

SUPPLEMENTARY DATA

Details of genetics testing of the index cases.

A panel of genes associated with Hypertrophic Cardiomyopathy (≥ 21 genes) was performed in 46 out of 47 probands, including: ACTC1, ALPK3, CSRP3, DES, FHL1, FHOD3, FLNC, GLA, LAMP2, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, PTPN11, TNNC1, TNNI3, TNNT2, TPM1, TRIM63, TTR. In 1 proband, Sanger sequencing of MYBPC3, MYH7, TNNT2, TNNI3, and TPM1 was performed. No additional likely pathogenic or pathogenic variants in the same or in other genes associated with the phenotype were identified.

All identified variants were confirmed by Sanger sequencing. Familial screening of the variant identified in the index case was also performed using Sanger sequencing, following standard protocols.

Microsatellite haplotype analysis

The analyzed STR markers were D11S1763, D11S1344, MYBPC3_CA, D11S4109 and D11S1765, distributed across chromosome 11 surrounding the MYBPC3 locus (table S1).

Genomic DNA was amplified by fluorescent PCR, and fragment analysis was performed using Applied Biosystems genetic analyzers with the Applied Biosystems™ LIZ500 internal size standard. Fragment separation profiles and allele sizing were analyzed using

the Microsatellite Analysis Software (MSA) module available on Thermo Fisher Cloud. Generated electropherograms were manually reviewed to confirm allele calling and genotype quality.

Affected individuals carrying the MYBPC3 p.Arg891Alafs*160 pathogenic variant and WT non-carrier controls were included in the study. All individuals were considered apparently unrelated.

Table S1. Genomic localization of STR markers surrounding MYBPC3.

Marker	Chromosomal region	Genomic position (hg38)
D11S1763	11p12	chr11:42,739,764-42,940,067
D11S1344	11p11.2	chr11:46,045,309-46,245,675
p.Arg891Alafs*160	11p11.2	chr11:47,335,943
MYBPC3_CA	11p11.2	chr11:47,340,066-47,340,329
D11S4109	11p11.2	chr11:47,479,854-47,680,126
D11S1765	11q12.2	chr11:61,011,068-61,011,3709

Results of the haplotype analysis surrounding MYBPC3

Microsatellite haplotype analysis using five STR markers flanking the MYBPC3 locus identified a conserved ancestral haplotype among apparently unrelated HCM patients carrying the pathogenic MYBPC3 p.Arg891Alafs*160 variant.

The analyzed markers were distributed across chromosome 11 surrounding MYBPC3, spanning from 11p12 to 11q12.2. A high degree of haplotype conservation was observed in the markers located closest to MYBPC3, particularly the intragenic marker MYBPC3_CA and the proximal marker D11S4109.

A conserved founder haplotype was identified in the majority of affected individuals. The frequency of the founder-associated alleles among variant carriers is shown in table S2.

Table S2. Frequency of founder-associated alleles among MYBPC3 variant carriers.

Marker	Frequency (%)
D11S1763	86.96
D11S1344	90.24
MYBPC3_CA	100
D11S4109	100
D11S1765	87.5

Complete conservation was observed for MYBPC3_CA and D11S4109 among affected individuals, whereas the distal markers D11S1763 and D11S1765 showed lower conservation frequencies, compatible with historical recombination events affecting the ancestral chromosome over successive generations.

WT individuals displayed substantial allelic heterogeneity across all analyzed markers and did not share the proximal disease-associated haplotype observed in variant carriers.

The pattern of maximal conservation in markers located closest to MYBPC3 together with progressive haplotype decay at distal loci strongly supports the existence of a shared ancestral chromosome and a founder effect underlying the recurrent MYBPC3 p.Arg891Alafs*160 variant in this cohort of HCM patients.

Clinical characteristics and outcomes for different founder MYBPC3tv

A systematic literature search was conducted in MEDLINE (PubMed) between February and April 2025 with the search terms: “Hypertrophic cardiomyopathy” (MeshTerm) and “founder” (title or abstract). The search was limited to the English language, and duplicate studies or repeated series from the same cohort were excluded. Sixty-three studies were identified (62 directly from the search terms + 1 identified from reference lists). Only studies with founder MYBPC3tv with 2 or more unrelated families and more than 15 carriers were included. Studies that did not provide the number of participants, the definition of events, the number of events, or follow up time data were excluded. The articles were peer-reviewed by two researchers working independently. A flow-chart with the process of the identification of the studies and the details of the exclusion reasons is presented in [figure S1](#).

Ultimately, 10 studies of different founder MYBPC3tv involving a total of 810 patients with clinical data were selected to compare with the variant presented in this study. The PRISMA guidelines were followed ([table S3](#)), and the review protocol was prospectively registered in PROSPERO (CRD420261407462).

Forest plot figures for annual SCD/aSCD rates and for a composite adverse cardiac event for different published founder MYBPC3tv are presented in [figure S2](#) and [figure S3](#).

Additional clinical characteristics and outcomes from different published founder MYBPC3tv are presented in figure S4.

Figure S1. Flowchart showing the process of identifying the studies

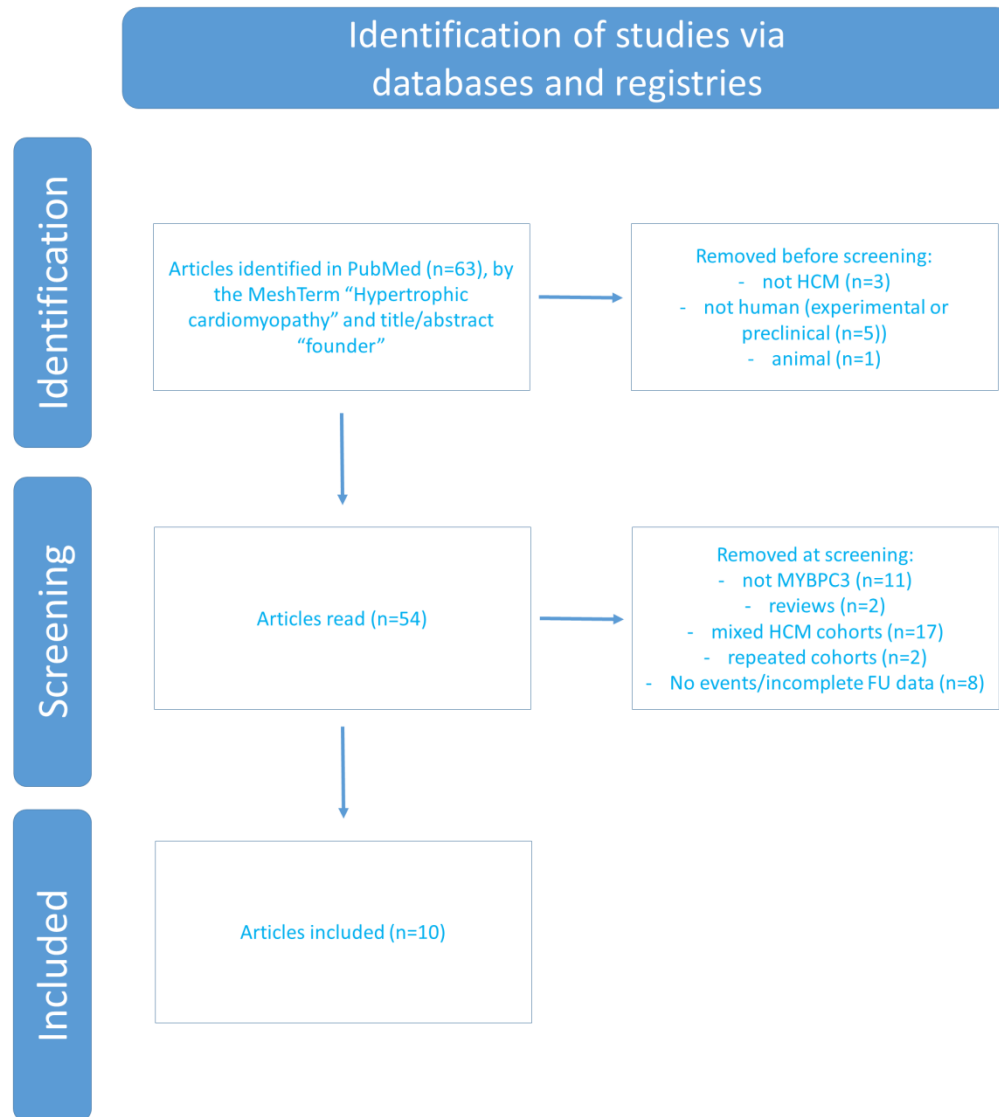


Table S3. PRISMA checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	

Section and Topic	Item #	Checklist item	Location where item is reported
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	

Section and Topic	Item #	Checklist item	Location where item is reported
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	
	23d	Discuss implications of the results for practice, policy, and future research.	
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Figure S2. Forest plot of the annual SCD/aSCD rates for different published founder MYBPC3tv variants.

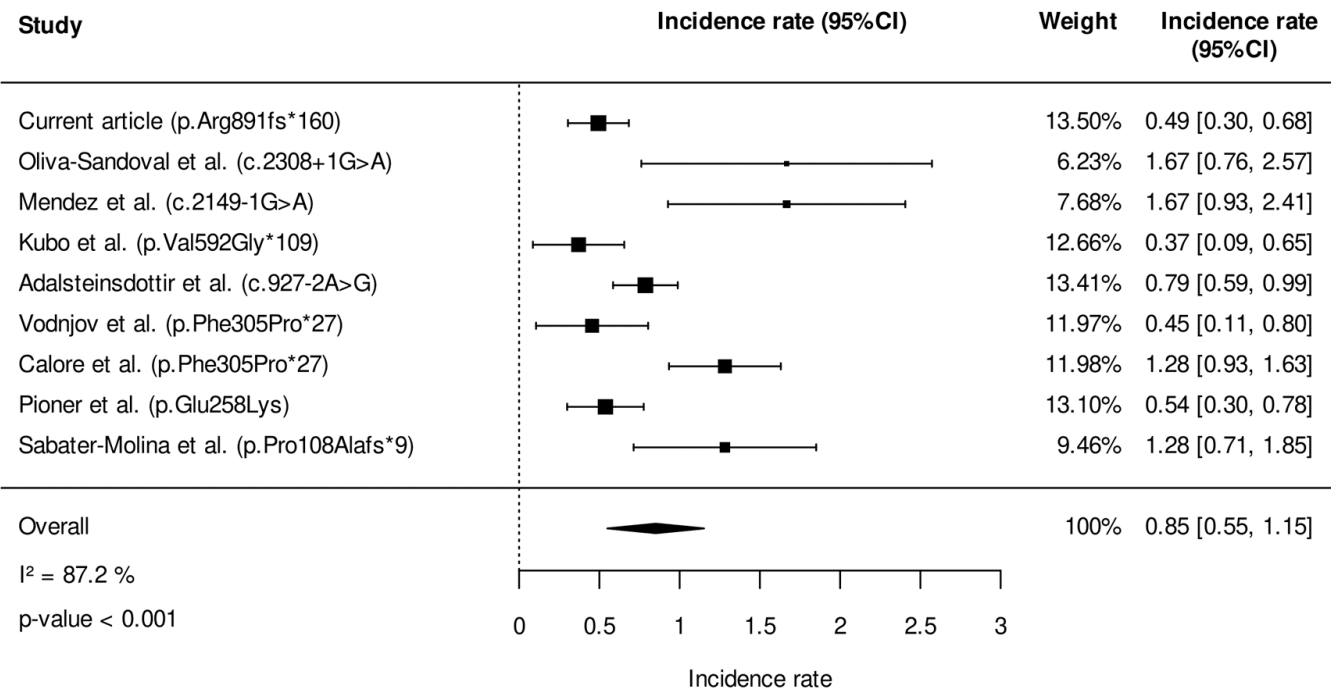


Figure S3. Forest plot of the annual adverse cardiovascular event rates for different published founder MYBPC3tv variants.

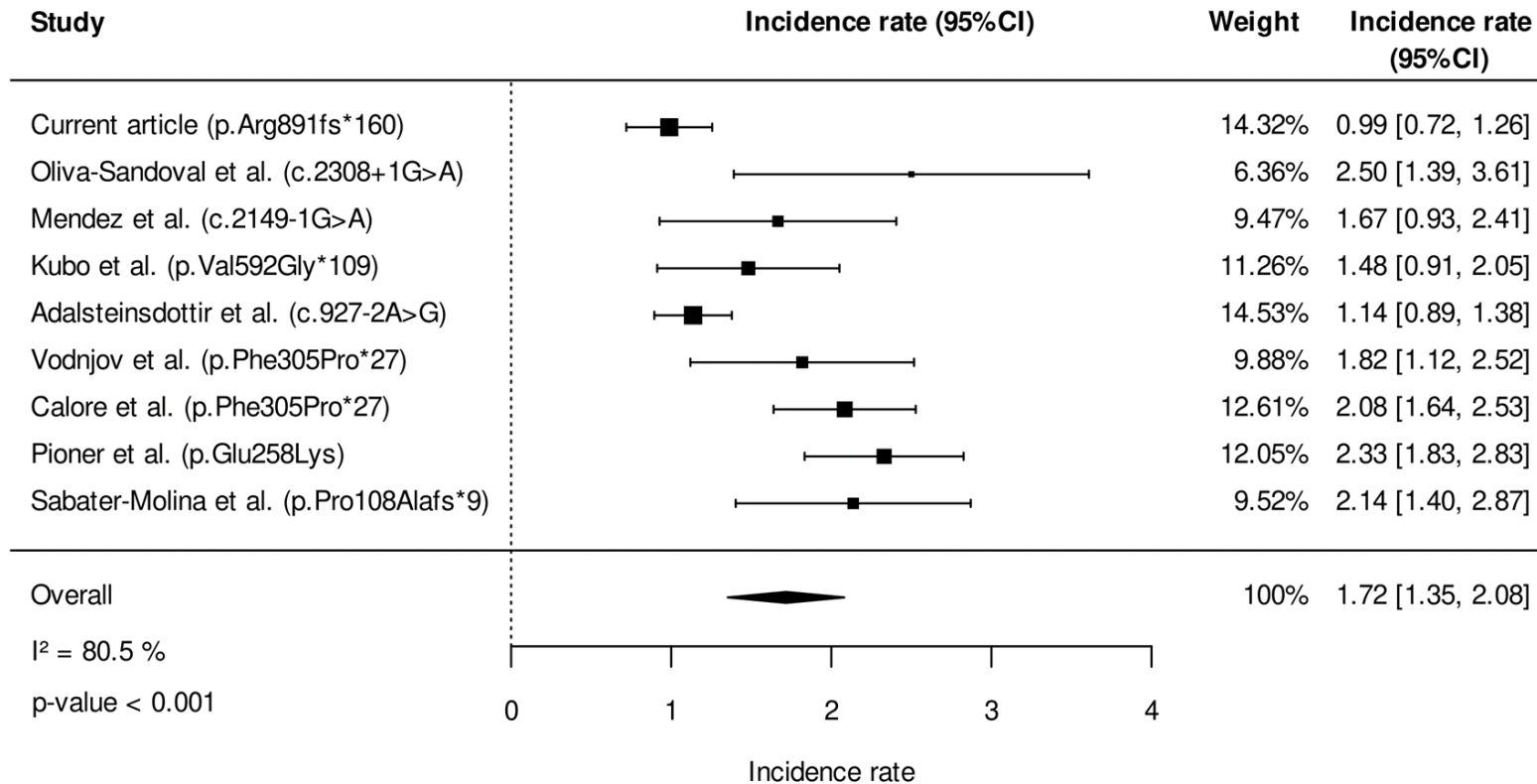


Figure S4. Clinical characteristics for different published founder MYBPC3tv variants and predicted MyBP-C truncated proteins.

Authors	Country / Year	Variant	Carriers (P R)	Females (%)	HCM (n, %)	Age (y)	Diag Age (y)	MLVH (mm)	LVdys (n, %)	HCM (n, %)	FU (y)	SD/aSD (n, %)	SD/aSD Annual Rate	ACE (n, %)	ACE Annual Rate	Protein (%)	Predicted MyBP-C Truncated Protein
Sabater-Molina et al. ²¹	Spain / 2017	p.Pro108Alafs*9	54 (13 41)	33	39 (82)	55	43	20	14 (40)	39 (82)	6	3 (8)	1,3	5 (26)	2,1	8,5	
Pioner et al. ³¹	Italy / 2023	p.Glu258Lys	93 (93 0)	33	93 (100)	NA	50	20	19 (21)	93 (NA)	6	3 (3)	0,5	13 (23)	2,3	20,2	
Lorca et al. ³²	Spain / 2019	p.Gly263*	39 (13 26)	50	34 (87)	57	54	20	3 (9)	34 (87)	5	0 (0)	0,0	0 (0)	0,0	20,6	
Calore et al. ³⁴	Italy / 2015	p.Phe305Pro*27	64 (19 45)	47	48 (75)	55	40	20	6 (13)	48 (75)	13	8 (17)	1,3	13 (35)	2,1	23,8	
Vodnjov et al. ³⁵	Slovenia / 2024	p.Phe305Pro*27	30 (18 12)	43	20 (67)	52	40	18	5 (28)	20 (67)	11	1 (5)	0,5	4 (20)	1,8	23,8	
Adalsteinsdottir et al. ²⁷	Iceland / 2014	c.927-2A>G	88 (12 76)	36	88 (NA)	NA	42	22	6 (7)	88 (NA)	13	9 (8)	0,8	13 (15)	1,1	25,2	
Waldmüller et al. ²⁸	India / 2003	p.Pro453Cysfs*21	15 (2 13)	27	11 (64)	46	34	25	NA	11 (64)	NA	3 (27)	-	4 (36)	-	35,6	
Kubo et al. ²³	Japan / 2005	p.Val592Gly*109	39 (15 24)	43	30 (77)	56	47	21	7 (18)	30 (77)	9	1 (3)	0,4	4 (13)	1,5	46,5	
Teirlinck et al. ²⁹	France / 2012	c.1928-2A>G	34 (9 25)	68	21 (62)	47	NA	NA	NA	21 (62)	NA	0 (0)	-	0 (0)	-	52,0	
Mendez et al. ³³	Spain / 2021	c.2149-1G>A	33 (8 25)	41	20 (62)	NA	37	21	NA	20 (62)	9	3 (15)	1,7	3 (15)	1,7	56,1	
Oliva-Sandoval et al. ²²	Spain / 2019	c.2308+1G>A	65 (18 47)	30	40 (62)	47	41	21	7 (16)	40 (62)	3	2 (5)	1,7	3 (8)	2,5	60,3	
Current article	Spain / 2025	p.Arg891fs*160	159 (47 112)	41	90 (56)	57	44	19	5 (8)	90 (56)	9	4 (4)	0,5	8 (18)	1,0	69,9	
Jaaskelainen et al. ³⁰	Finland / 2013	p.Gln1061*	94 (35 59)	34	73 (78)	49	NA	22	NA	73 (78)	NA	0 (0)	-	0 (0)	-	83,3	
Total			807 (302 505)	40	607 (73)	52	44	21	72 (18)	607 (70)	8	37 (7)	0,9	70 (16)	1,7	-	

ACE, adverse cardiac events; BSA, body surface area; Diag Age, age at diagnosis; FU, follow-up; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; LVdys, left ventricular dysfunction; MLVH, maximal left ventricular hypertrophy; MYBPC3tv, MYBPC3 truncating variant; P/R, proband/relative ratio; SCD/aSCD, sudden cardiac death/aborted sudden cardiac death.

In this supplementary material, forest plots are provided with the estimated annual rate for SCD or aborted SCD and for a global cardiac adverse event to facilitate comparisons (figures S2 and S3).

Definitions of times of starting follow-up, if taken as the date of diagnosis or the date of first evaluation in the referral center, and inclusion or exclusion of sudden death equivalents like resuscitated cardiac arrest or ICD therapies have been shown to influence calculation of annual event rates and might explain some of the observed differences between series.¹

HCM patients from our cohort (p.Arg891Alafs*160) had a significantly lower annual SCD rate than other series, e.g. Oliva-Sandoval et al (c.2308+1G>A), Mendez et al (c.2149-1G>A), Calore et al (p.Phe305Pro*27) and Sabater-Molina et al. (p.Pro108Alafs*9). Similarly, the annual ACE rate in our cohort was also significantly lower than Oliva-Sandoval et al. (c.2308+1G>A), Calore et al (p.Phe305Pro*27), Pioner et al (p.Glu258Lys) and Sabater-Molina et al (p.Pro108Alafs*9). The data indicate fewer life-threatening cardiac events in p.Arg891Alafs*160 affected carriers compared to most of the pathogenic MYBPC3tv.

Part of the complexity of addressing genotype-phenotype association can be observed in the only two described series with the same MYBPC3tv. p.Phe305Pro*27 was first described in Italy² and then in Slovenia.³ The Italian study found that the variant was highly penetrant (high probability of being HCM affected in carriers aged ≥20 years old) and significantly associated with an arrhythmic profile in probands with p.Phe305Pro*27 compared to carriers of other MYBPC3 pathogenic or likely pathogenic variants. The Slovenian study with the same MYBPC3 p.Phe305Pro*27 variant failed to replicate any of these findings.³

The discrepancies between series may be the consequence of different populations, methodological differences, definitions of events, and calculation of follow-up times. The ratio of probands/relatives (P/R ratio) is informative regarding family size and extent of pedigree evaluation. Probands, as demonstrated here, often express more severe disease with worse prognosis versus other affected relatives. Despite published data supporting this hypothesis, the differences in P/R ratio have not been specifically addressed. Analysis from our extended cohort of founder families, including 145 probands and 202 affected relatives (unpublished data) showed that globally the annual rate of SCD or equivalent was 4.4 (95%CI: 2.16-9.12, $p<0.0001$) times higher in probands compared to HCM affected relatives, while the annual ACE rate was 2.0 (95%CI: 1.19-3.43, $p<0.001$) (table S4 and table S5).

Table S4. Annual SCD rates from a local cohort of founder's families with *MYBPC3*tv (includes probands/relative ratio for SCD).

	No.	SCD	FU (y)	SCD annual rate	Ratio P/R SCD
Probands	145	25	6.70	2.57	4.41
Relatives	202	11	9.33	0.58	

FU, follow-up; P/R, proband/relative; SCD, sudden cardiac death; y, years.

Table S5. Annual ACE rates in a local cohort of founder families with *MYBPC3*tv (including the proband-to-relative ratio for ACE).

	No.	ACE	FU (y)	ACE annual rate	Ratio P/R ACE
Probands	145	33	6.46	3.52	2.02
Relatives	202	26	7.39	1.74	

ACE, adverse cardiac events; FU, follow-up; P/R, proband/relative; y, years.

Outcomes in HCM regarding genotype

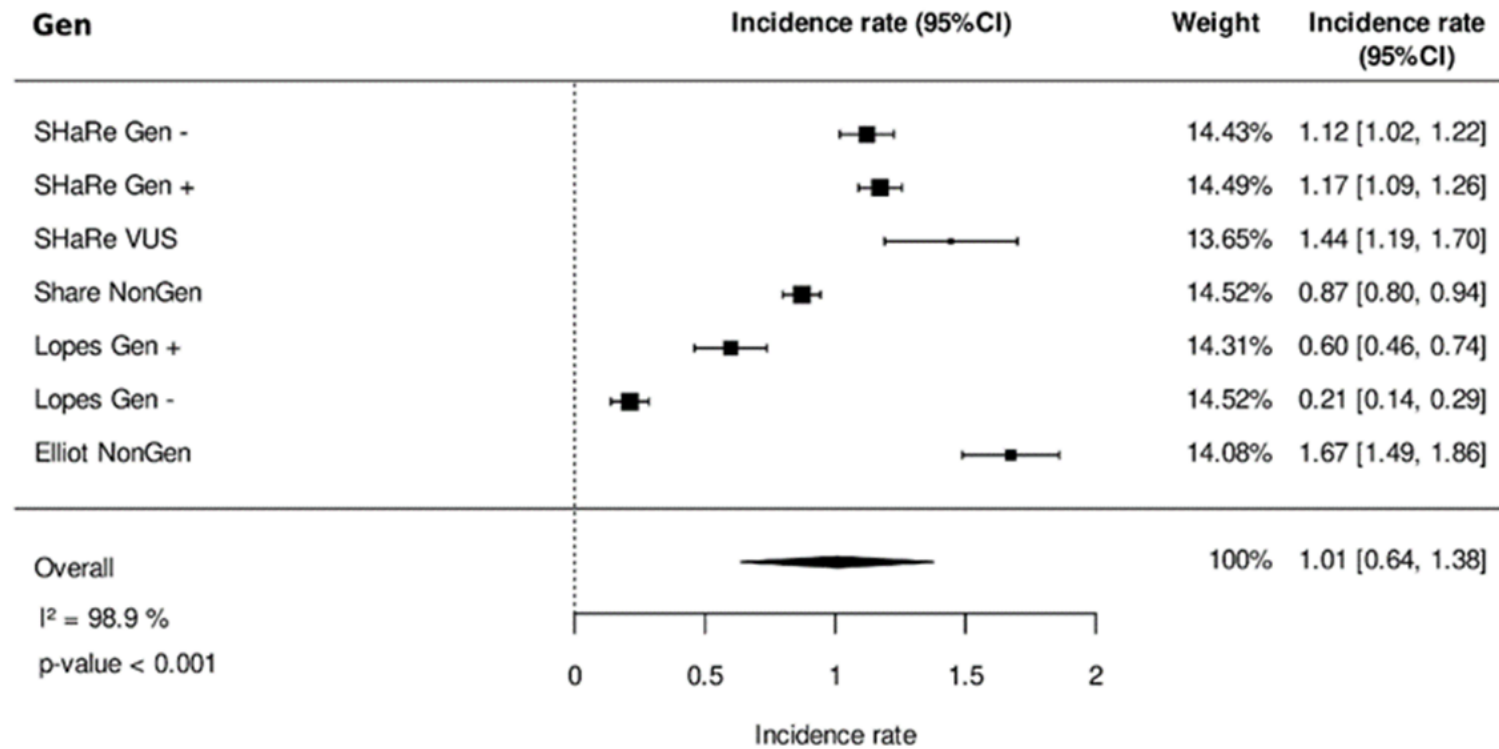
There is an open debate on whether the identification of a pathogenic or likely pathogenic genetic variant in an HCM-associated gene is related to an increased risk of SCD. Although some authors and research groups have provided data to support the use of genetic data for risk stratification of HCM patients, current recommendations do not consider its use as an independent variable in this regard.^{4,3}

Genotype (positive and negative) and non-genotyped forest plots published in HCM series for SCD and adverse cardiovascular events are provided in the supplementary material (figure S5 and figure S6).

The SCD/aSCD annual rate for all MYBPC3tv series is similar to that presented for large nongenotyped HCM populations reported in the literature (1.02, 95% CI 0.76 to 1.26).¹

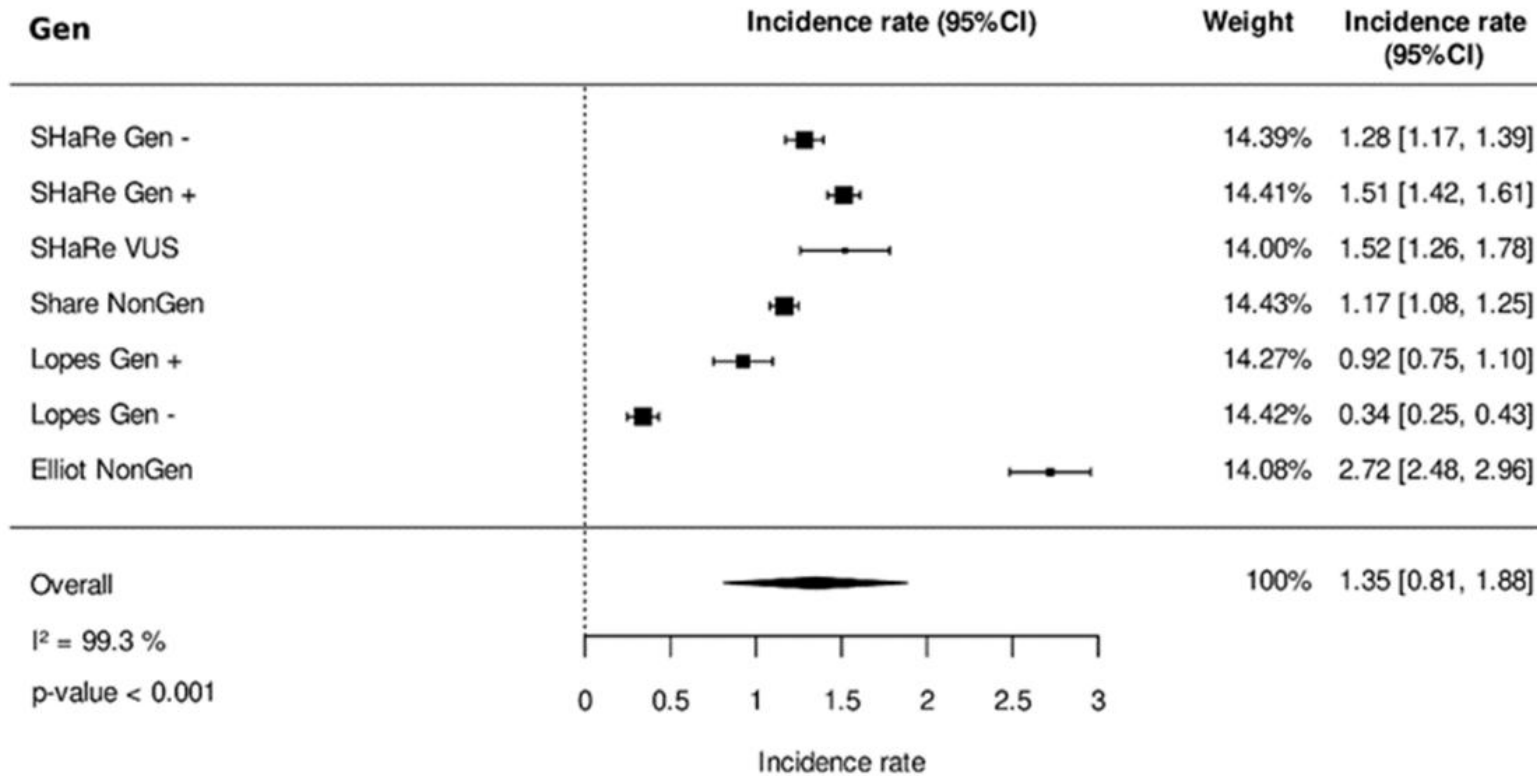
Early historical series that included a large non-genotyped HCM population reported an SCD annual rate of 1.67 (95%CI, 1.49 to 1.86) and more recent large gene negative HCM cohorts reported rate of 1.12 (95%CI, 0.87 to 1.42).

Figure S5. Forest plot of the annual SCD/aSCD rates from published HCM cohorts grouped by genotype.



SCD/aSCD, sudden cardiac death/aborted sudden cardiac death.

Figure S6. Forest plot of the annual adverse cardiovascular event rates from published HCM cohorts grouped by genotype.



The systematic review of published series of MYBPC3tv demonstrated heterogeneity in the cohorts that might explain differences in estimated annual event rates. In particular, the ratio between probands and affected relatives seems to be relevant for risk estimation. Probands often expressed more severe disease, which was associated with a worst prognosis compared with other affected relatives.

Correlation between maximal LVH and age, sex and BSA

Scatter plots showing the relationship between maximal LV wall thickness (MLVWT) and age (figure S7), and MLVWT and BSA (figure S8) in individuals carrying the MYBPC3 p.Arg891Alafs*160 variant. Boxplot showing the distribution of MLVWT regarding sex (figure S9).

Figure S7. Correlation between maximal left ventricular hypertrophy and age.

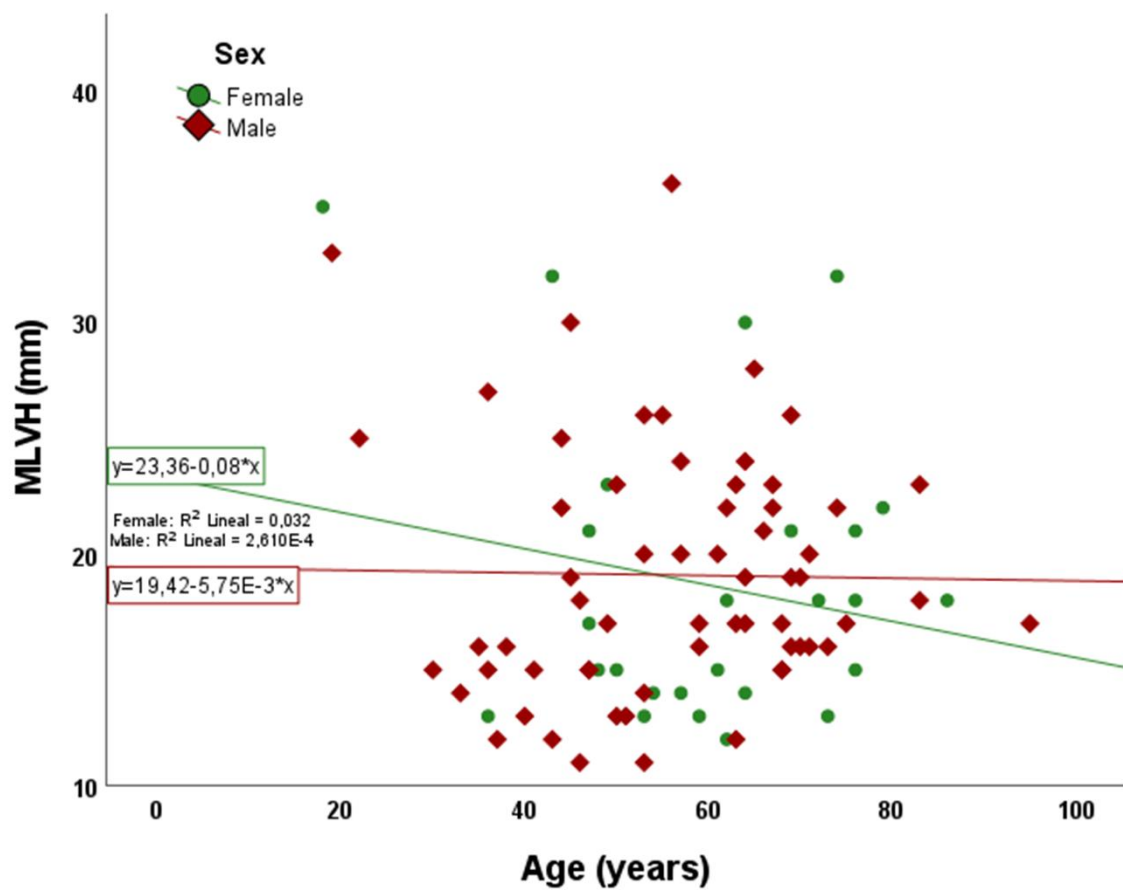


Figure S8. Correlation between maximal left ventricular hypertrophy and BSA.

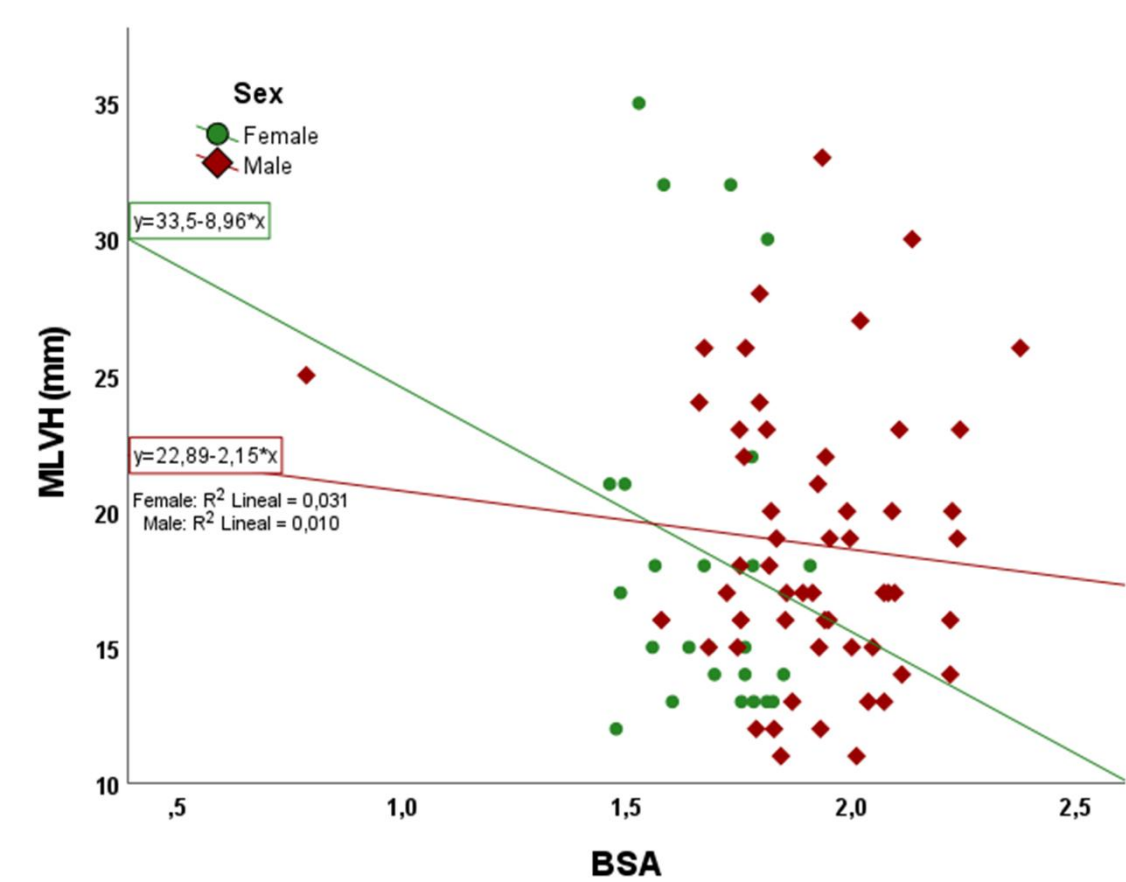
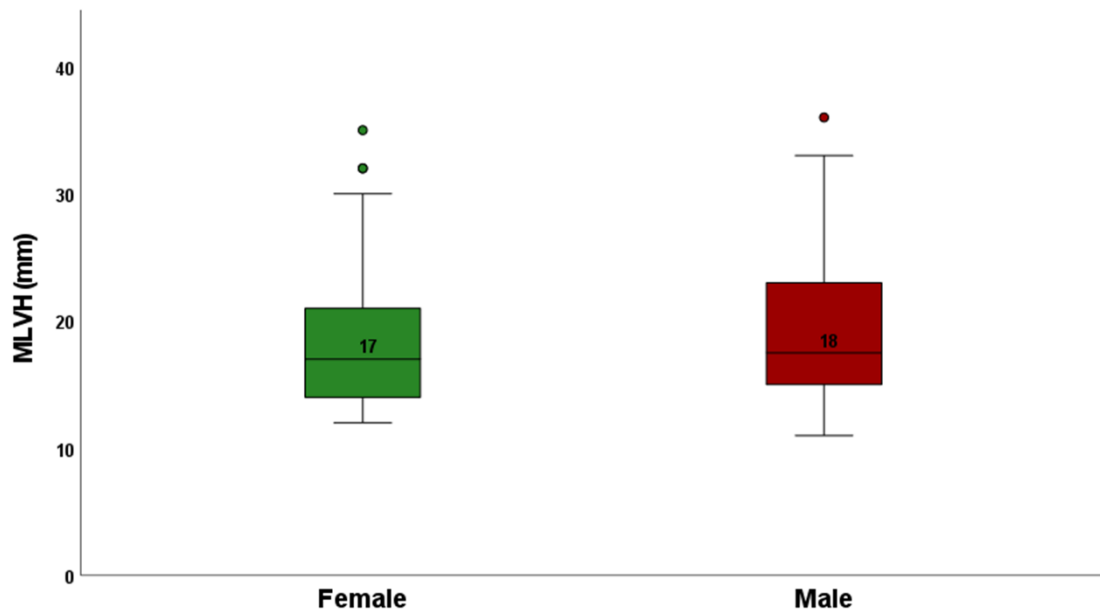


Figure S9. Correlation between maximal left ventricular hypertrophy and sex.



References of the supplementary data

1. Ho CY, Day SM, Ashley EA, et al. Genotype and Lifetime Burden of Disease in Hypertrophic Cardiomyopathy: Insights from the Sarcomeric Human Cardiomyopathy Registry (SHaRe). *Circulation*. 2018;138:1387-1398.
2. Calore C, De Bortoli M, Romualdi C, et al. A founder *MYBPC3* mutation results in HCM with a high risk of sudden death after the fourth decade of life. *J Med Genet*. 2015;52:338-347.
3. Vodnjov N, Maver A, Teran N, Peterlin B, Toplišek J, Writzl K. Clinical Outcome of Hypertrophic Cardiomyopathy in Probands with the Founder Variant c.913_914del in *MYBPC3*: A Slovenian Cohort Study. *J Cardiovasc Transl Res*. 2025;18:110-120.
4. Jiménez-Jáimez J, Alcalde Martínez V, Jiménez Fernández M, et al. Clinical and Genetic Diagnosis of Nonischemic Sudden Cardiac Death. *Rev Esp Cardiol*. 2017;70:808-816.