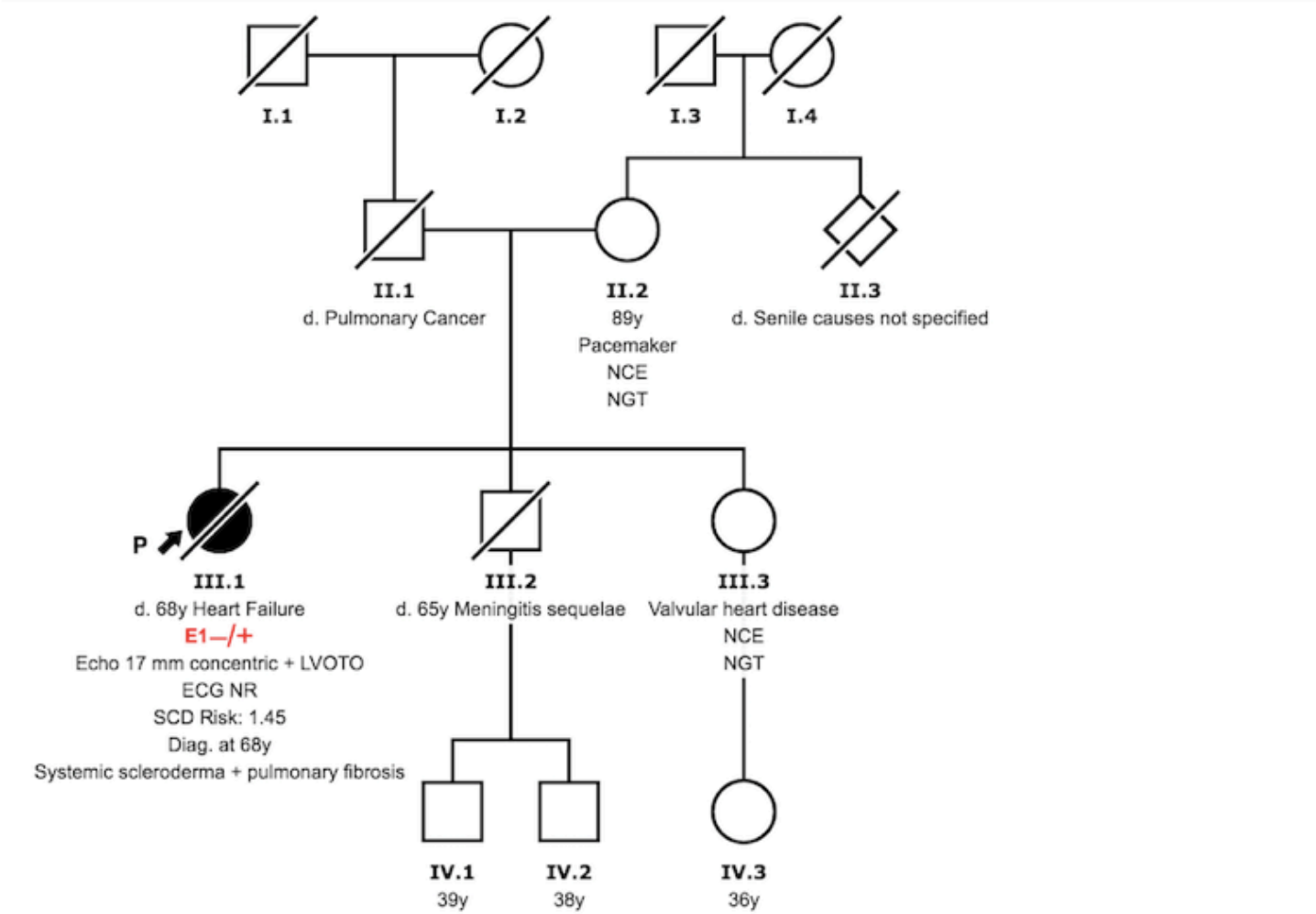
E1 TPM1 (g.63335090G>T, c.62G>T, p.Arg21Leu)

"+/+" = Homozygous, "-/+" = Heterozygous, "+" = Hemizygous, "-/-" = Not found, "-" = Not found

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.3	M	Yes (Homoz)	No	HCM septal	71	75	I	-	-	22	NSTV	-	-	+ (zero at rest; 120 exercise)	-		**Echo**: LA 48mm, Diastolic dysfunction II, mitral and aortic insufficiency **Holter:** NSVT	
III.5	F	Obligate carrier		NCE														
III.3	M	Obligate carrier		NCE														
I.8	M	Obligate carrier		NCE														Unspecified Death 71 y
I.9	F	Obligate carrier		NCE														
II.5	F	NGT		HCM Septal	?	70	?	+	-	?	?	?	?		?		Pacemaker implanted (Bradi-tachy synd)	Death 70 y sepsis

Abn BP, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation. E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

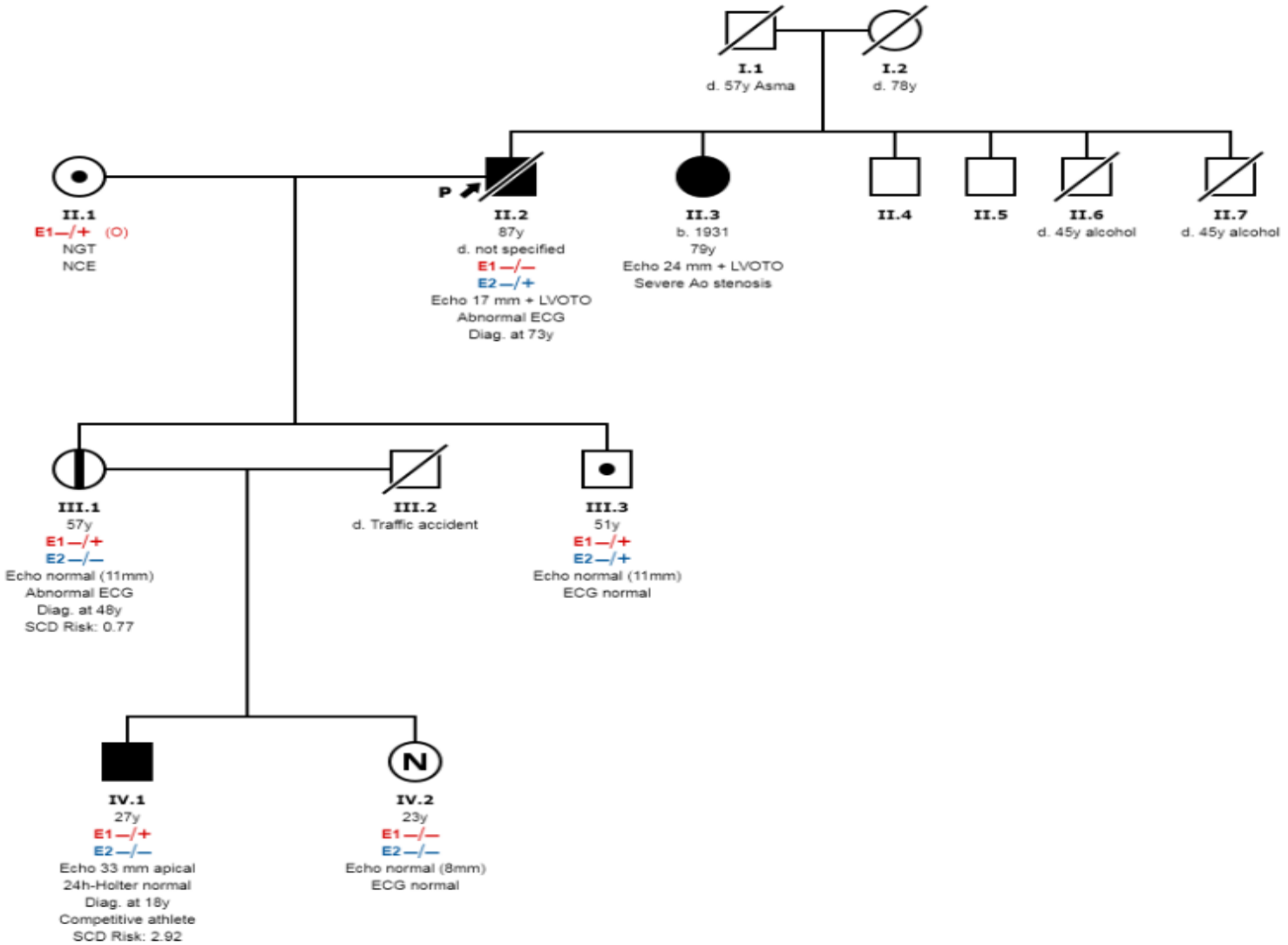
Pedigree #2



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/ FV	Syncope	Abn BP Resp	LVOTO (peak grad)	LV Dysf (EF)	Events	Additional features	Others
III.1	F	Yes	No	HCM concentric	68	68	IV	-	-	17	-	-	-	+ (53)	-(75)	Heart failure death 68 y	**Echo:** LA 43 mm, diastolic dysfunction I, moderate tricuspid regurgitation. PSAP 70 mmHg	Pulmonary fibrosis, systemic scleroderma; NO systolic dysfunction was reported

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #3



LOD SCORE 0.16

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.2	M	**No**	*MYH7* Thr1019 Asn (+?)	HCM septal	73	83	II	+	-	16	-	-	-	+ (30-55 at rest, >250 Valsalva).	-(>55)		**ECG** LVH, anterolateral negative T waves	
III.1	F	Yes	No	Not affected (?)		57	I	-	-	11	-	-	-	-	-(83)		**Echo:** Diastolic dysfunction I. **ECG**: pathologic Q waves	
III.3	M	Yes	*MYH7* Thr1019 Asn (+?)	Not affected		51	I	-	-	11	-	-	-	-	-(61)		**ECG** normal	
IV.1	M	Yes	No	HCM atypical	18	27	I	-	-	33	-	-	-	-	-(67)		**ECG** high QRS voltage, pathological Q waves. LA 40 mm	Competitive athlete football
II.1	F	Obligate carrier	?	NCE														

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu.;E2, other variant (see table). The index case is described in the gray line.

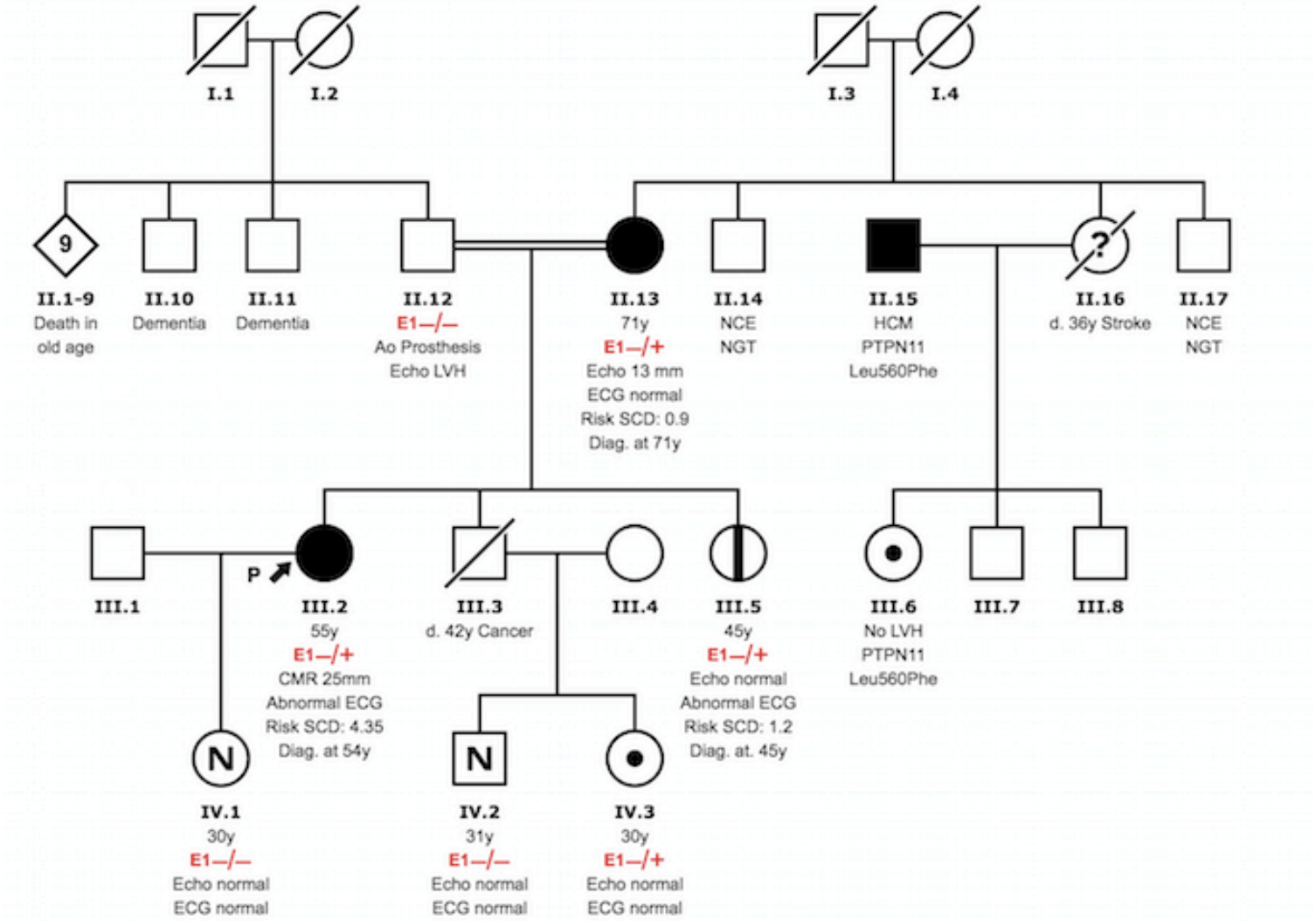
Comments Pedigree #3:

1. Complex genotype: The single p.Arg21Leu carrier (III.3) with the additional variant (*MYH7* p.Thr1019Asn) in this family was unaffected at age 51 years. Both variants have been described as having late/incomplete penetrance.

We consider that ***MYH7* p.Thr1019Asn** is a likely rare pathogenic variant (present in 4 heterozygous carriers in the gnomAD database). It has been reported in the literature in a French family of African descendent in which 4 carriers had dilated cardiomyopathy and 5 carriers were unaffected. In our center, we have identified it in some DCM cases, but mainly in multiple HCM patients. In some cases, the variant was identified in association with another sarcomere variant, as in this family.

>> In this family, the index case (II.2) was studied by NGS panel; however, the familial variant in *MYH7* was not identified in other family members (III.1 and IV.1) by Sanger sequencing. Therefore, individual III.1 was tested with an NGS panel, which allowed the identification of the *TPM1* p.Arg21Leu.

Pedigree #4



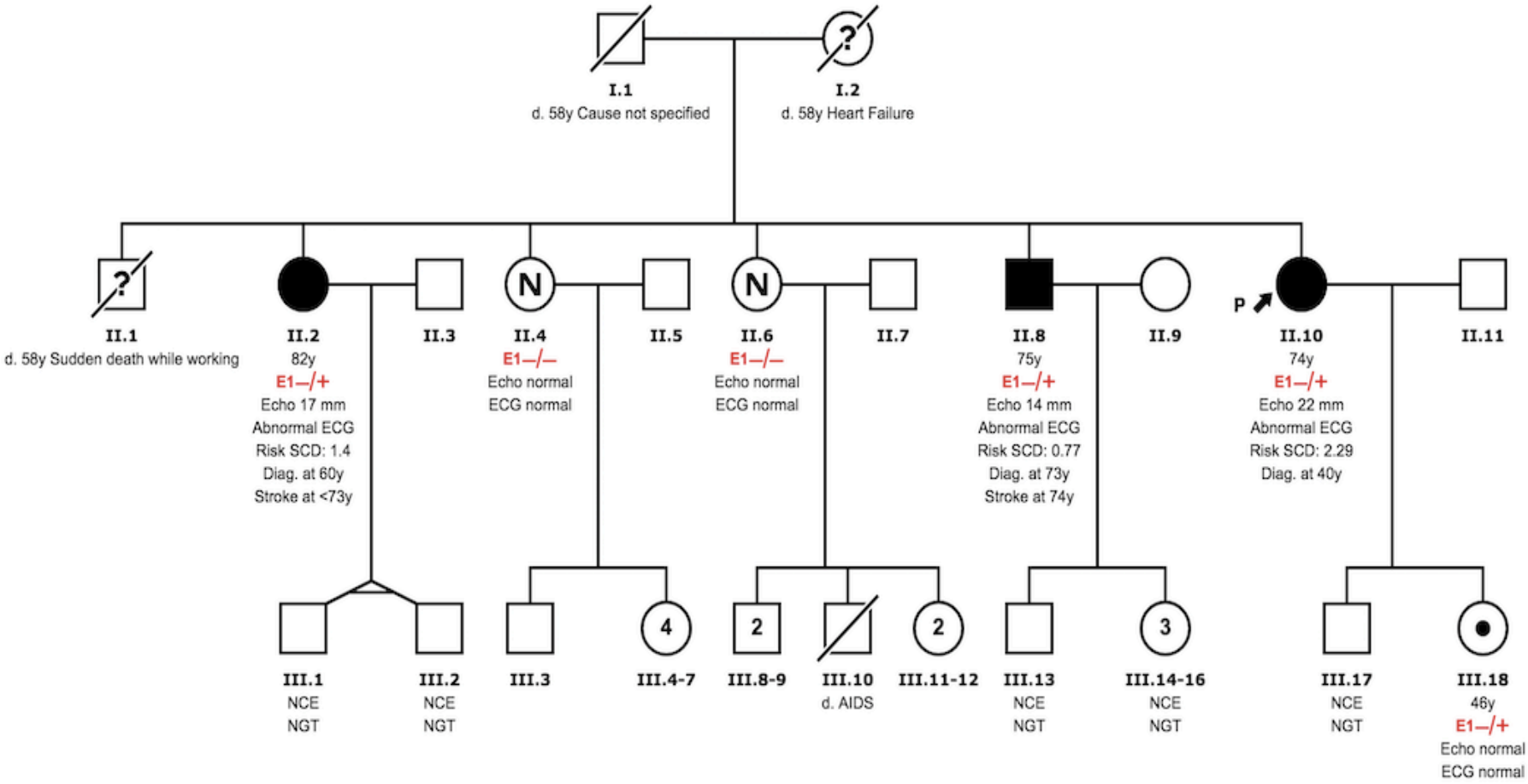
LOD SCORE: 0.68

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
III.2	F	Yes	No	HCM septal	54	58	II	+	-	25	-	+	-	-	-(60)		**MRI:** LA 45 mm, diastolic dysfunction, LV mass 173g. **ECG:** negative T waves, pathologic Q waves	SAH
III.5	F	Yes		Not affected (?)	45	45	I	-	-	13	-	-	-	-	-(>55)		**ECG:** Abnormal progression of R wave V_1-V_3	
II.13	F	Yes		HCM	71	71	I	-	-	13	-	-	-	-	-(>55)		**ECG** normal. **Echo:** Mild Ao, mild RT	SAH
IV.3	F	Yes		Not affected		30	I	-	-	-	-	-	-	-	-		**ECG** normal **Echo** normal	
II.16	F	NGT		?		36										Stroke-related death 36-y		

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; SAH, systemic arterial hypertension; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

PS: At the pedigree image: Patients II.15 and III.6, who were carriers of a PTPN11 variant, and not of the TPM1 variant, were not included in our study population.

Pedigree #5

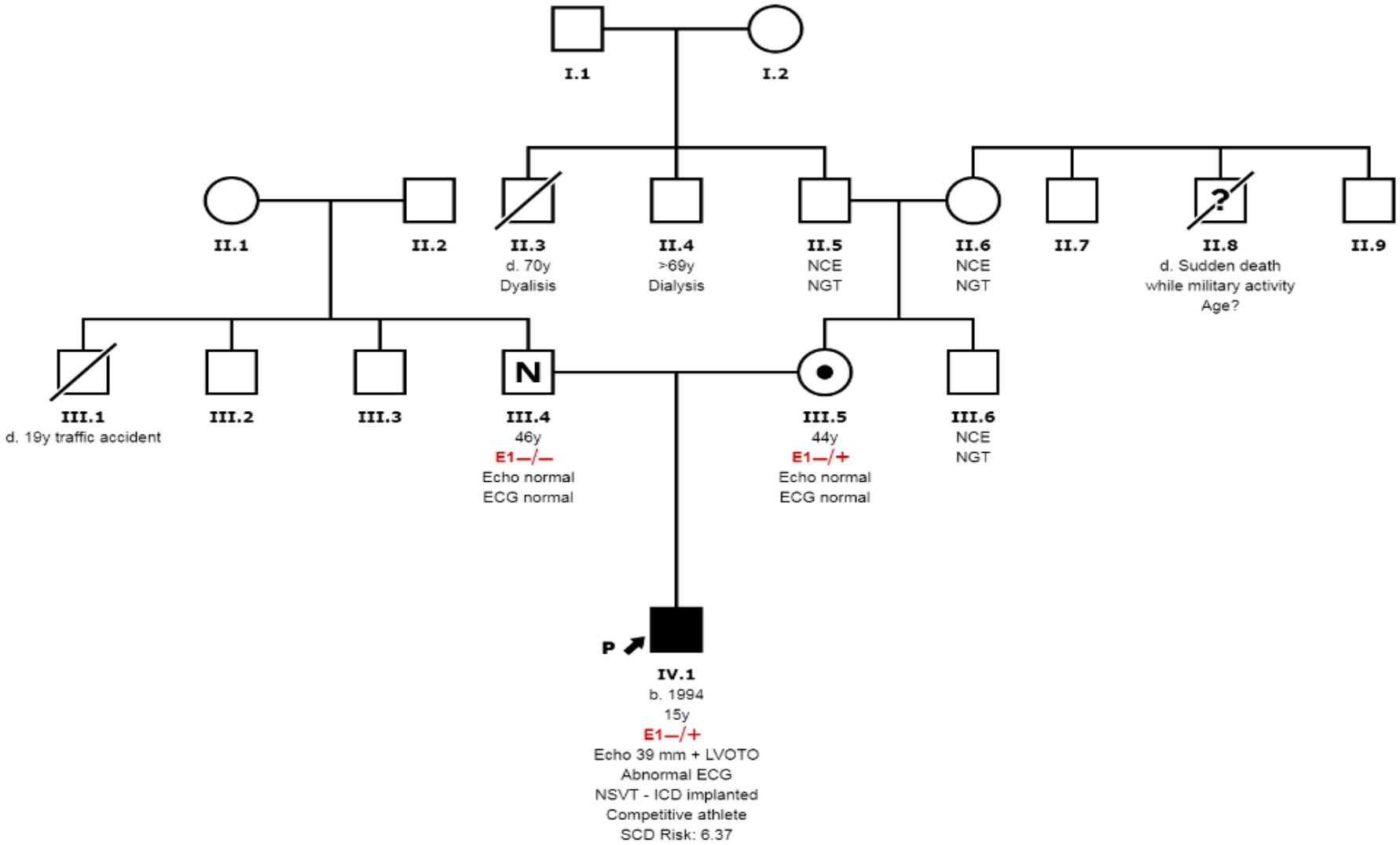


LOD SCORE: 0.17

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.10	F	Yes	No	HCM septal	40	74	II	+	+	22	-	-	-	+ (50)	-(78)		**ECG:** High QRS voltage, negative T waves/AF. **MRI:** LA: 57 mm, LGE septal (RV-Apex)	
II.2	F	Yes		HCM septal	60	82	I	-	+	17	-	-	-	-	-(70)	Stroke at <73y	**ECG:** High QRS voltage, pathologic Q waves. **Echo:** LA: 57 mm	
II.8	M	Yes		HCM septal	73	75	I	-	+	14	-	-	-	-	-(79)	Stroke at 74y	**ECG:** Pathologic Q waves	
I.2	F	NGT		?		58									+	Heart failure death 58y		
II.1	M	NGT		?		58										Sudden death 58-y while working		
III.18	F	Yes		Not affected		46												

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation.

Pedigree #6

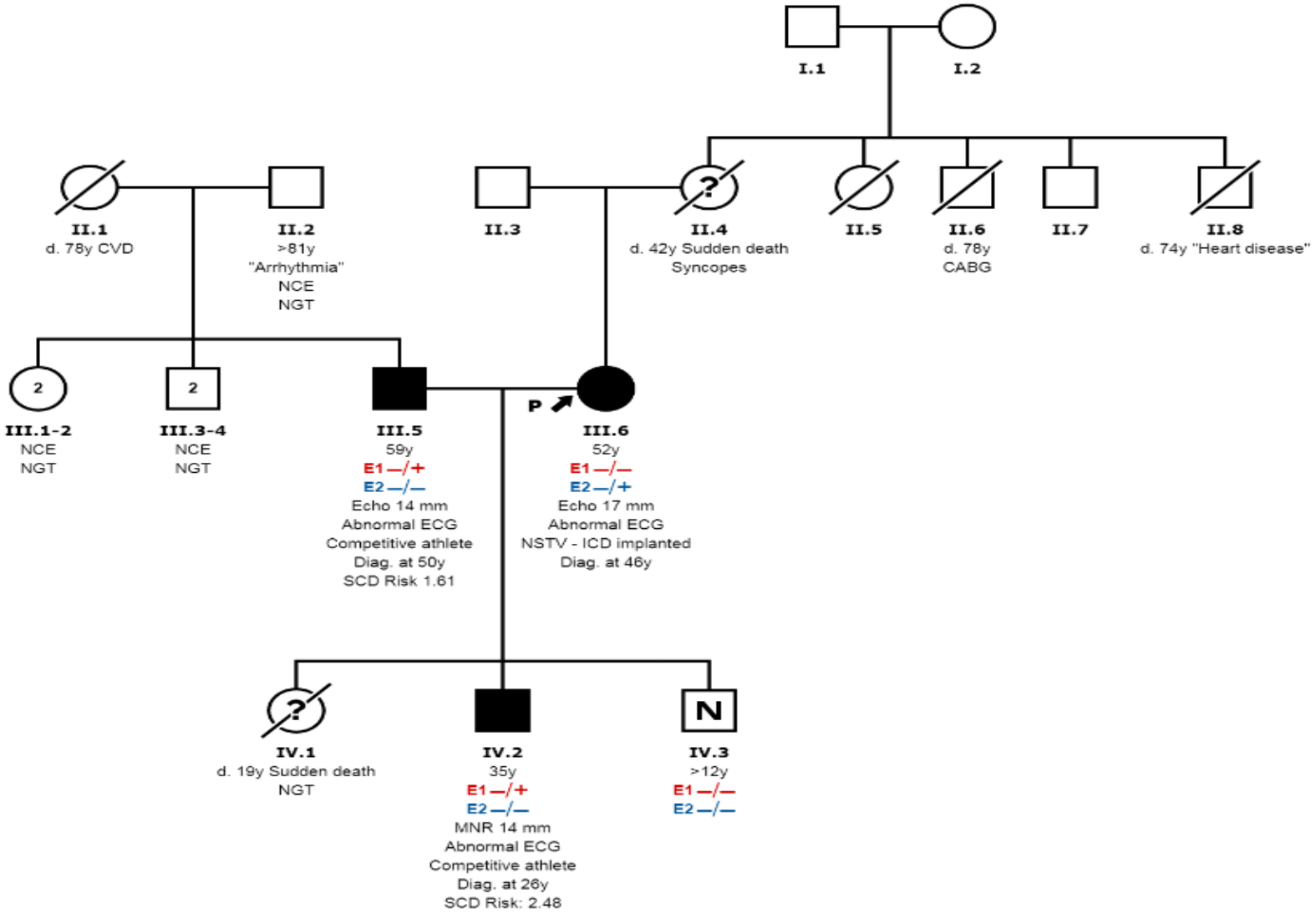


Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	**Others**
IV.1	M	Yes		HCM septal	15	22	II	-	+	39	+	-	-	+ (35 exerc)	- (78)		**ECG:** pathologic Q wave, high QRS voltage. **Holter:** VEB rare, 1 episode of NSVT (8 complex) **MRI:** LA 41 mm, mild LVOTO, basal fibrosis and midseptal, apical.	Competitive athlete football ICD implanted 22 y
III.5	F	Yes	No	Not affected		44	-	-	+	-	-	-		-	-			
III.4	M	No		Not affected		46	-	-	-	-	-	-		-	-			
II.8	M	?		?		21										Sudden death - age 21 y		During military activity

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction);

max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VEB, ventricular ectopic beats; VT/VF, ventricular tachycardia/ventricular fibrillation. E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #7



LOD SCORE: 0.17

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
III.6	F	**No**	*MYH7* Gly741 Arg (+++)	HCM septal	46	52	I	-	+	17	+	-	-	-	-(61)		**ECG:** Low QRS voltage, inv T waves, pathol. Q waves **MNR:** Late enhancement by fibrosis **Holter:** NSVT	ICD implanted 47 y
III.5	M	Yes		HCM septal	50	59	II	-	-	14	-	-	-	-	-(58)		**ECG:** Pathologic Q waves	Competitive athlete – football
IV.2	M	Yes	No	HCM septal	26	35	I	-	+	14	-	-	-	-	-(75)		**ECG:** High QRS voltage, pathologic Q waves	Competitive athlete – football
IV.1	F	NGT		SCD		19			+		+					Sudden death – 19 y		

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VEB, ventricular ectopic beats; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p;Arg21Leu; E2, other variant (see table). The index case is described in the gray line;

Comments pedigree #7:

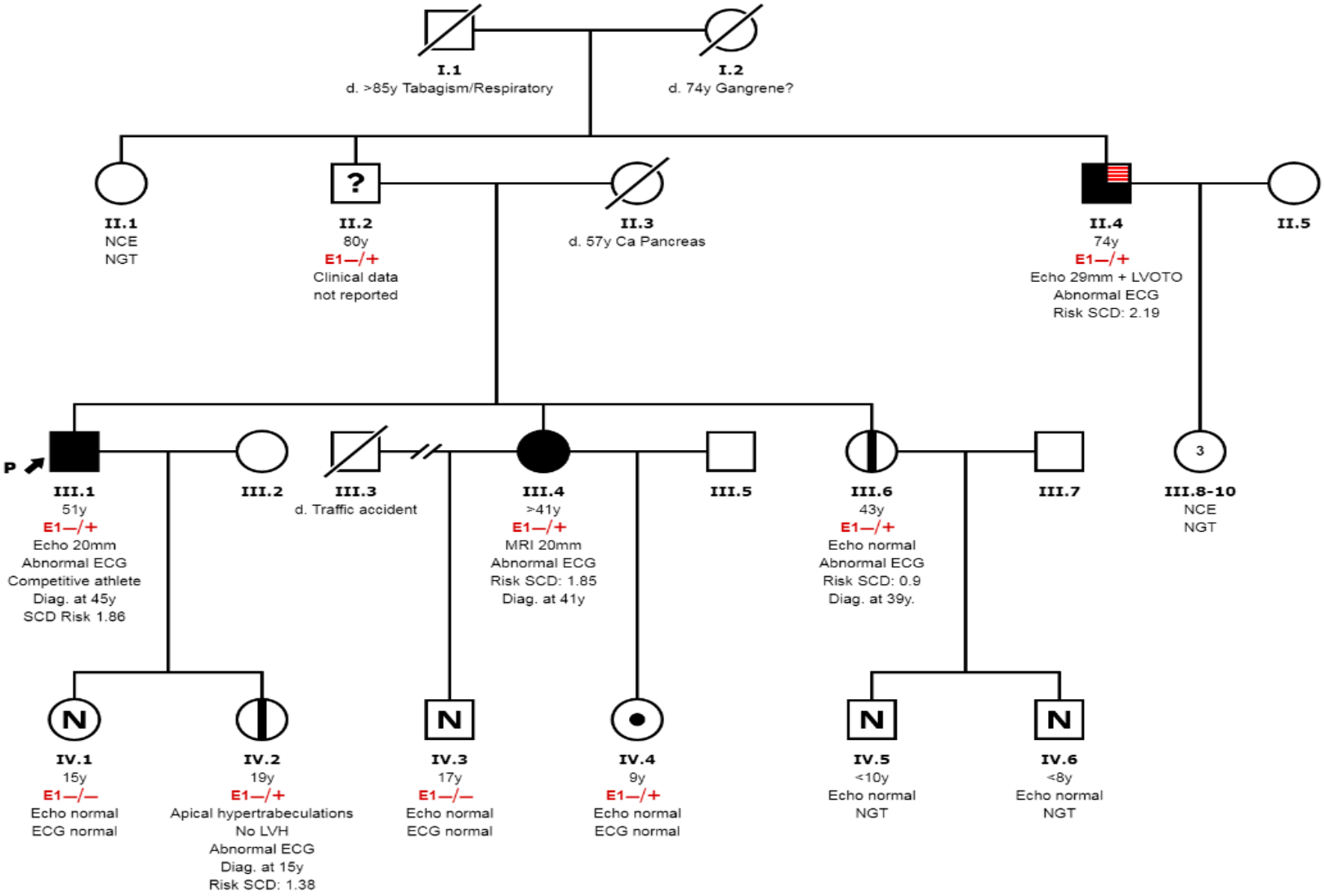
1. Complex genotype: Two pathogenic variants are associated with HCM in this family, but there is no carrier with both variants. The index case (III.6) had been evaluated after her daughter's (IV.1) sudden death, with a previous family history of sudden death in her mother (II.4). We could not determine the daughter's (IV.1) genotype; she could be carrier of both or of only 1 of the family's variants.

We consider ***MYH7* p.Gly741Arg** to be a pathogenic variant. Gly741 is a hotspot residue with 3 likely pathogenic missense variants affecting multiple HCM patients (73 cases from 41 families in total).

PS: The sudden cardiac death reported in patient II.4 was not included in our survival analysis.

>> In this family, the index case (III.6) was studied by NGS panel; however, the familial variant in *MYH7* was not identified in her son (IV.2) by Sanger sequencing. Therefore, individual IV.2 was tested with an NGS panel, which allowed the identification of the *TPM1* p.Arg21Leu.

Pedigree #8



LOD SCORE: 1.12

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV dysf (EF)	Events	Additional features	Others
III.1	M	Yes	No	HCM septal	45	51	I	-	-	20	-	-	-	-	-(78)		**ECG:** negative T waves V_{1-6}. **MRI:** LA 40 mm, extensive LGE (6 segments)	Competitive athlete
III.4	F	Yes		HCM septal	41	>41	I	-	-	20	-	-	-	-	-(78)		**ECG:** negative T waves V_{1-3}, pathologic Q waves V_{5-6} **MRI**: No report of LGE	
III.6	F	Yes		Not affected (?)		43	I	-	-	-	-	-	-	-	-		**ECG**: altered R wave V_{1-3}	
II.4	M	Yes		HCM septal	48	74	I	+	-	29	-	-	-	+(60)	-(55)		**Echo:** mild mitral insufficiency, moderate ascending aortic dilatation (47 mm) **ECG:** Paroxysmal atrial fibrillation, *in*	**Limb-girdle muscular dystrophy**

																	sinus rhythm: high QRS voltages, abnormal repolarization, VEB, LA 51mm **MRI**: Systolic function borderline, LGE antero septal, basal and medial	
IV.2	F	Yes		Not affected (?)		19	I	-	-	-	-	-	-	-	-(62)		**Echo:** apical hypertrabecular (without criteria for NC) **ECG:** pathologic Q wave inf and V_{3-6} **Holter:** Rare ectopic ventricular beats multifocal	
IV.4	F	Yes		Not affected		9	-	-	-	7	-	-	-	-	-			
II.2	M	Yes		?													**No data available**	

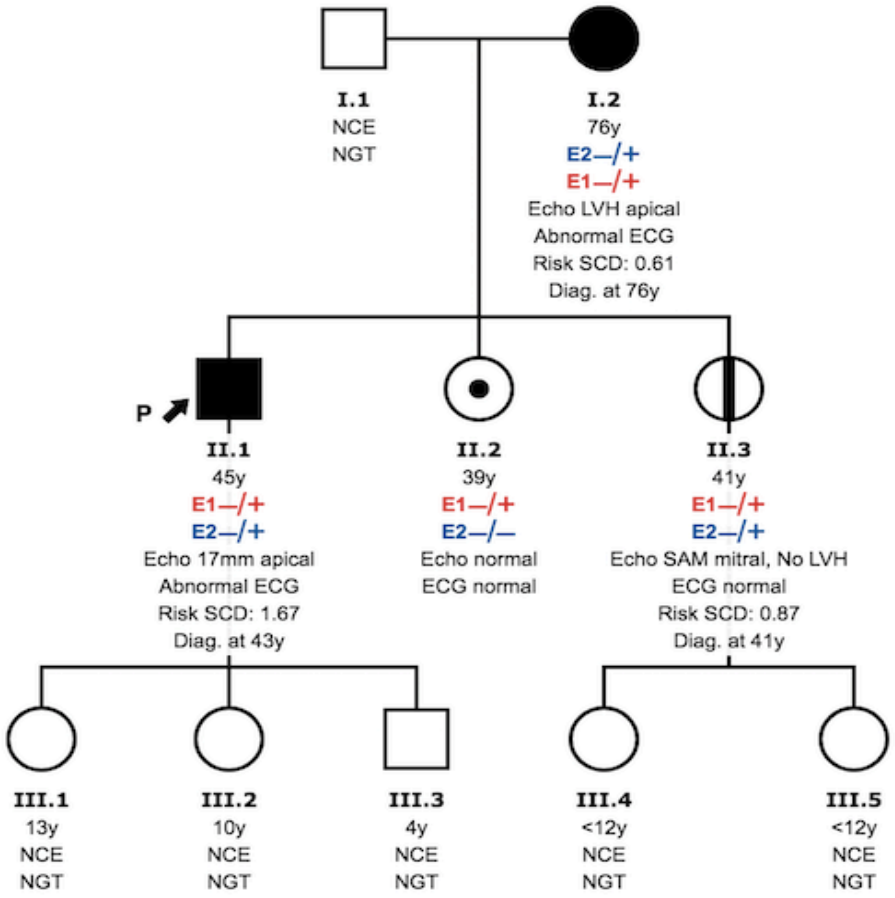
Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH,

left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p;Arg21Leu. The index case is described in the gray line.

Comments pedigree #8:

1. Competitive sports: Individual III.1 showed low SCD risk by ESC calculator, but showed extensive myocardial fibrosis (6 affected cardiac segments).

Pedigree #9



LOD SCORE: 0.34

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.1	M	Yes	*TNNT2* Arg278Cys (+++)	HCM Apical	43	45	I	-	-	17	-	-	-	-	-(62)		**Echo:** LA 40 mm. **MNR:** LGE mesobasal, anterior septal, basal anterior, mesoinferior	
II.2	F	Yes	No	Not affected		39	-	-	-	-	-	-	-	-	-			
II.3	F	Yes	*TNNT2* Arg278Cys (+++)	Not affected (?)		41	I	-	-	-	-	-	-	-	-		**Echo** SAM mitral **ECG** normal **Echo:** SAM mitral	
I.2	F	Yes	*TNNT2* Arg278Cys (+++)	HCM apical	76	76	I	-	-	+	-	-	-	-	-		**ECG:** negative T waves, high voltages. **MNR:** no LGE	

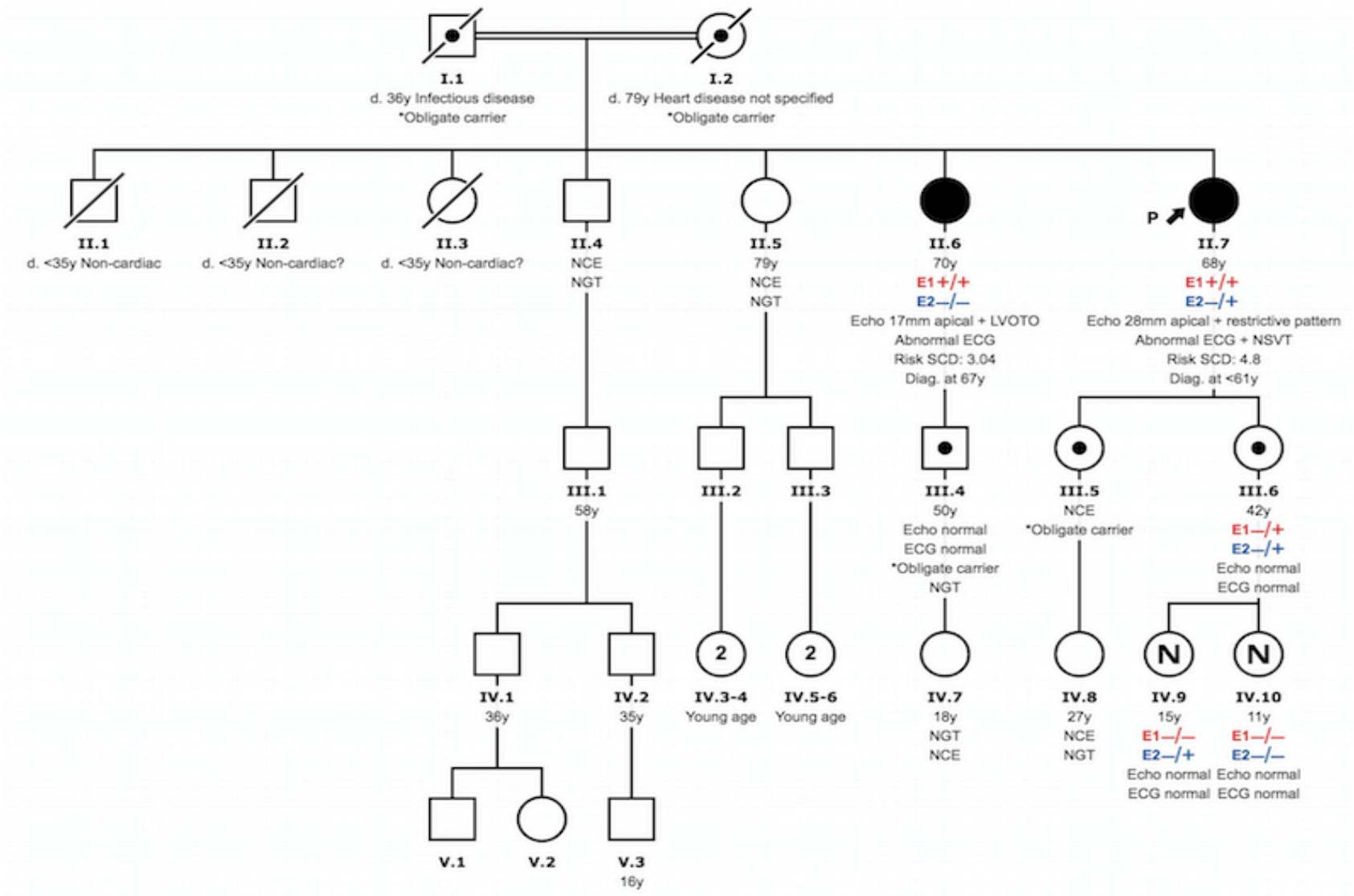
Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; SAH, systemic arterial hypertension; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line

Comments pedigree #9:

1. **Complex genotypes:** Individuals II.1, II.3 and I.2 were carriers of a pathogenic *TNNT2* variant, and expressed relatively mild phenotypes.

   We consider ***TNNT2* p.Arg278Cys** to be a pathogenic HCM-associated variant. It has been identified in more than 220 carriers from > 130 families. The variant appears to be associated with late/incomplete penetrance and mild-moderate hypertrophies. This incomplete penetrance explains the presence of this variant in a relevant number of individuals in control populations (98 heterozygous carriers in the gnomAD database). Another 3 missense variants affecting the same amino acid p.Arg278Pro/Leu/His have also been identified in multiple HCM patients with a similar clinical profile. Among the > 130 families carrying variants in Arg278, we have reported 9 sudden deaths in carriers and 17 sudden deaths in first/second-degree relatives without genetic testing.

Pedigree #10



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.7	F	Yes (Homoz)	*MYBPC3* Asp75Asn (+?)	HCM Apical	<61	68	III	-	-	28	+	-	-	-	-(63)		**Holter:** NSVT **Echo:** Restrictive pattern **MRI:** LA 54 mm, biventricular LGE (subepicardical, medium mural, RV and apical). LVH medium ventricular, right ventricular and severe apical	obesity
II.6	F	Yes (Homoz)	No	HCM Apical	67	70	II	-	-	17	-	-	-	+ (118 at rest)	-(64)		**Holter**: rare isolated VEBs **ECG**: mild abnormal repolarization **Echo:** LA 46 mm, diastolic	

																	dysfunction II, mitral regurgitation	
III.6	F	Yes	*MYBPC3* Asp75Asn (+?)	Not affected		42	I	-	-	-	-	-	-	-	-			
I.2	F	Obligate carrier	?	Cardiac disease not specified		79										Death at 79 y – heart disease		
I.1	M	Obligate carrier	?	NCE		36												
III.4	M	Obligate carrier		Not affected		50	I	-	-	-	-	-	-	-	-			
III.5	F	Obligate carrier		NCE														

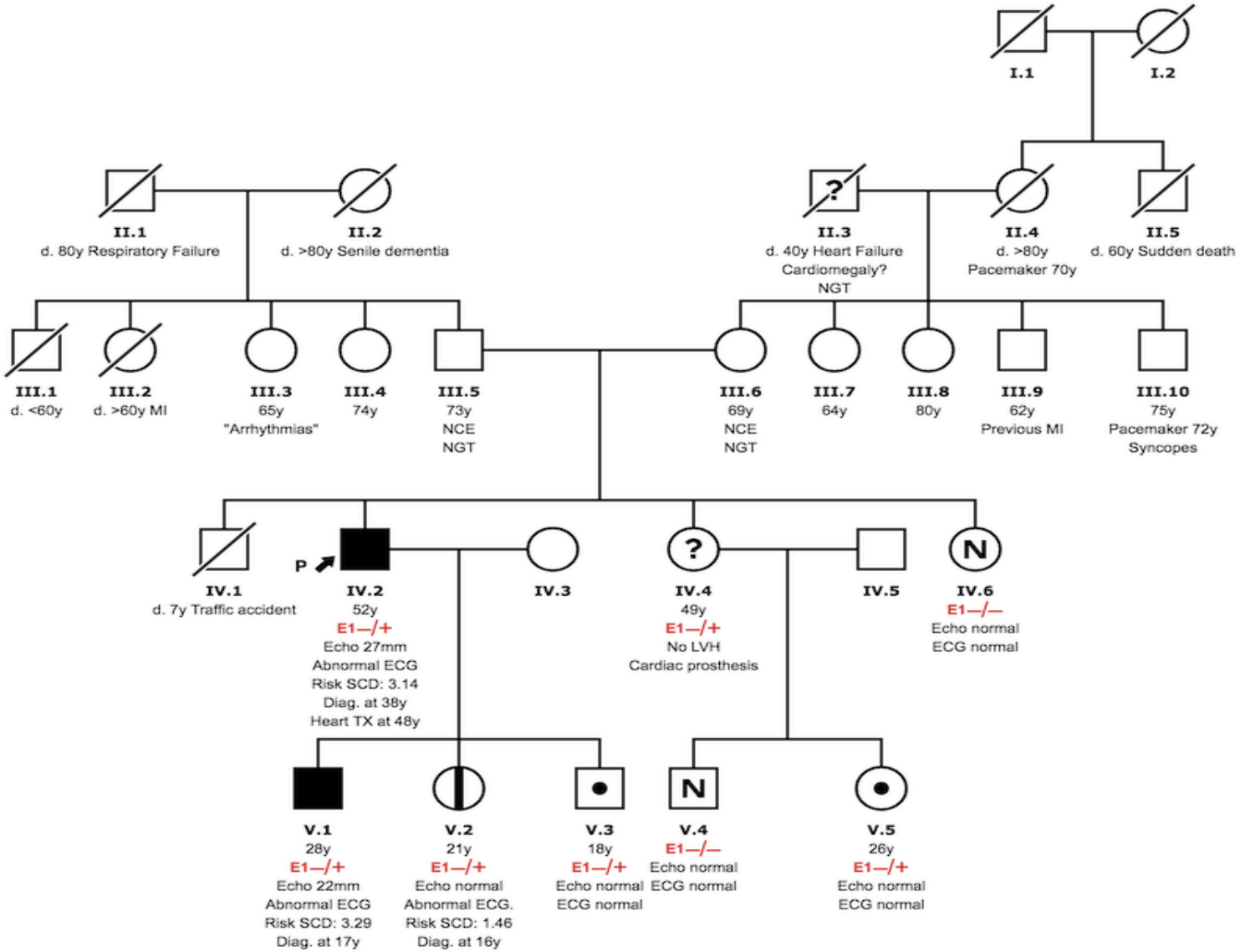
Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VEB, ventricular ectopic beats; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu; E2, other variant (see table); the index case is described in the gray line;

Comments pedigree #10:

1. Complex genotypes: *TPM1* p.Arg21Leu homozygous sisters (II.7 and II.6) showed severe phenotypes although diagnosed at advanced ages. The homozygous carrier II.7 had a more marked phenotype that may be related to the presence of an additional likely pathogenic variant in *MYBPC3*. In contrast, his 42-year-old daughter (III.6), clinically unaffected, was a compound heterozygous carrier of *TPM1* p.Arg21Leu and *MYBPC3* p.Asp75Asn variants.

We consider ***MYBPC3*** **p.Asp75Asn** to be a likely rare pathogenic variant (4 carriers in the gnomAD database). This variant has been identified in HCM index cases, and familial studies showed the presence of both affected and unaffected carriers, suggesting late/incomplete penetrance.

Pedigree #11

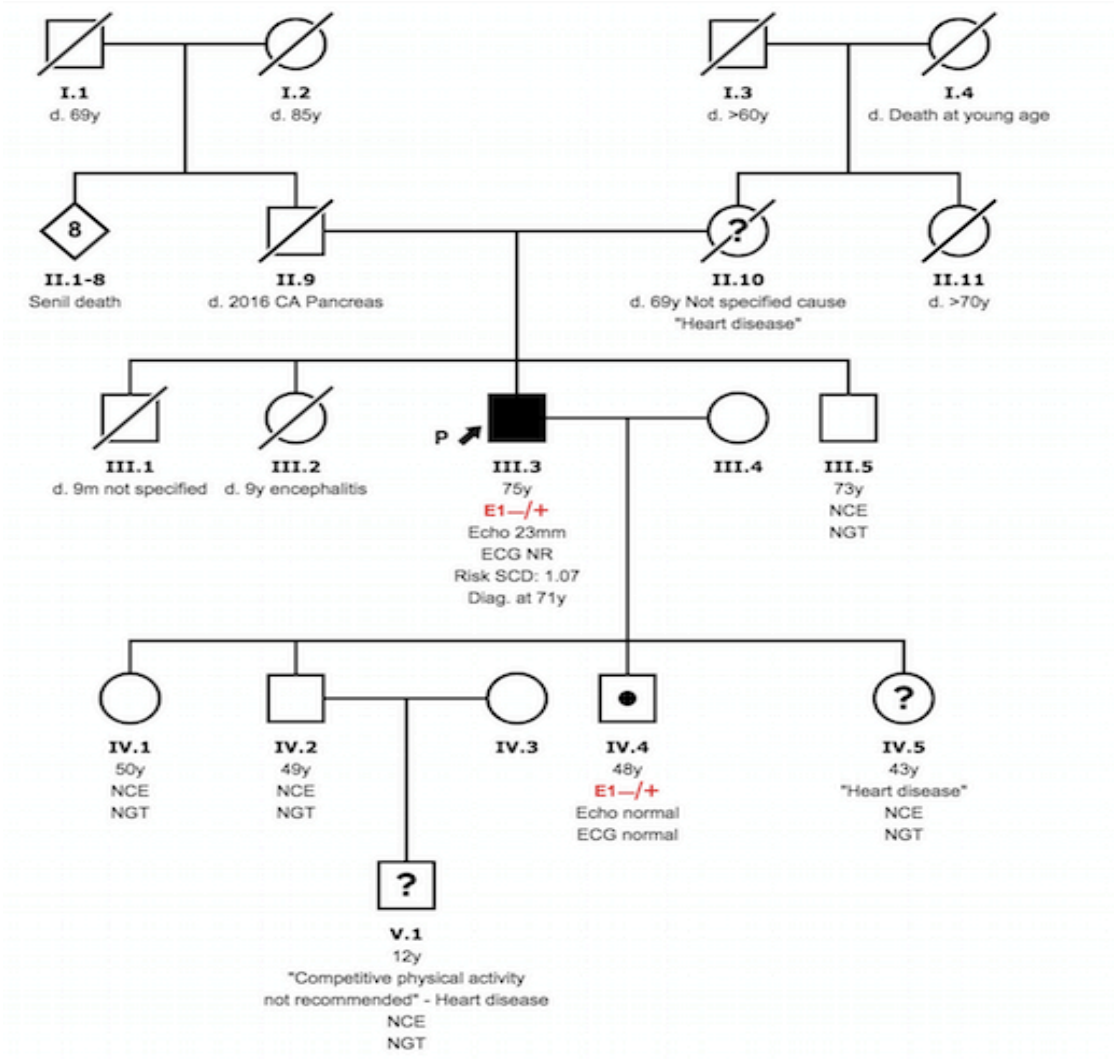


LOD SCORE: 0.17

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FH SD	Max LVH	TV/ FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
IV. 2	M	Yes	No	HCM septal	38	52	III-IV	+	-	27	-	-	-	-	-(65)	Heart transplant at 48 y	**Echo:** LA 58mm, diastolic dysfunctio n II. **ECG:** Atrial Flutter	
IV. 4	F	Yes		?		49												Valvular cardiac disease - prosthesis
V.1	M	Yes		HCM septal	17	28	I	-	-	22	-	-	-	-	-(72)		**Echo:** LA 46mm **ECG:** negative T waves, high QRS voltages	
V.2	F	Yes		Not affected (?)		21	I	-	-	11	-	-	-	-	-		**ECG:** high QRS voltage, Q waves	
V.3	M	Yes		Not affected		18												
V.5	F	Yes		Not affected		26												
II.3	M	NGT		?		40										Heart failure death at 40y		

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

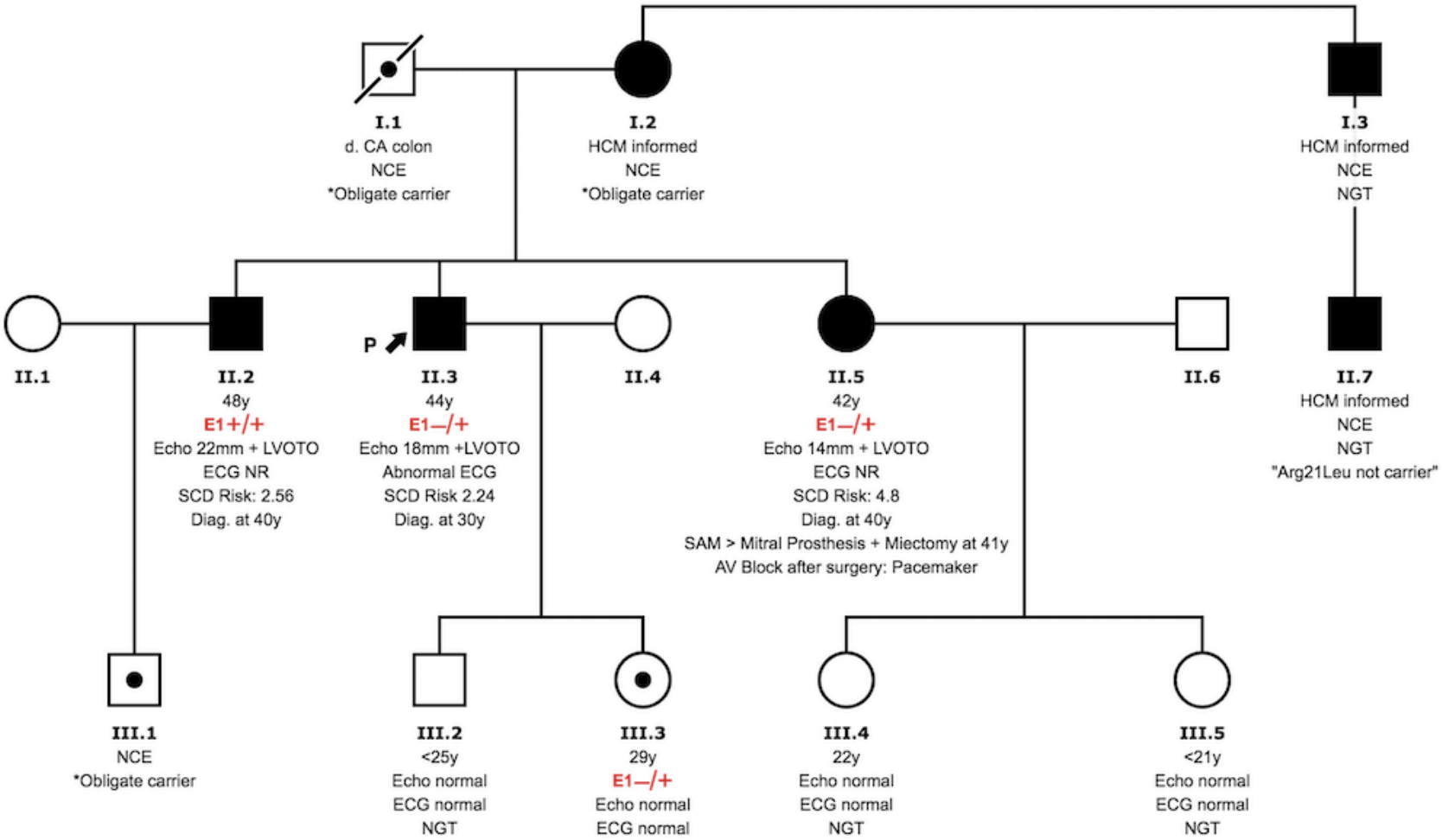
Pedigree #12



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn Vasc Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
III.1	M	Yes	No	HCM septal	71	75	I	-	-	23	-	-	+	-	-(67)			
IV.4	M	Yes		Not affected		48												
II.10	F	NGT		Heart disease not specified		69												Death at 69 y – unspecified cause.
IV.5	F	NGT		Heart disease not specified		43												
V.1	M	NGT		Heart disease not specified		12												"Competitive physical activity not recommended"

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #13



LOD SCORE: 0.34

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FH SD	Max LVH	TV/ FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.3	M	Yes	No	HCM septal	30	44	I	-	-	18	-	-	+	+(60)	-(68)		**Echo:** LA 40mm, diastolic dysfunction I. **ECG**: pathologic Q wave, abnormal repolarization, high QRS voltages	
II.2	M	Yes (Homozy)		HCM septal	38	48	I	-	-	22	-	-	-	+(40)	-(70)		**Echo**: LA 45 mm	
II.5	M	Yes		HCM septal	40	42	III	+	-	14	-	+	-	+(75)	-(68)		**Echo:** elongated mitral valve leaflet, mitral insufficiency	Myectomy and mitral valvular prosthesis at 41 y Pacemaker (AV block) after surgery

III.3	F	Yes		Not affected		29												
I.1	M	Obligate carrier		NCE														
I.2	F	Obligate carrier		HCM reported – NCE		71												
III.1	M	Obligate carrier		NCE														

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; AV, atrioventricular; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. E2, other variant (see table). The index case is described in the gray line.

Comments pedigree #13:

There is the chance of 2 HCM-genetic etiologies in this family. Individual II.7 was studied in another center and reported as being affected by hypertrophic cardiomyopathy, but *"p.Arg21Leu noncarrier".* No details about the genetic testing or phenotype were reported in this patient.

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FH SD	Max LVH	TV/ FV VE	Syncope	Abn Vasc Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
	M	Yes	No	HCM apical	69	72	I	-	-	14	-	-	-	-	-(67)		**Echo**: LA 46 mm. **MRI:** LVH 11 mm septal, LGE septum inferior apical	PTCA Right coronary artery.

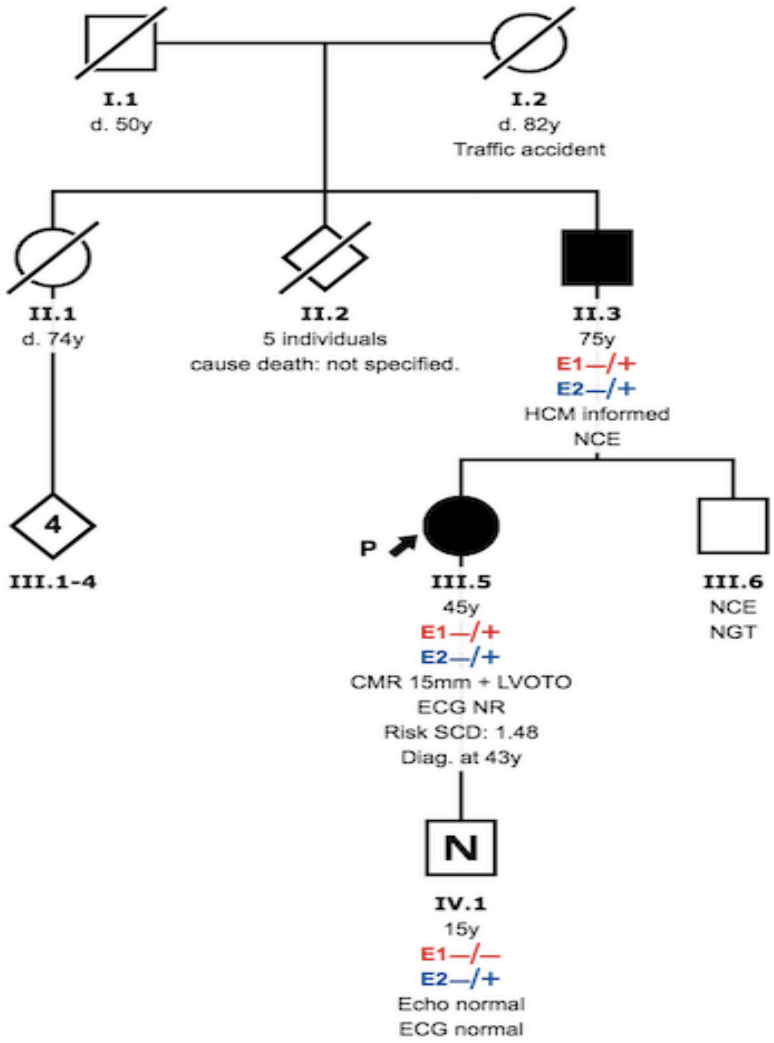
Pedigree #14

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; PTCA, percutaneous transluminal coronary angioplasty; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation. The index case is described in the gray line.

SCD risk: 1.04

No pedigree was reported (no pedigree image).

Pedigree #15



LOD SCORE 0.23

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.1	F	Yes	*MYL3* Met173Val (+?)	HCM septal	43	45	II	-	-	15	-	-	-	+(>30)	-(66)		**MRI**: Mild intramural LGE in the hypertrophy areas and in the insertion of the RV with LV	SAH.
II.3	M	Yes	*MYL3* Met173Val (+?)	HCM reported		75				+								

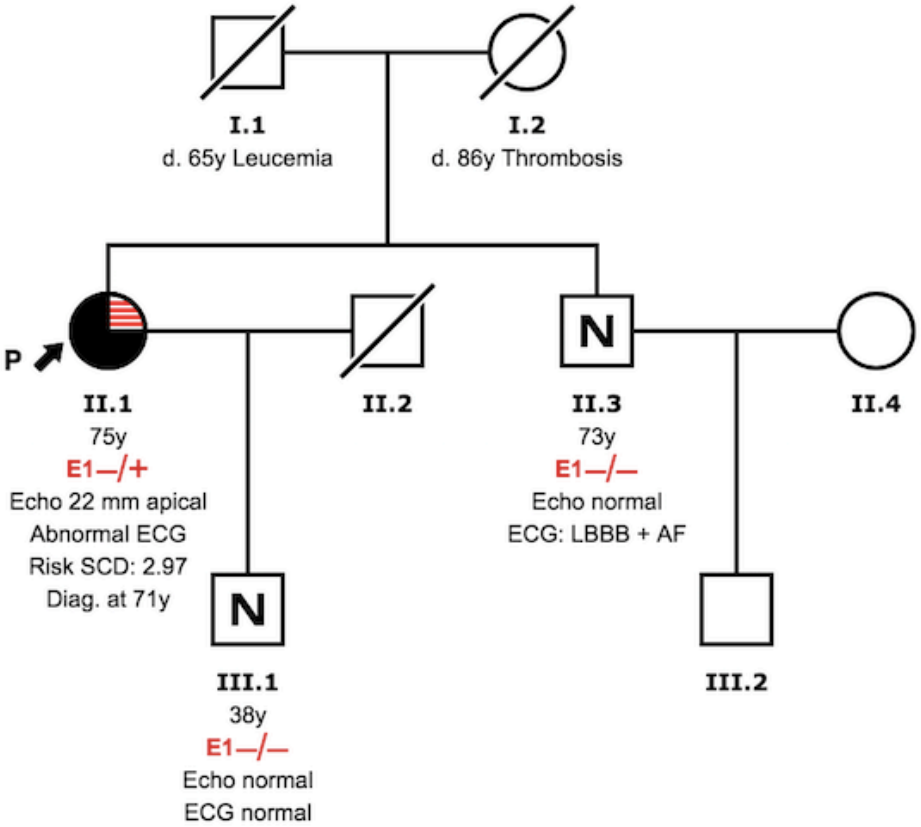
Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), left ventricle systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SAH, Systemic arterial hypertension; RV, Right ventricle; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p;Arg21Leu; E2, other variant (see table). The index case is described in the gray line.

Comments pedigree #15:

1. Complex genotypes: There are 2 carriers with complex genotype in this pedigree, but individual II.3 was studied in another center and no clinical details could be obtained.

We consider ***MYL3*** **p.Met173Val** to be a likely pathogenic variant (+?) that has been associated with the development of hypertrophic cardiomyopathy (28 carriers from 14 families—half of them either unaffected of with unknown phenotype; consequently, familial cosegregating has not been clearly documented). It is present in a single individual from the gnomAD database (control). A functional study suggesting a damaging effect has been published. Data suggest that this variant could be associated with late disease onset and incomplete penetrance.

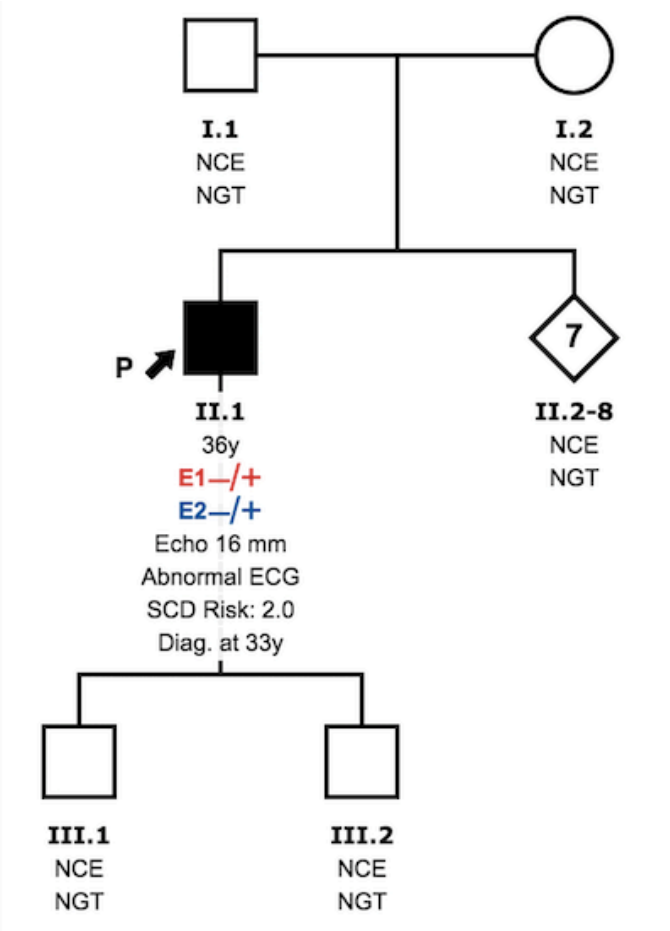
Pedigree #16



Id.	Sex	TPM1 Arg21Leu	Other mutat	Pheno type	Age at Dx	Age , y	NYHA	AF	FH SD	Max LVH	TV/ FV VE	Sync ope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.1	F	Yes	No	HCM apical	71	75	II	+	-	22	-	+	-	-	-(82)		**Echo:** LA 47 mm, moderate mitral insufficiency and mild tricuspid insufficiency. **ECG:** *in sinusal* negative T waves, high QRS voltage. Atrial flutter/ fibrillation	SAH **Retinitis pigmentosa (bilateral blindness)**

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SAH, Systemic arterial hypertension; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #17



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.1	M	Yes	*MYH7* Lys351Asn (?)	HCM septal	33	36	I	-	-	16	-	-	-	-(17)	-(84)		**Echo:** LA 42 mm **ECG:** normal	Mild SAH

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SAH, Systemic arterial hypertension; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. E2, other variant (see table). The index case is described in the gray line.

Comments:

1. **Complex genotype:** The young age at diagnosis of this carrier (II.1) could be related to the presence of the MYH7 variant; however, he did not have a severe phenotype.

We consider ***MYH7* p.Lys351Asn** to be a variant of unknown clinical significance (?). It has been described to date in this single HCM-patient, and also in a single carrier from the gnomAD population (control). Two missense variants located at the same amino acid (p.Lys351Glu/Thr) have been identified in HCM cases (6 unrelated carriers).

Pedigree #18

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
	M	Yes	No	HCM septal	13	32	III	-	-	51	-	+	+	+(140)	-(55)		**Echo:** LA 47 mm, RA enlarged, severe mitral regurgitation, SAM. Wave S <8 (tissue Doppler) **ECG:** High QRS voltages	Myectomy. Primary prevention–ICD implanted

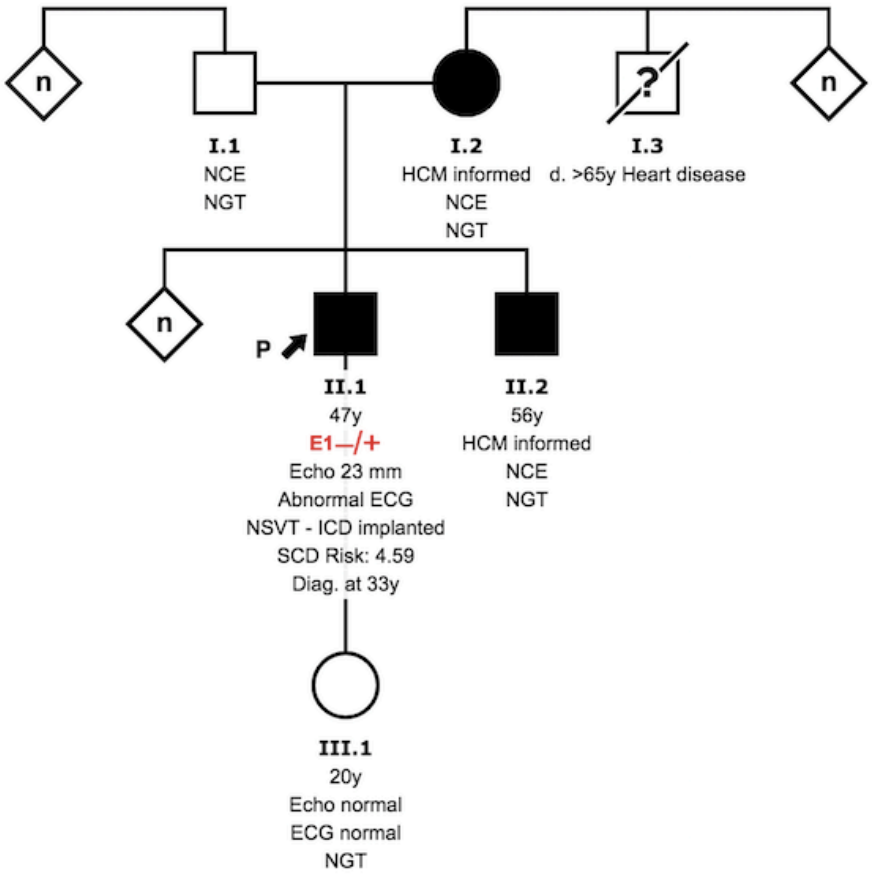
Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF),

systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

SCD Risk: 9.94

No pedigree was reported (no pedigree image).

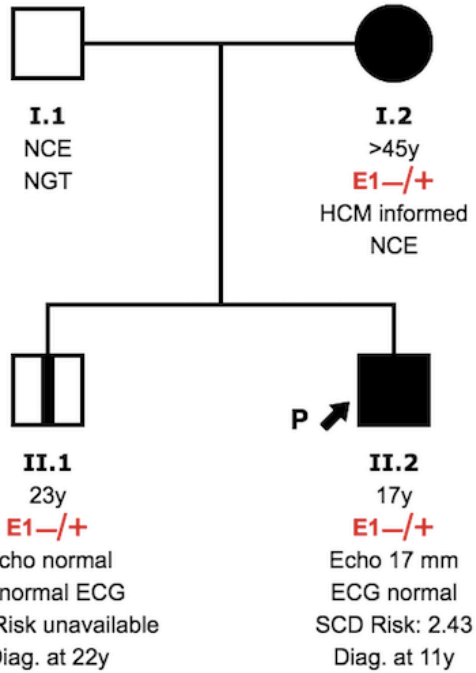
Pedigree #19



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV / FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.1	M	Yes	No	HCM septal	33	47	I	-	-	23	+	No	No	-	-(>55)		**ECG:** First-degree AVB, high QRS voltage	Primary prevention – ICD implanted
II.2	M	NGT		HCM reported		56												
I.2	F	NGT		HCM reported		?												
I.3	M			Heart disease not specified		>65										Heart disease death ➢		

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #20



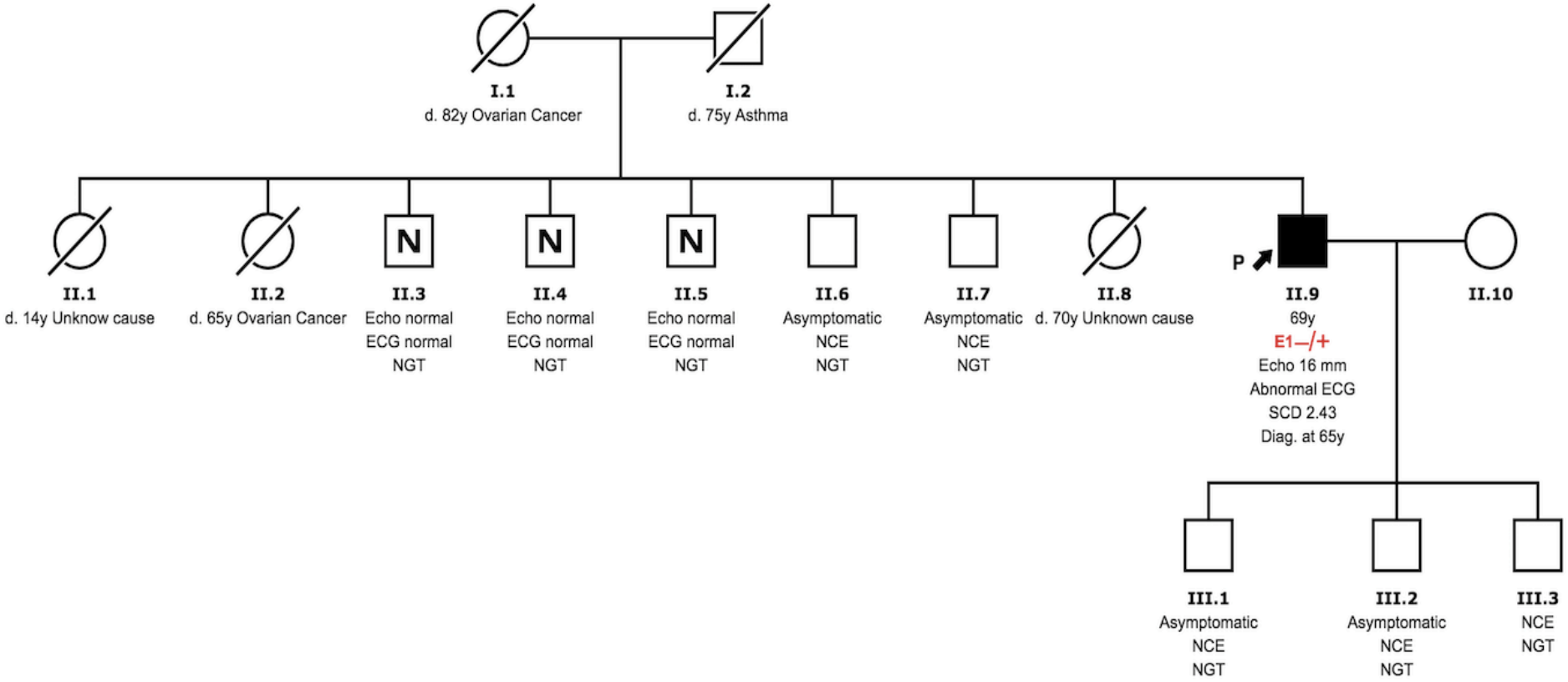
LOD SCORE: 0.17

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV / FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.2	M	Yes	No	HCM septal	11	17	I	-	-	17	-	No	No	-	-(77)			
I.2	F	Yes		HCM reported	<45	>45												
II.1	M	Yes		HCM	22	23	I	-	-	-	-	No	-	-	-		**ECG:** High voltages	

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF),

systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

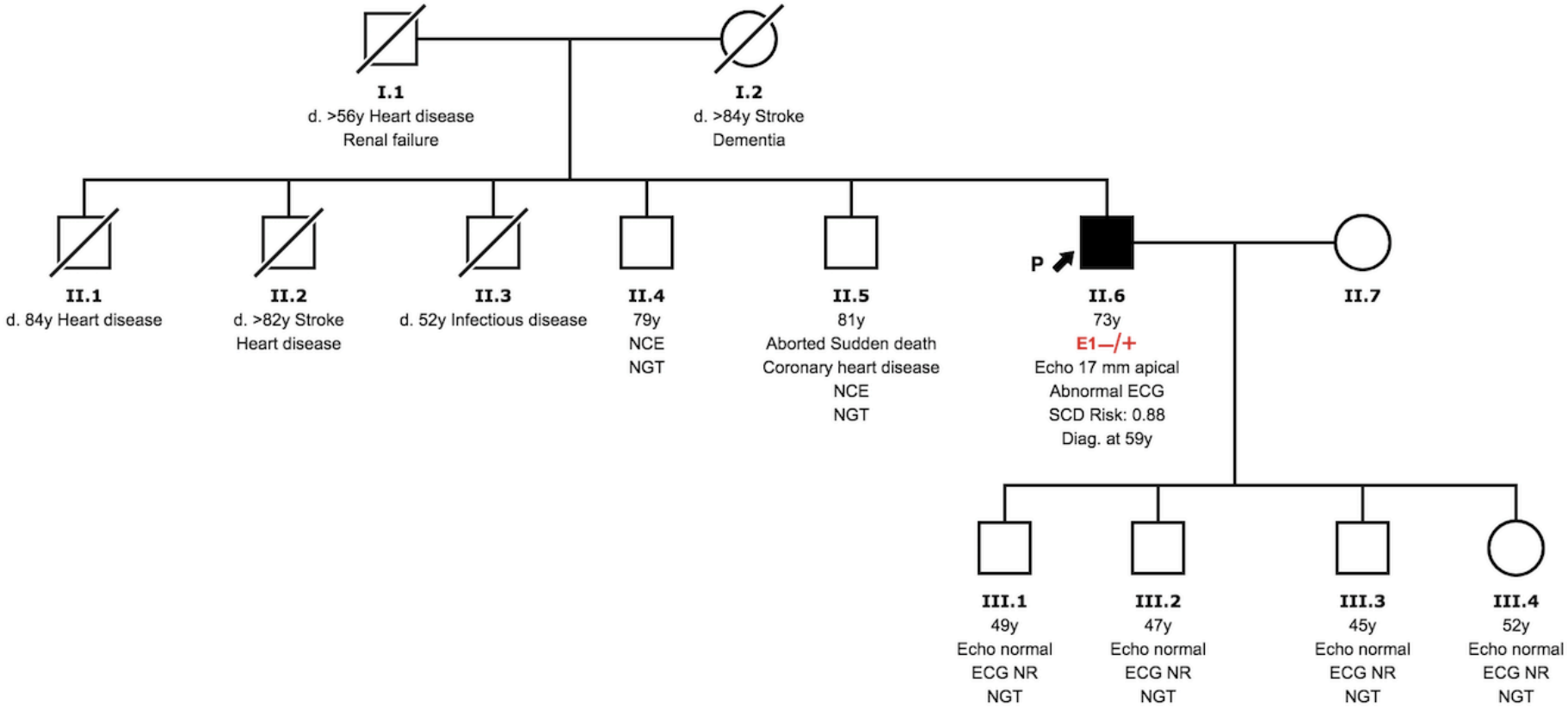
Pedigree #21



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.9	M	Yes	No	HCM septal	65	69	I	-	-	16	-	No	No	-	-(72)		**Echo:** LA dilatation, diastolic dysfunction I. **ECG:** High QRS voltages, pathologic Q waves, negative T waves.	

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

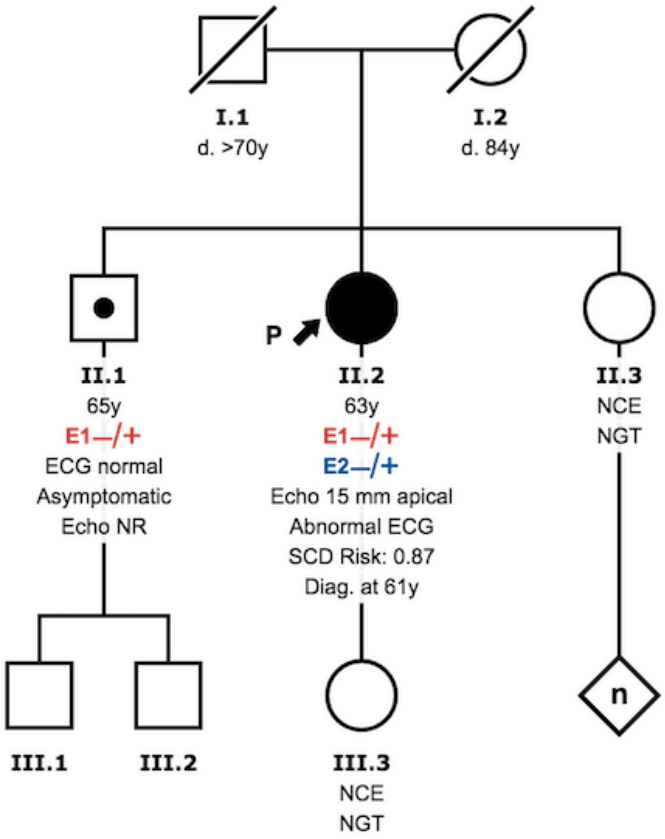
Pedigree #22



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II6	F	Yes	No	HCM apical	59	73	I	-	-	17	-	-	-	-	-(77)		**MRI**: No late gadolinium enhancement **ECG**: QRS high voltages, negative T waves V_2-V_6, DI-II **Holter:** VEBs >200/24 h	
I.1	M				56											Heart disease death at 56 y		

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); Max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VEB, Ventricular ectopic beats; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #23



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II2	F	Yes	*MYH7* Leu1333 Val (?)	HCM apical	61	63	I	-	-	15	-	-	-	-	-(58)		**ECG:** High voltages, LVH and right ventricle hypertrophy, short PR?	

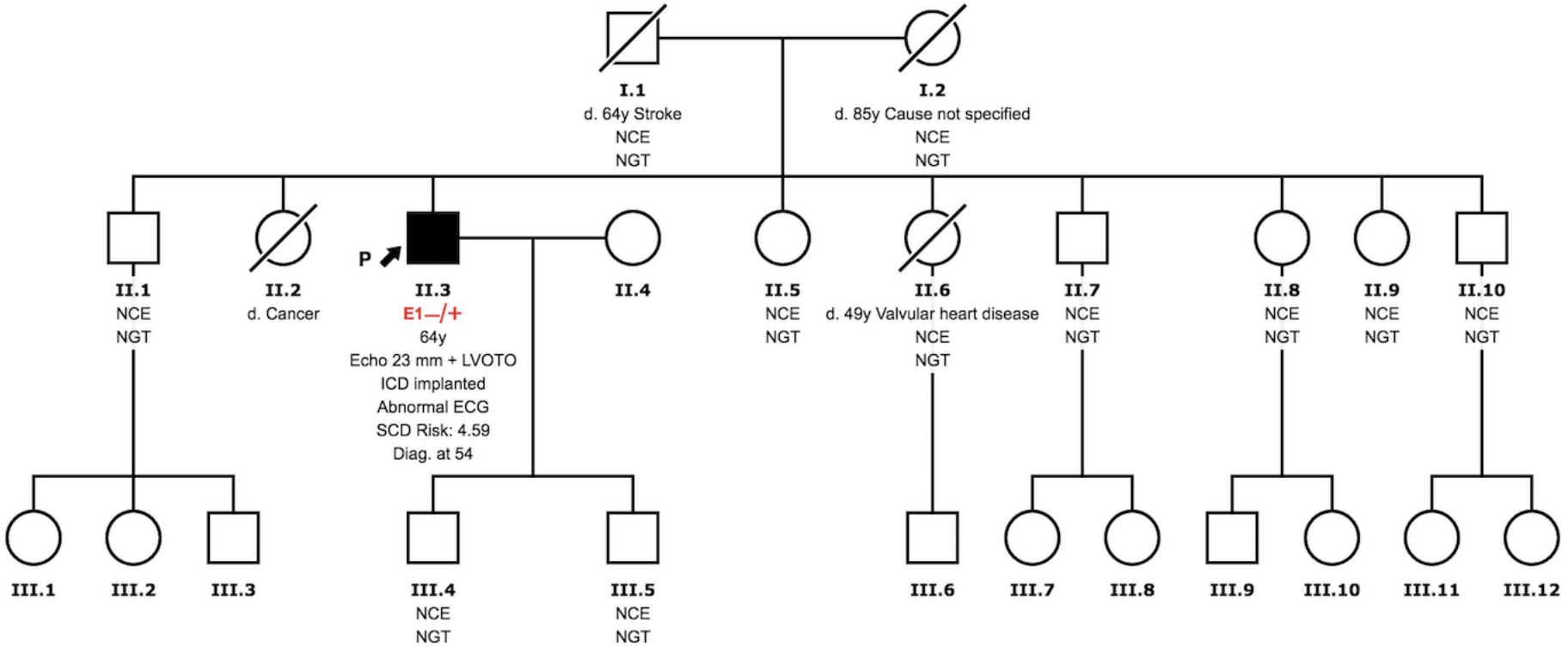
Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); Max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu; E2, other variant (see table). The index case is described in the gray line.

Comments:

1. **Complex genotype:** Patient II.2, with a mild phenotype, carried an additional *MYH7* variant of uncertain significance.

We consider ***MYH7* p.Leu1333Val** is a variant of unknown clinical significance (?). This is the third HCM index case we have identified with the variant, and we have identified it in another index with DCM phenotype. It has been reported in 4 heterozygous individuals from the gnomAD database. These data could suggest late/incomplete penetrance for the variant, but its pathogenicity is still uncertain.

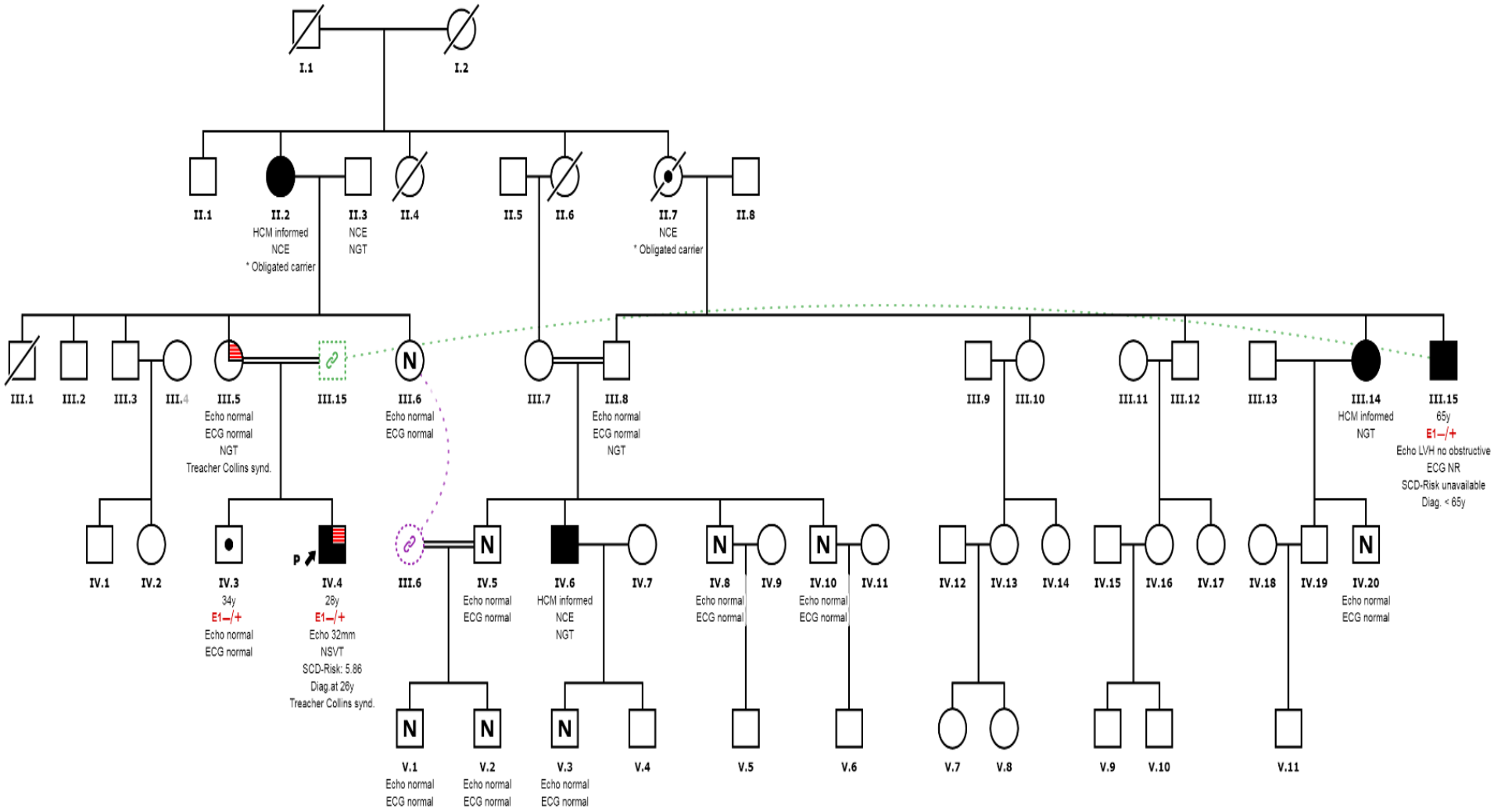
Pedigree #24



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.3	M	Yes	No	HCM septal	54	64	I	-	-	23	-	+	+	+(88)	-(60)			SAH severe ICD implanted
I.1	M	NGT		NCE		64										Stroke-related death at 64 y		
II.6	F	NGT		Heart disease		49										Valvular heart disease—death at 49 y		

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #25



LOD SCORE: 0.17

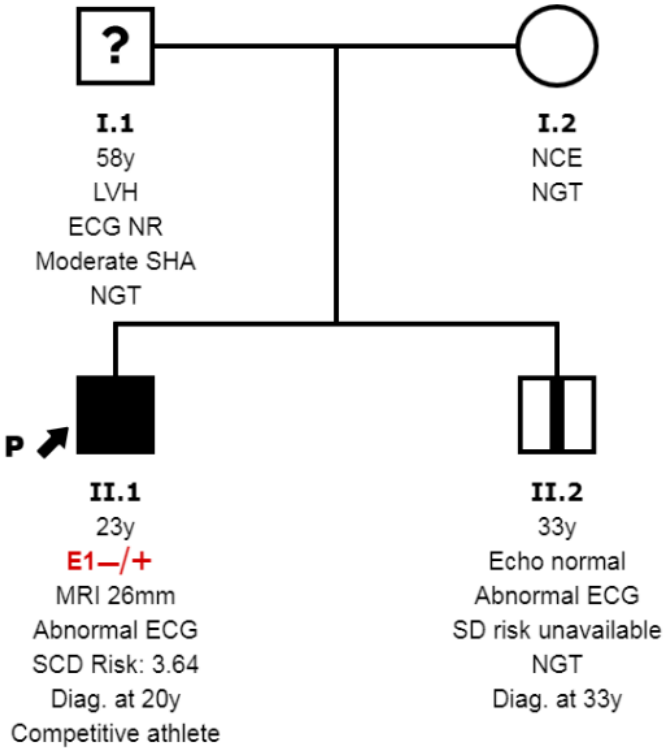
Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
IV.4	M	Yes	No	HCM septal	26	28	I	-	-	32	+	-	-	-	-(70)		**Echo:** LA 35mm	**Treacher-Collins syndrome**
III.15	M	Yes		HCM	<65	65	?	?	-	+	?	?	?	-	-			
IV.3	M	Yes		Not affected		34	I	-	-	-	-	-	-	-	-			
II.2	F	Obligate carrier		HCM reported														
II.7	F	Obligate carrier		NCE														

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Comments pedigree #26:

This pedigree has a carrier (II.3) with severe phenotype diagnosed at a young age, who also had a diagnosis of Treacher-Collins syndrome. Cardiac involvement has not been reported among the clinical features of this syndrome. His older brother is a clinically unaffected *TPM1* p.Arg21Leu carrier.

Pedigree #26



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.1	M	Yes	No	HCM septal	20	23	I	-	-	26	-	-	-	-	-		**ECG:** High voltages, abnormal repolarization. **MRI:** LGE 2 segments, LA 41 mm	Football practice from the age of 9 to 22 y
II.2	M	NGT		Not affected (?)		33	I	-	-	-	-	-	-	-	-		**ECG:** High voltages, abnormal repolarization	
I.1	M	NGT		?		58	I	-	-	+	-	-	-	-	-			Moderate SAH

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SAH, Systemic arterial hypertension; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #27

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
	F	Yes	No	HCM septal	60	69	II	-	-	21	-	-	-	-	-		**ECG:** High voltages. **MRI:** LGE anteroseptal, anterior mid-basal segment. LA 48 mm	mild SAH

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SAH, Systemic arterial hypertension; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

SCD risk: 1.73

No pedigree was reported (no pedigree image).

Pedigree #28

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn BP Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
	M	Yes	*MYH7* Tyr582Cys (?)	HCM septal	56	57	II-III	-	-	17	-	-	-	+ (110)	-(61)		**ECG:** High voltages. **MRI:** No fibrosis, LA 45 mm, mild-moderate degenerative mitral regurgitation	

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

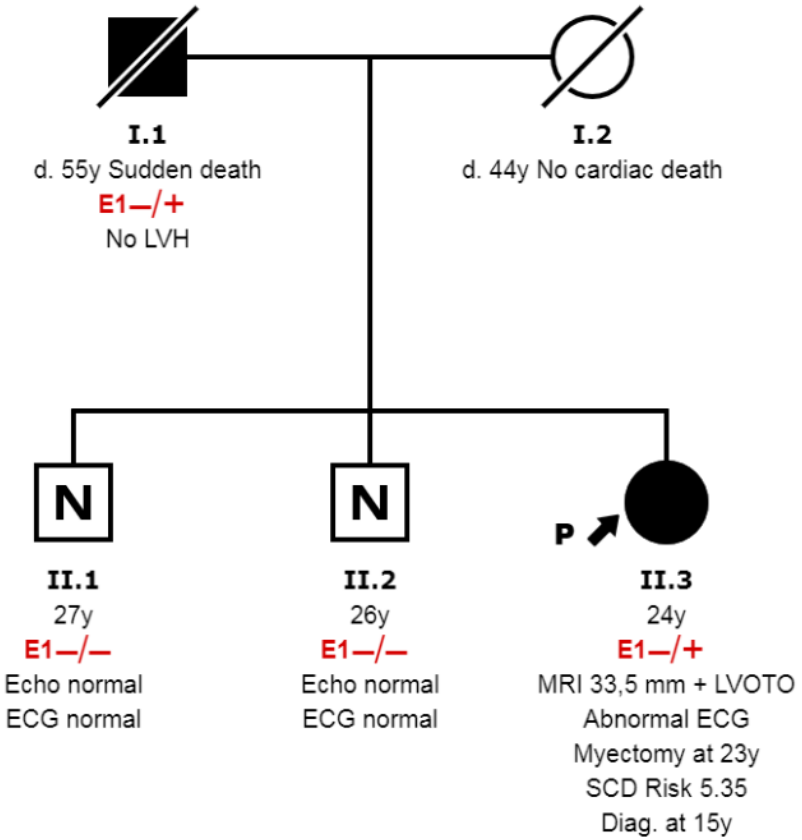
SCD risk: 2.39

No pedigree was reported (no pedigree image).

Comments:

1. **Complex genotype:** Carrier of an additional variant of uncertain significance in MYH7 with mild phenotype and low SCD risk. No myocardial fibrosis in MRI. We consider ***MYH7* p.Tyr582Cys** to be a variant of unknown clinical significance (?). To date, it has been identified only in this single patient. The variant is absent in control populations. It is located within a protein domain in which many other variants have been associated with HCM.

Pedigree #29

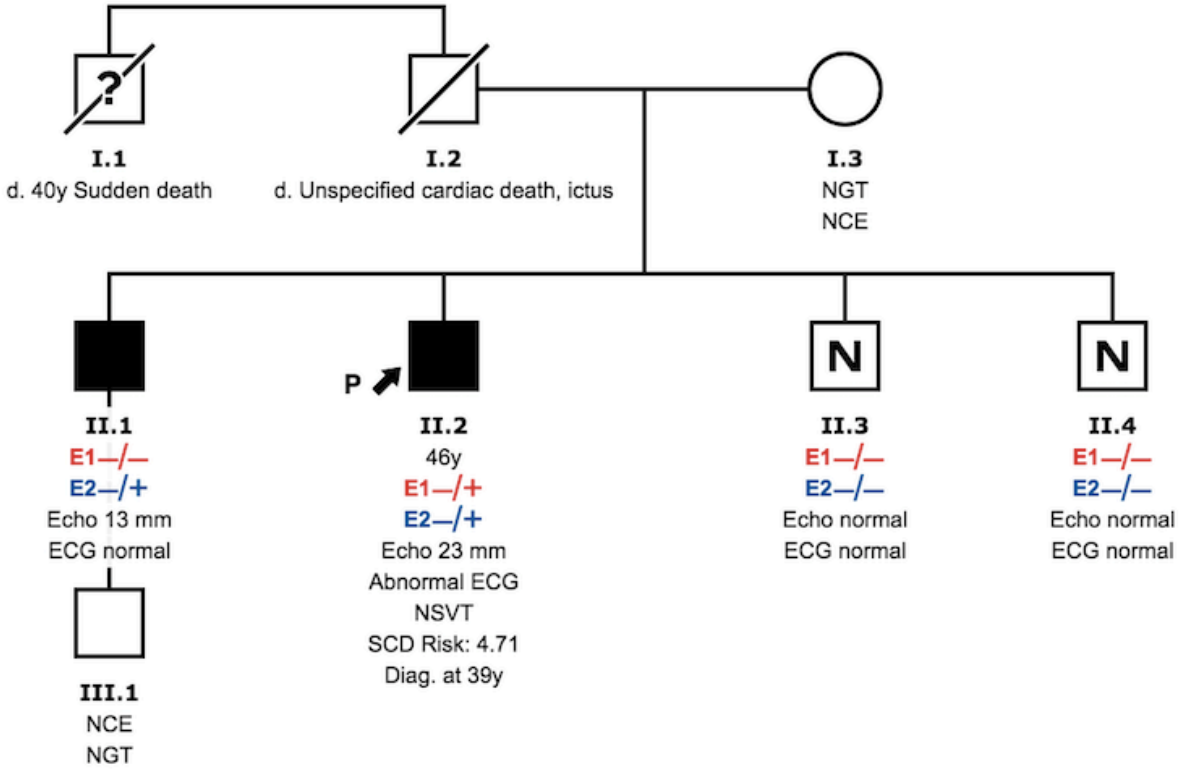


LOD SCORE: 0.23

Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn Vasc Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.3	F	Yes	No	HCM septal	15	24	II	-	+	33.5	-	-	-	+ (90)	-(72)		**ECG:** Q waves **MRI:** LGE in all segments, LA 32 mm	Myectomy at 23y
I.1	M	Yes	No	Sudden Death		55				No						Sudden death at 55y		Autopsy. reported without LVH. Investigated with NGS panel in another center

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line.

Pedigree #30



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn Vasc Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.2	M	Yes	*TPM1* Met281 Val (+++)	HCM septal	39	46	I	-	+	23	+	-	-	- (20)	- (77)		**MRI:** LGE > 3 segments **Holter:** NSVT 3 beats	
I.1	M	?	?			40										Sudden death at 40 y		

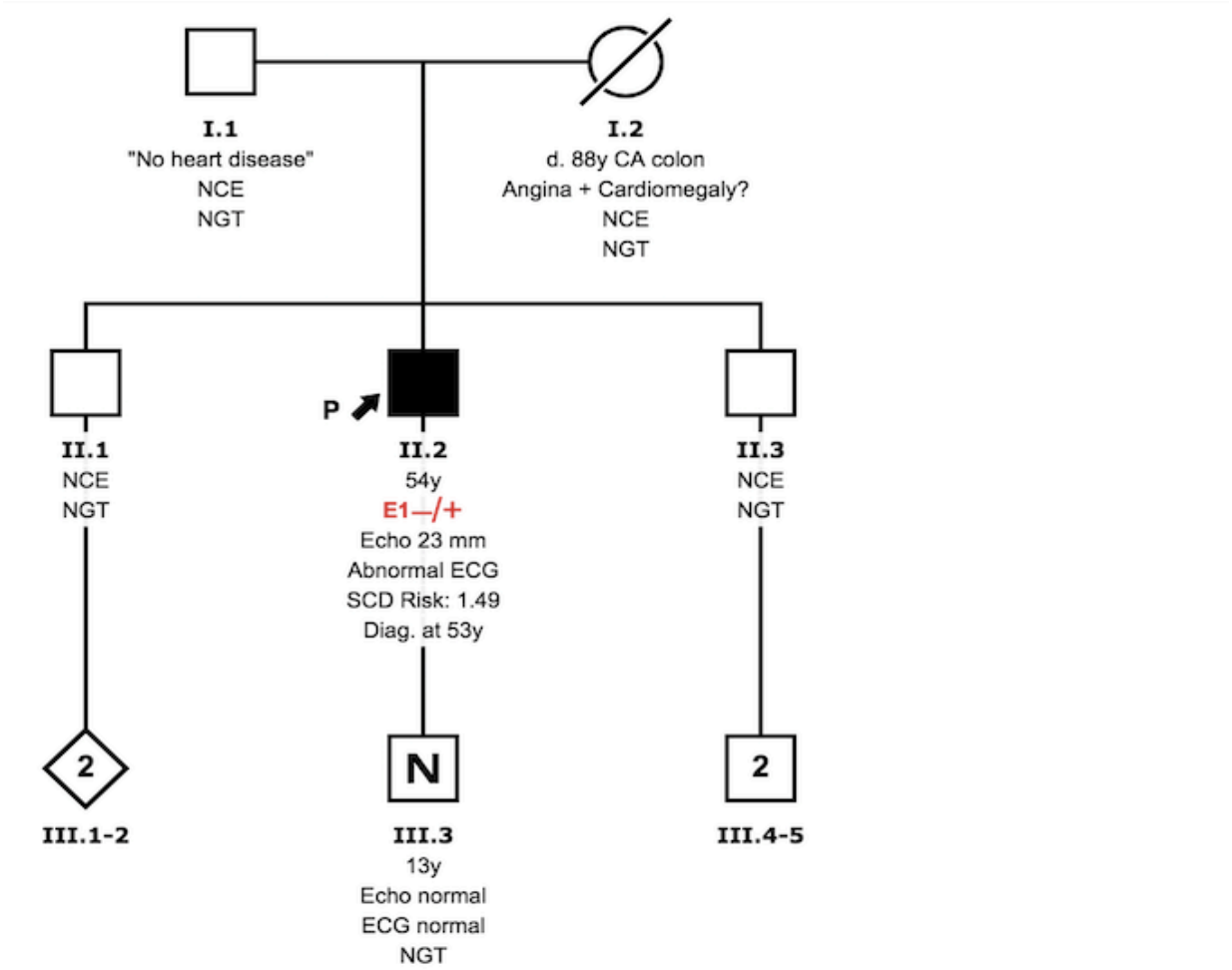
Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SAH, systemic arterial hypertension; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu; E2, other variant (see table). The index case is described in the gray line.

Comments pedigree #30:

1. Complex genotype: Two pathogenic variants in TPM1 were identified in this family. Only individual II.2 carried both variants. Compared to his brother (II.1), who carried only the *TPM1* p.Met281Val, the clinical expression of the index case was more severe and could suggest a synergic effect of the 2 variants. The paternal uncle (I.1) had a sudden death at 40 years of age; we cannot determine if he had only 1 of the family's variants or both.

We consider ***TPM1*** **p.Met281Val** to be a rare pathogenic variant (8 individuals in gnomAD). This variant has been identified in at least 25 HCM-pedigrees (30 affected carriers and 8 unaffected relatives). Familial cosegregation has been documented in at least 1 large family. Clinical data of the carriers suggest late/incomplete penetrance (only 5 carriers were diagnosed under the age of 45 years). Another rare variant affecting the same amino acid (p.Met281Thr) has been identified in 5 HCM patients (from 4 pedigrees).

Pedigree #31



Id	Sex	TPM1 Arg21Leu	Other mutat	Phenotype	Age at Dx	Age, y	NYHA	AF	FHSD	Max LVH	TV/FV	Syncope	Abn Vasc Resp	LVOTO (Peak grad)	LV Dysf (EF)	Events	Additional features	Others
II.2	M	Yes	No	HCM septal	53	54	I	-	-	23	-	-	-	- (23)	- (80)		**Holter:** clear	Multiple sclerosis

Abn BP resp, abnormal blood pressure response on exercise; AF, atrial fibrillation; Dx, diagnosis; DCM, dilated cardiomyopathy; ECG, electrocardiogram; Echo, echocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; Id, individual; ICD, implanted cardiodefibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy; LVOTO (peak grad), left ventricle outflow tract obstruction (gradient in mmHg); LV dysf (EF), systolic dysfunction (ejection fraction); max LVH, left ventricular hypertrophy (maximal ventricular wall thickness in mm); MRI, magnetic resonance imaging; NYHA, New York Heart Association functional class; NCE, no clinical evaluation; NGT, no genetic testing; NSVT, nonsustained ventricular tachycardia; SAH, systemic arterial hypertension; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation; E1, TPM1 p.Arg21Leu. The index case is described in the gray line;

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