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Cardiac myosin binding protein-C variants in paediatric-onset hypertrophic cardiomyopathy: natural history and clinical outcomes

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ABSTRACT

Background:

Variants in the cardiac myosin-binding protein C gene (*MYBPC3*) are a common cause of hypertrophic cardiomyopathy (HCM) in adults and have been associated with late-onset disease, but there are limited data on their role in paediatric-onset HCM. The objective of this study was to describe natural history and clinical outcomes in a large cohort of children with HCM and pathogenic/likely pathogenic (P/LP) *MYBPC3* variants.

Methods and Results:

Longitudinal data from 62 consecutive patients diagnosed with HCM under 18 years of age and carrying at least one P/LP *MYBPC3* variant were collected from a single specialist referral centre. The primary patient outcome was a major adverse cardiac event (MACE).

Median age at diagnosis was 10 (IQR: 2-14) years, with twelve patients (19.4%) diagnosed in infancy. Forty-seven (75%) were male and 31 (50%) were probands. Median length of follow-up was 3.1 (IQR: 1.6-6.9) years. Nine patients (14.5%) experienced a MACE during follow-up and five (8%) died. Twenty patients (32.3%) had evidence of ventricular arrhythmia, including 6 patients (9.7%) presenting with out-of-hospital cardiac arrest. Five year freedom from MACE for those with a single or two *MYBPC3* variants was 95.2% (95% CI: 78.6-98.5) and 68.4% (95% CI: 40.6-88.9), respectively (hazard ratio 4.65, 95% CI: 1.16-18.66, p=0.03).

Conclusions:

MYBPC3 variants can cause childhood-onset disease, which is frequently associated with life-threatening ventricular arrhythmia. Clinical outcomes in this cohort vary substantially from aetiologically and genetically mixed paediatric HCM cohorts described previously,

highlighting the importance of identifying specific genetic subtypes for clinical management of childhood HCM.

KEYWORDS

Cardiomyopathy; child; sudden death; sarcomere

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease in adults, with a prevalence of 1 in 500[1]. In contrast, childhood-onset disease is rare, with estimated prevalence rates from population-based studies of ~3 per 100,000[2, 3]. HCM is most commonly inherited as an autosomal dominant trait, caused by mutations in genes encoding components of the cardiac sarcomere in up to 60% of cases, even in young children[4-6]. Around 70% of HCM-causing variants occur in one of two genes: β-myosin heavy chain (*MYH7*) or myosin-binding protein C (*MYBPC3*)[7]. While substantial phenotypic heterogeneity and age-related penetrance are recognised in most sarcomeric HCM-causing gene variants, *MYBPC3* variants in particular have been reported to cause relatively late-onset disease with a milder phenotype[8, 9].

Previous paediatric HCM cohort studies have described aetiologically mixed patient groups, with little focus on specific genotypes[10-12]. Although individual case reports describe childhood onset disease and sudden cardiac death (SCD) caused by compound heterozygous or homozygous *MYBPC3* variants[13-19], there have been no previous studies systematically characterising *MYBPC3* variants as a cause of HCM in children, particularly in heterozygosity. The aim of this study was to describe the natural history and clinical outcomes in a large cohort of consecutive children diagnosed with HCM and carrying variants in *MYBPC3*.

METHODS

Patients

All consecutive children diagnosed with HCM under the age of 18 years with at least one variant in *MYBPC3* classified as pathogenic or likely pathogenic (P/LP) at the time of testing

evaluated at the Great Ormond Street Hospital Centre for Inherited Cardiovascular Diseases between 1998 and 2018 were included in this study. Diagnosis of HCM was made where left ventricular wall thickness was more than two standard deviations greater than the body surface area corrected predicted mean, not solely explained by abnormal loading conditions[20]. Individuals carrying a variant in *MYBPC3* who did not meet diagnostic criteria for HCM (phenotype-negative mutation carriers) were excluded, since the study aim was to describe paediatric-onset disease phenotypes and outcomes, rather than paediatric carriers of *MYBPC3* variants.

Clinical evaluation

All patients underwent systematic clinical evaluation at baseline and throughout follow-up, until transition to adult services at 18 years. Anonymised clinical data were collected at baseline, during 6-12 monthly follow-up and at the most recent clinical review, including: demographics; family history; symptoms; medical therapy; genetic test results; resting 12-lead electrocardiogram (ECG); 2D and Doppler echocardiogram; and, where available, cardiopulmonary exercise testing (CPET); cardiac magnetic resonance imaging (cMRI); and ambulatory ECG monitoring.

Echocardiographic measurements were made according to current guidelines[21]. Maximal left ventricular wall thickness (MLVWT) was defined as the greatest thickness in any single segment, measured on 2D echocardiography at end diastole in the parasternal short-axis view in 4 places at basal and mid-ventricular level (anterior and posterior septum, lateral and posterior wall) and 2 places at apical level (anterior and posterior septum), as previously described[22]. Left atrial diameter was measured in the parasternal long-axis view using 2D or M-Mode. Left ventricular outflow tract obstruction (LVOTO) was defined as a Doppler-

derived pressure gradient >30mmHg on echocardiography[20]. Non-sustained ventricular tachycardia (NSVT) was defined as three or more consecutive beats with a rate faster than 120bpm, self-resolving within thirty seconds[20] and recorded by either ambulatory ECG or by indwelling monitoring device.

Clinical outcomes

The primary patient outcome was a major adverse cardiac event (MACE), defined as death (SCD or heart failure-related death), cardiac transplantation, haemodynamically-compromising sustained ventricular arrhythmia or appropriate therapy from an implanted cardioverter defibrillator (ICD). ICD therapy was considered appropriate where a defibrillation shock was triggered by documented ventricular tachycardia or fibrillation, according to information stored by the device. Information relating to the clinical outcomes of patients transitioned to adult services was sourced from adult cardiology centres.

Genetic Evaluation

Genetic sequencing methods varied according to era, type of test requested (diagnostic or predictive) and individual laboratory conducting testing. Targeted testing of HCM genes was performed using direct Sanger sequencing (3-11 genes) prior to 2011. After 2011, next-generation sequencing was more widely available (21-104 gene panels). Pathogenicity of all variants was reclassified using current American College of Medical Genetics (ACMG) guidelines[23]. Additional variants occurring in other genes previously associated with inherited heart muscle disease were also recorded, where reported. Patients carrying more than one variant with a potential impact on cardiac phenotype were considered to have “complex”

genotypes for the purpose of analysis. Genetic variants are described following the Human Genome Variation Society (HGVS) recommendations[24].

Statistical Analysis

R Studio software version 1.2.1335 was used for statistical analysis of clinical data[25]. Z-scores were used to describe echocardiogram and cMRI measurements relative to corresponding mean values in children of the same body size[26]. Mean values (±SD) were calculated for continuous variables and median values with interquartile ranges (IQR) were calculated for skewed data. Normal distribution was determined using the Shapiro Wilk normality test. The Welch Two Sample t-test was used to compare the means of normally distributed numerical data and the Wilcoxon rank sum test with continuity correction for non-normally distributed numerical data, with one-way analysis of variance used to compare three groups. Pearson's Chi-squared test with Yates' continuity correction and Fisher's exact test were used for comparing independent categorical variables. Survival analysis was undertaken using Kaplan Meier curves with log rank analysis and univariate Cox proportional hazard regression analysis. A p-value of <0.05 was considered statistically significant.

Ethics Approval Statement

The study was approved by the Great Ormond Street Hospital/University College London Institute of Child Health Joint Research and Development Office before data collection commenced (local reference: 18HL01/19HL04). The study was conducted using anonymised, retrospective data, and patient consent was therefore waived in line with local approval.

RESULTS

Clinical Characteristics

Sixty-two patients from 59 families with disease-causing *MYBPC3* variants were identified. Median age at diagnosis was 10 years (IQR: 2-14) (Supplemental Figure 1). Twelve patients (19.4%) were diagnosed in infancy (below 1 year of age). Forty-seven patients (75%) were male, 31 (50%) were the proband in the family and 15 (24%) had a family history of SCD. Twenty-six patients (41.9%) were diagnosed through clinical screening due to family history, 18 (29%) incidentally (following detection of a murmur, during investigation of another health condition or during cardiac screening programmes in the community), 11 (17.7%) due to symptoms, and 6 (9.7%) following presentation with an out-of-hospital cardiac arrest (OOHCA). Fifteen patients (24.2%) were diagnosed prior to 2000, 13 (21%) between 2000 and 2009, and 34 (54.8%) from 2010 onwards. Where sufficient data relating to family history were available, family history of childhood-onset HCM was identified in six families (11.5% of those with information available). In four cases these paediatric relatives presented with SCD. The baseline clinical and echocardiographic characteristics are summarised in Table 1.

Baseline cMRI and CPET data are summarised in Supplementary Table 1. Briefly, 14 patients (22.6%) underwent baseline and follow-up cMRI. At the start of follow-up, median indexed left ventricular mass was 83g/m2 (IQR: 66-119) and mean ejection fraction was 72.4±6.5%. Late gadolinium enhancement (LGE) was observed in 6 of 11 patients (54.5%) who received contrast at baseline. One additional patient without LGE at baseline developed this during follow-up. Thirty-two patients (51.6%) underwent CPET. No patients developed arrhythmia during exercise; 10 children developed ST segment depression or T-wave

changes, of which nine were patients carrying more than one variant in *MYBPC3*. Mean peak VO_2 was 33.4±10.4ml/kg/min.

Genetic testing strategy and results

All patients had undergone genetic testing which identified at least one variant in *MYBPC3* (see Supplementary Table 2 for full list of variants). Forty-one patients (66.1%) underwent diagnostic genetic panel testing, one underwent whole exome sequencing and twenty patients (32.3%) underwent predictive testing for a familial variant. Fifty patients (80.6%) carried a single *MYBPC3* variant and twelve patients (19.4%) carried two distinct genetic changes in *MYBPC3*. Nine patients (18%) with a single *MYBPC3* variant were found to carry an additional genetic variant in another gene of interest: *MYH7* (n=2), *TNNT2* (n=2), *FLNC*, *GLA*, *JUP*, *MYH6*, *ANKRD1*, *BRAF* and *MAP2K1*. One patient carried two additional variants in *MYH7* and *ANKRD1* and one patient carried two additional variants in *TNNT2* and *JUP*. A total of twenty-one patients (33.9%) therefore had a "complex" genetic status, carrying more than one variant with a potential impact on cardiac phenotype.

After reclassification against current ACMG criteria, 40 (64.5%) patients carried a primary *MYBPC3* variant classified as pathogenic, 19 (30.6%) as likely pathogenic and 3 (4.8%) as variants of uncertain significance (VUS). Amongst the 12 patients carrying two variants in *MYBPC3*, the second variant was classified as pathogenic in 3, likely pathogenic in 6, and VUS in 3.

Among patients with a single *MYBPC3* variant, 20 (48.8%) were missense substitution variants, 2 (4.9%) were nonsense substitution variants, 11 (26.8%) were insertions or deletions of nucleotides within the gene and the remaining 8 (19.5%) were intronic/splice-site variants. Amongst the 21 patients with complex genetic status, the breakdown of primary

MYBPC3 variants was: 10 missense, 1 nonsense, 5 insertions/deletions and 5 intronic/splice-site. In the 12 patients with a second genetic variant in *MYBPC3*, 11 of these were missense variants and 1 was a frameshift variant. A total of 62 exonic *MYBPC3* variants were identified across the cohort, with 19 (30.6%) of these in exons 16 and 17 (see Figure 1), corresponding to the C3 functional domain of the cMyBP-C protein (residues 449-539), thought to be required for flexibility of the N-terminal region and consequently important for interaction with myosin S2 or actin[27].

Three patients carried a single *MYBPC3* variant classified as a VUS under ACMG criteria, but felt by the clinical team to be likely pathogenic, based on a combination of the clinical and laboratory information available at the time. These patients were all genetic probands, diagnosed at a mean age of 4.72±6.4 years and with mean MLVWT Z-score at baseline of 13.4±8.5. In all three cases, the *MYBPC3* variant segregated with affected first degree relatives and was not identified in undiagnosed family members. None of these patients experienced adverse clinical outcomes during follow-up.

Clinical outcomes

Median length of follow-up was 3.1 years (IQR: 1.6-6.9). Fifty-one patients (82%) were alive at last clinic review. Six patients (9.7%) were lost to follow-up after transition to adult services. Clinical outcomes are summarised in Table 2. Nine patients (14.5%) experienced MACE during follow-up and five (8%) died: three of these were SCDs, one was a pulseless electrical activity cardiac arrest during a catheter procedure following transplantation and one death occurred in a patient on the cardiac transplant waiting list. Whole cohort survival free from MACE is illustrated in Figure 2a. Twenty patients (32.3%) had evidence of ventricular arrhythmia [OOHCA (n=6); SCD (n=3); appropriate ICD therapy (n=5); or NSVT (n=10)].

None of the patients diagnosed during infancy experienced MACE during follow-up (Figure 2b).

Baseline echocardiographic data for patients with and without sustained ventricular arrhythmia are compared in Table 3, where sustained ventricular arrhythmia includes patients experiencing SCD, appropriate ICD therapy and OOHCA, but excludes those with only NSVT. Patients with sustained ventricular arrhythmia had significantly higher mean end-systolic diameter (27.1±5.5mm vs 18.8±5.8mm; p=0.00086), higher mean end-diastolic LV diameter (40.9±5.1mm vs 34.9±8.1mm; p=0.0072) and lower mean fractional shortening (34.2±10.8% vs 45.1±8.3%; p=0.012). These differences were also statistically significant at the end of follow up: 34.1±6.4mm vs 23.1±5.3mm (p=0.007), 45.7±6.0mm vs 39.3±6.8mm (p=0.04) and 26.7±6.8% vs 41.3±6.8% (p=0.002) respectively. None of the patients with resting LVOT at baseline or at the end of follow-up experienced sustained ventricular arrhythmia. Resting LVOTO developed between baseline and follow-up in 2 individuals.

Among patients with a single *MYBPC3* variant, 1 patient with a missense variant had MACE (5%), compared to 3 (14.3%) with other variant types (p=0.63). No baseline echocardiographic parameters were significantly different in patients with missense variants when compared patients with other variant types (data not shown).

Single vs complex genotypes

Eight patients (66.7%) with two *MYBPC3* variants experienced ventricular arrhythmia, compared to 12 patients (24%) with a single *MYBPC3* variant (p=0.013). Excluding NSVT, 6 patients (50%) with two variants experienced ventricular arrhythmia, compared to 5 patients (10%) with a single variant (p=0.005).

Nine patients (75%) with two *MYBPC3* variants underwent ICD implantation compared to 14 patients (28%) with a single variant (p=0.007). Five year freedom from MACE for those with a single or two *MYBPC3* variants was 95.2% (95% CI: 78.6-98.5) and 68.4% (95% CI: 40.6-88.9), respectively (hazard ratio 4.65, 95% CI: 1.16-18.66, p=0.03) (see Figure 2c). There was no statistically significant difference in MACE between patients carrying a single *MYBPC3* variant (n=4; 9.8%) and those with an additional variant of interest in a different gene (n=1; 11.1%) (p>0.999). Exclusion of those individuals with a second *MYBPC3* variant classified as a VUS from the two *MYBPC3* variants group did not affect the findings; there was no statistically significant relationship between the pathogenicity of secondary *MYBPC3* variants and likelihood of a patient experiencing a MACE during follow-up (p=0.48). Of note, among the three patients with a secondary *MYBPC3* variant classified as a VUS, one presented with an OOHCA. Data regarding *MYBPC3* variant phase was available for five of the patients with two *MYBPC3* variants and this confirmed that the variants were carried in *trans* in these individuals. Familial genetic testing in the other families was either incomplete, or results were unavailable.

Probands vs non-probands

Supplemental Table 3 shows the differences between probands and non-probands. There was no significant difference between probands and non-probands in relation to survival (see Figure 2d). Five year freedom from MACE for probands and non-probands was 84.7% (95% CI: 62.2-93.5) and 94.9% (95% CI: 68.8-99.3) respectively (hazard ratio 1.03, 95% CI: 0.24-4.31, p=0.97). Eight probands (25.8%) experienced ventricular arrhythmia excluding NSVT, compared to 3 non-probands (9.7%) (p=0.18).

DISCUSSION

To our knowledge, this study describes the largest paediatric cohort with *MYBPC3*-associated HCM reported to date. The principal finding is that *MYBPC3* variants, even in heterozygosity, can cause HCM in young children, often with a severe and highly arrhythmogenic phenotype, in contrast to the notion that such variants are associated with late-onset disease.

MYBPC3 as a cause of childhood HCM

While early studies of HCM suggested that *MYBPC3* variants were primarily associated with late-onset disease[8, 9], more recent data have demonstrated significant phenotypic heterogeneity, even amongst members of the same family[28-38]. Our results provide further evidence for this and extend the spectrum of *MYBPC3* disease, showing that HCM caused by *MYBPC3* variants can present during childhood. This phenotypic heterogeneity suggests that additional genetic and epigenetic modifiers may play an important role in disease progression.

Probands were diagnosed earlier than non-probands and exhibited more severe disease phenotypes at baseline. Non-probands were primarily diagnosed through family screening while probands were more likely to be diagnosed due to symptoms. Despite this, there was no significant difference in survival or outcomes between probands and non-probands.

Our data suggest that early-onset disease is not limited to probands or to individuals with a family history of early-onset disease. Current European HCM guidelines[20] recommend that routine HCM screening should commence at the age of 10 years. In the present cohort, ten patients attending for family screening reached diagnostic criteria for HCM before the age of 10. Together with previously published data[22, 39], our data suggest that HCM screening

should commence at an earlier age. This is reflected in the updated American HCM guidelines[40], which now advocate clinical screening in children from the time that HCM is diagnosed in a family member, regardless of the child's age.

Clinical features of paediatric MYBPC3 variant carriers

Across the cohort, significant and progressive left ventricular hypertrophy (LVH) was observed, with phenotypes characterised by non-obstructive, arrhythmic disease. In contrast, left atrial dilatation was rare and haemodynamically significant resting LVOTO was less widespread than has been described in previous adult and paediatric HCM studies [12, 41, 42].

A major finding in this study is the high proportion of patients experiencing either ICD therapy, SCD, OOHCA or non-sustained VT. This is in keeping with findings in adults[31], and suggests that arrhythmia is a common phenotype in both adult and paediatric MYBPC3-related HCM, with implications for SCD prevention strategies.

Importantly, there was no significant correlation between variant type or location and clinical phenotypic severity or outcomes. This is in keeping with recent findings in 1316 individuals with HCM caused by *MYBPC3* variants (including 163 diagnosed below the age of 18) from the SHaRe Registry[43]. Together, these data suggest that genotype-phenotype correlations in HCM are dependent on additional as yet unidentified genetic and epigenetic factors.

In keeping with previous studies of adult HCM[31, 44], a distinct gender imbalance was observed in this paediatric *MYBPC3* cohort. Four of the five deaths occurred in male patients, all three SCDs occurred in males and all patients presenting with OOHCA were male. The only female death occurred in a patient carrying two *MYBPC3* variants.

In adults with HCM, disease penetrance appears consistently higher and diagnosis generally occurs at an earlier age in males, but female patients, once diagnosed, are more likely to develop heart failure symptoms with increased mortality[44-47]. Findings in the present cohort are consistent with this, suggesting that male *MYBPC3* variant carriers are more likely to present during childhood. While clinical outcomes in male paediatric patients were significantly worse than in females, this may simply represent the same disease process with earlier onset in males. Further long-term studies are required to fully explore sex differences in *MYPBC3* HCM. The underlying reasons for the male-female disparity in HCM remain unclear, but recent evidence implicates modifier genes on the sex chromosomes or sex hormones which may prevent or delay development of hypertrophy[44, 45]. Oestrogen, progesterone and androgen receptors which are present in the heart tissue may mediate sex-specific effects in the cardiovascular system, and there is evidence that oestrogen receptors play a role in the development of hypertrophy in animal models[48, 49]. Microvascular density has also been shown to vary between males and females and may be associated with likelihood of cardiac fibrosis and with markers of diastolic function[50].

Previous studies have indicated poor outcomes, including increased risk of death or transplantation, in children diagnosed with HCM during infancy[10, 12]. In contrast, none of the twelve patients diagnosed during infancy in the present study experienced MACE during follow-up, and only three of these patients presented due to symptoms. This difference may be explained by the fact that previous studies have included patients presenting with underlying metabolic disease or malformation syndromes, highlighting the importance of the underlying aetiology in determining outcomes in infantile HCM.

Effect of complex genetic status

MYBPC3 variants in homozygosity or compound heterozygosity have previously been associated with very early onset and severe disease with poor clinical prognosis[13, 14, 16, 18, 19], and the effect of gene dosage on disease severity in *MYBPC3* HCM has been described in adult cohorts and family studies[15, 17, 51-54]. The findings in the present study that patients carrying a second variant in *MYBPC3* were significantly more likely to experience ventricular arrhythmia than those patients carrying a single *MYBPC3* variant and had significantly worse clinical outcomes are consistent with this.

Our data contrast with previous findings of severe, infant-onset disease in patients with compound heterozygous *MYBPC3* variants, since all but one of the patients with a second *MYBPC3* variant were diagnosed after the first year of life. This suggests that additional MYBPC3 variants can play a role in clinical disease expression and penetrance beyond infancy, most likely in addition to other genetic and epigenetic factors.

Genome-wide association studies have recently demonstrated the existence of numerous novel susceptibility loci for HCM which may play an important role in disease expression and outcomes. The presence of common genetic variation at one or more of these loci may explain the variable disease expression observed in carriers of pathogenic sarcomeric variants[55]. Epigenetic factors may also influence HCM phenotype development by acting on signalling cascades, membrane receptors and transcription factors, or through proteomic upstream regulators of disease pathomechanisms, post-translational gene expression regulators and histone modification[56-58].

Confirmed variant pathogenicity was not always necessary for the apparent gene dosage effect to be observed, since increased risk of poor clinical outcomes was observed in patients carrying two recognised pathogenic *MYBPC3* variants, as well as in those with a second *MYBPC3* variant of uncertain pathogenicity. Indeed, some of the most severe phenotypes

were observed in children carrying two *MYBPC3* variants, with one variant having been inherited from each parent. The normal or very mild cardiac phenotypes detected in the parents of these individuals demonstrates that undetected secondary *MYBPC3* variants (including VUS) may be of clinical importance as disease modifiers in some families affected by *MYBPC3* HCM.

Limitations

Missing and inconsistent clinical data is a limitation of the retrospective study design. In particular, different genetic testing techniques and protocols across the different eras in this study mean that additional variants in other genes of interest may have not been detected in those patients who had only undergone Sanger sequencing. Furthermore, 20 patients underwent predictive testing for a single familial variant, which may have failed to identify additional variants of potential relevance. Data relating to variant phase in the patients carrying a second *MYBPC3* variant was not available for all patients, limiting our ability to interpret the true relevance of secondary *MYBPC3* variants.

Recruitment of the cohort from a single specialist referral centre may result in recruitment bias and may have skewed the cohort towards individuals with more severe and difficult-to-manage disease; however, the fact that over 50% of the cohort were referred through family screening or following an incidental finding suggests that the cohort is likely to be representative of the wider *MYBPC3*-related paediatric HCM population.

CONCLUSIONS

This study demonstrates that children with *MYBPC3* variants can develop early-onset HCM which can be associated with life-threatening ventricular arrhythmias, in contrast to previous reports of *MYBPC3* as a late-onset HCM gene. Outcomes in the present cohort varied significantly from the aetiologically and genetically mixed paediatric HCM cohorts described previously. These observations indicate the importance of distinguishing genetic subtypes of paediatric disease for clinical management and in future research.

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COMPETING INTERESTS:

There are no competing interests for any author.

CONTRIBUTORSHIP STATEMENT:

EF, GN and JPK designed the study. EF, GN, VA, KD, MC, JPO, PS, KM, RM, HF, LRL, EC and JPK were involved in data acquisition, analysis and interpretation. EF, GN, VA, KD, MC, JPO, PS, KM, RM, HF, LRL, EC and JPK were involved in drafting, reviewing and revising of the manuscript and have approved the final version. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ETHICS APPROVAL STATEMENT:

The study was approved by the Great Ormond Street Hospital/University College London Institute of Child Health Joint Research and Development Office before data collection commenced (local reference: 18HL01/19HL04). The study was conducted using anonymised, retrospective data, and patient consent was therefore waived in line with local approval.

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TABLES

Table 1. Baseline cohort characteristics

	Whole cohort (n=62)	Two *MYBPC3* variants (n=12)	Single *MYBPC3* variant plus variant(s) in other genes of interest (n=9)	Single *MYBPC3* only (n=41)	p-values
Median age at diagnosis/start of follow-up	10 yrs (IQR: 2-14)	9 yrs (IQR: 6-13.5)	10 yrs (IQR: 2-12)	10 yrs (IQR:2-14)	0.93
Median age at last follow-up review	15 yrs (IQR: 11-17)	15.4 yrs (IQR: 10.6-17.9)	14.5 yrs (IQR: 10.4-17.5)	16.2 yrs (IQR: 13.4-18.3)	0.65
Reported symptoms					
Any symptoms	21 (33.9%)	4 (33.3%)	2 (22.2%)	15 (36.6%)	0.85
Chest pain	11 (17.7%)	1 (8.3%)	2 (22.2%)	8 (19.5%)	0.70
Dyspnoea	9 (14.5%)	2 (16.7%)	1 (11.1%)	6 (14.6%)	>0.999
Palpitations	7 (11.3%)	2 (16.7%)	0	5 (12.2%)	0.60
Medications					
Any medication	21 (33.9%)	6 (50%)	4 (44.4%)	11 (26.8%)	0.42
Beta-blockers	21 (33.9%)	6 (50%)	4 (44.4%)	11 (26.8%)	0.25
Disopyramide	1 (1.6%)	0	0	1 (2.4%)	>0.999

Verapamil	2 (3.2%)	0	0	2 (4.9%)	>0.999
Amiodarone	1 (1.6%)	0	0	1 (2.4%)	>0.999
ACE inhibitors	2 (3.2%)	2 (16.7%)	0	0	0.05
Baseline echocardiogram					
Median MLVWT	17.5mm (IQR: 12-25)	20mm (IQR: 14.5-23.5)	17.0mm (IQR: 9-19)	17.0mm (IQR: 11.5-25.5)	0.95
Median MLVWT Z-score	10.3 (IQR: 6.6-16.5)	12.8 (IQR: 11-17.8)	9.75 (IQR: 6.9-15.2)	9.45 (IQR: 5-16-7)	0.29
Mean left atrium diameter	31.4mm ± 6.8	29.7mm ± 5.2	29.8mm ± 6.5	32.2mm ± 7.2	0.39
Mean left atrium diameter Z-score	1.1 ± 1.36	1.0 ± 1.0	1.0 ± 1.6	1.1 ± 1.5	0.9
Median left ventricular outflow tract gradient	6mmHg (IQR: 5-11)	6mmHg (IQR: 4.5-7.5)	8mmHg (IQR: 6-8)	7mmHg (IQR: 5-24.8)	0.29
Left ventricular outflow tract gradient ≥30mmHg	7 (11.3%)	0	1 (11.1%)	6 (15%)	0.75
Median lateral E/E' ratio	8.1 (IQR: 6.5-13.7)	13.6 (IQR: 8.9-20.3)	6.6 (IQR: 5-12.3)	7.9 (IQR: 6.5-10.6)	0.10
Median septal E/E' ratio	13.9 (IQR: 10-17.2)	16.9 (IQR: 14.8-24.1)	13.7 (IQR: 12.4-14)	12.3 (IQR: 8.4-17)	**0.011**

Lateral E/E' ratio ≥10	12 (37.5%)	5 (71.4%)	2 (40%)	5 (25%)	**0.035**
Septal E/E' ratio ≥10	24 (75%)	8 (100%)	4 (80%)	12 (63.2%)	0.196
Mean LVESD	20.3mm ± 6.5	21.9mm ± 8.3	17.4mm ± 6.1	20.5mm ± 6.0	0.82
Mean LVESD Z-score	-2.1 ± 2.1	-1.6 ± 2.6	-2.8 ± 2.2	-2.2 ± 1.8	0.55
Mean LVEDD	35.8mm ± 8.0	34.2mm ± 8.2	33.1mm ± 9.3	36.9mm ± 7.5	0.23
Mean LVEDD Z-score	-1.8 ± 1.4	-1.9 ± 1.8	-1.7 ± 1.2	-1.8 ± 1.4	0.76

P values indicate statistical comparison of all three patient groups: two *MYBPC3* variants/single *MYBPC3* variant plus variant(s) in other genes of interest/single *MYBPC3* variant only

ACE = angiotensin-converting enzyme; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; MLVWT = maximal left ventricular wall thickness

Table 2. Clinical outcomes

Outcome Category	Clinical outcome	n	%
Arrhythmias	Out-of-hospital cardiac arrest	6	9.7%
	Non-sustained ventricular tachycardia	10	16%
	Appropriate ICD therapy	5	8%
	Atrial arrhythmia	4	6.5%
Procedures	ICD implantation	23	37%
	Pacemaker implantation	1	1.6%
	LVAD implantation	1	1.6%
	Cardiac transplantation	2	3.2%
	Surgical relief of outflow tract obstruction	3	4.8%
Deaths	Death during follow-up	5	8%
	SCD	3	4.8%
	Complications of procedures/interventions	2	3.2%
	Heart failure death	0	0%
Patients reaching composite MACE endpoint during follow-up		9	14.5%

ICD = implantable cardioverter defibrillator; LVAD = left ventricular assist device; MACE = major adverse cardiac event; SCD = sudden cardiac death

Table 3. Comparison of baseline variables in patients with and without sustained ventricular arrhythmia

Echocardiogram variables	Arrhythmia	No arrhythmia	p-value
Median MLVWT (mm)	20.0 (IQR: 18-21.8)	16 (IQR: 11-26)	0.49
Median MLVWT Z-score	12.3 (IQR: 9.9-13)	12.1 (IQR: 6.4-17.5)	0.577
Mean LA diameter (mm)	33.3 ± 6.6	30.9 ± 6.8	0.39
Mean LA diameter Z-score	1.4 ± 1.3	0.9 ± 1.4	0.37
Median LVOT (mmHg)	8.5 (IQR: 5.8-9.5)	6 (IQR: 5-24.8)	0.8995
Median lateral E/E'	13.6 (IQR: 12.2-20.3)	7.6 (IQR: 6-9.7)	**0.032**
Median septal E/E'	16.9 (IQR: 15.2-18.9)	12.5 (IQR: 8.7-16.1)	0.12
Mean LVESD (mm)	27.1 ± 5.5	18.9 ± 5.8	**0.00086**
Mean LVESD Z-score	-0.1 ± 2.0	-2.6 ± 5.8	**0.0062**
Mean LVEDD (mm)	40.9 ± 5.1	34.9 ± 8.1	**0.0072**
Mean LVEDD Z-score	-0.6 ± 1.1	-2.0 ± 1.4	**0.0052**
Mean fractional shortening (%)	34.2 ± 10.8	45.1 ± 8.3	**0.012**

Sustained ventricular arrhythmia includes all patients experiencing appropriate ICD therapy, out-of-hospital cardiac arrest or sudden cardiac death, but excludes those with only NSVT.

ICD = implantable cardioverter defibrillator; LA = left atrium; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVOT = left ventricular outflow tract; MLVWT = maximal left ventricular wall thickness; NSVT = non-sustained ventricular tachycardia

FIGURE LEGENDS:

Figure 1: Distribution of *MYBPC3* exonic variants – a histogram illustrating the location of *MYBPC3* exonic variants identified within the cohort. 19 (30.6%) variants were located in exons 16 and 17, corresponding to the C3 functional domain of the cMyBP-C protein and thought to be important for interaction with myosin S2 or actin[26].

Figure 2a: Whole cohort survival free from MACE (major adverse cardiac event): Kaplan-Meier curve to show whole cohort survival free from composite MACE endpoint over the course of diagnosed follow-up.

Figure 2b: Kaplan-Meier curve to show survival free from composite MACE endpoint over the course of diagnosed follow-up for patients diagnosed in infancy versus those diagnosed in later childhood

Figure 2c: Kaplan-Meier curve to show survival free from composite MACE endpoint over the course of diagnosed follow-up for patients with a single *MYBPC3* variant versus those with additional *MYBPC3* variant

Figure 2d: Kaplan-Meier curve to show survival free from death or equivalent event over course of diagnosed follow-up for probands versus non-probands

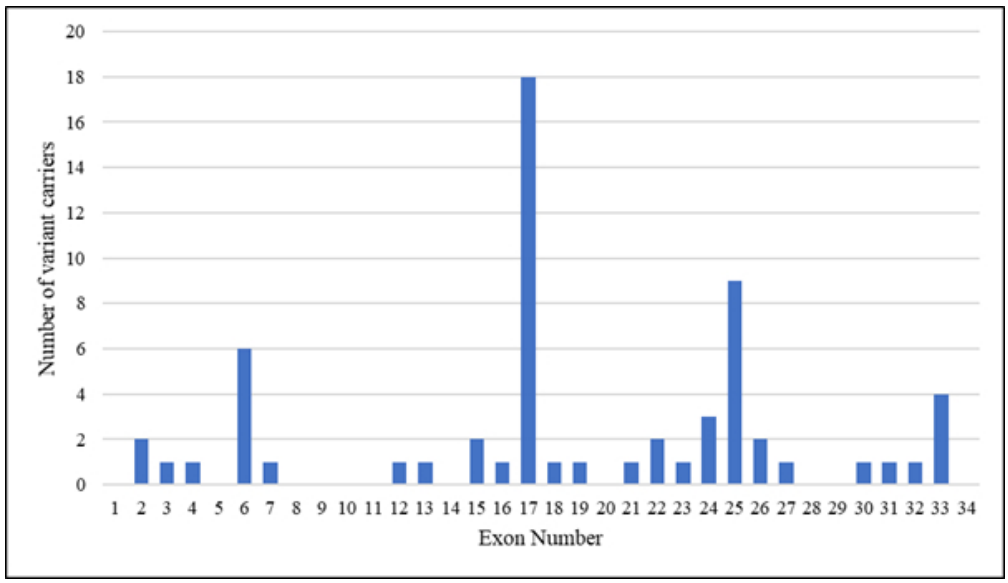


Figure 1: Distribution of MYBPC3 exonic variants – a histogram illustrating the location of MYBPC3 exonic variants identified within the cohort. 19 (30.6%) variants were located in exons 16 and 17, corresponding to the C3 functional domain of the cMyBP-C protein.

159x91mm (96 x 96 DPI)

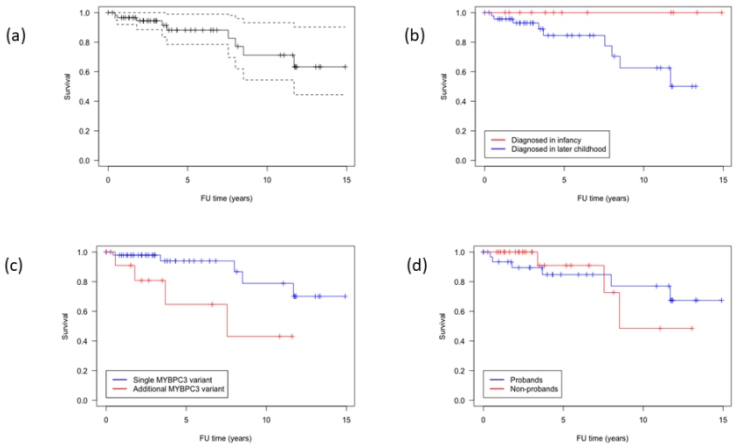
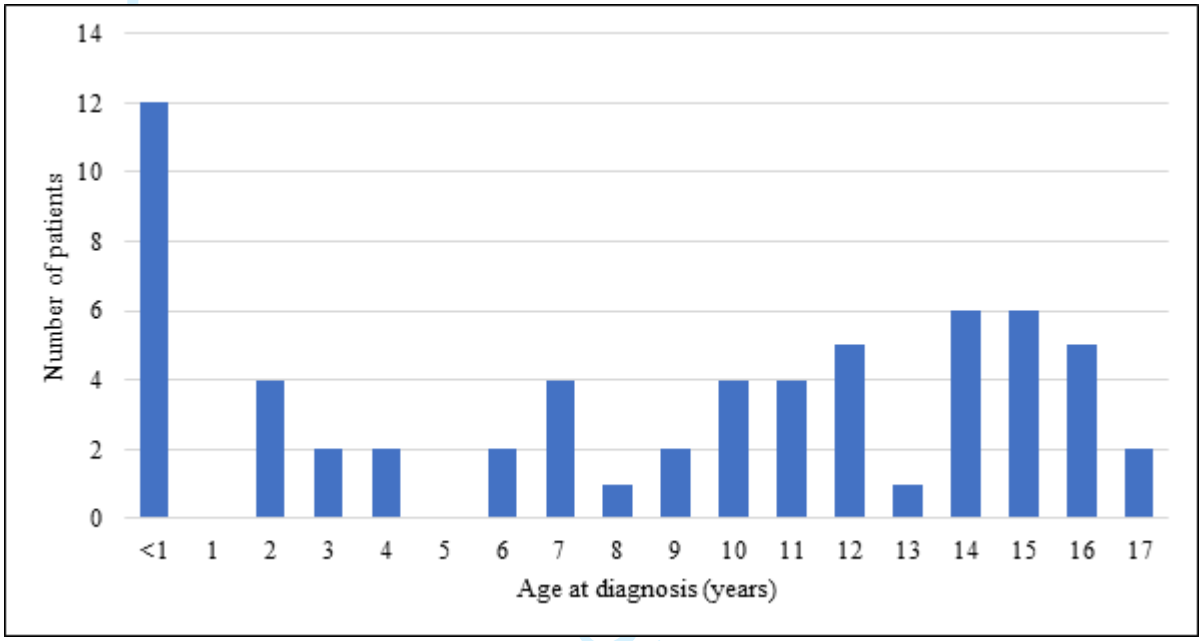


Figure 2a: Whole cohort survival free from MACE (major adverse cardiac event): Kaplan-Meier curve to show whole cohort survival free from composite MACE endpoint over the course of diagnosed follow-up. Figure 2b: Kaplan-Meier curve to show survival free from composite MACE endpoint over the course of diagnosed follow-up for patients diagnosed in infancy versus those diagnosed in later childhood. Figure 2c: Kaplan-Meier curve to show survival free from composite MACE endpoint over the course of diagnosed follow-up for patients with a single MYBPC3 variant versus those with additional MYBPC3 variant. Figure 2d: Kaplan-Meier curve to show survival free from death or equivalent event over course of diagnosed follow-up for probands versus non-probands.

338x190mm (96 x 96 DPI)

SUPPLEMENTAL MATERIAL

Supplemental Figure 1: Age at diagnosis (whole cohort) – a histogram to illustrate the ages at which patients were diagnosed. Of note, twelve patients (19.4%) were diagnosed during infancy (below 1 year of age).



Supplemental Table 1. Cardiac MRI and CPET data at baseline

Cardiac MRI findings (n= 14)	
Variable of interest	
Mean indexed LV mass (g/m^2)	100.0 ± 48.2
Median indexed LV mass (g/m^2)	83 (IQR: 66-119)
Mean MLVWT (mm)	19.1 ± 9.1
Median MLVWT (mm)	15 (IQR: 14-26)
No of patients with late gadolinium enhancement	6/11
Mean EF%	72.4% ± 6.5
Median EF%	72.5% (IQR: 67-76.8)
Mean LVESV (ml)	33.5 ± 17.3
Mean LVESV Z-score	-0.5 ± 1.2
Median LVESV (ml)	31 (IQR: 22-47)
Mean LVEDV (ml)	105.6 ± 39.8
Mean LVEDV Z-score	-0.1 ± 1.4
Median LVEDV (ml)	103 (IQR: 81-127)
Cardiopulmonary exercise test findings (n= 32)	
Variable of interest	
No of patients with arrhythmia during exercise	0
Mean peak VO_2 (ml/kg/min)	33.4 ± 10.4
Median peak VO_2 (ml/kg/min)	31.85 (IQR: 26.2-39.75)
Mean systolic BP at rest (mmHg)	106.2 ± 12.9
Median systolic BP at rest (mmHg)	108 (IQR: 97-115)
Mean peak systolic BP (mmHg)	138.6 ± 26.3
Median peak systolic BP (mmHg)	138 (IQR: 120-162)
Mean systolic BP at peak exertion (mmHg)	138.5 ± 27.4
Median systolic BP at peak exertion (mmHg)	138 (IQR: 116.3-163)

BP = blood pressure; CPET = cardiopulmonary exercise test; EF = ejection fraction; LV = left ventricular; LVEDV = left ventricular end-diastolic volume; LVESV = left ventricular end-systolic volume; MLVWT = maximal left ventricular wall thickness; MRI = magnetic resonance imaging

Supplemental Table 2. Genetic variants identified in the cohort

Patient No	*MYBPC3* nucleotide change	*MYBPC3* protein change	ACMG Classification[1]	Additional variants of interest	ACMG classification of additional variants[1]	Phase of MYBPC3 variants	Panel size	Proband?
1	c.1790G>A	p.(Arg597Gln)	Likely pathogenic				Predictive test	Y
2	c.927-2A>G	Predicted abnormal splicing	Pathogenic	*BRAF* c.707A>C; p.(Asn236Thr)	VUS		Predictive test	N
3	c.1168del	p.(His390MetfsTer16)	Pathogenic				Predictive test	N
4	c.1484G>A	p.(Arg495Gln)	Pathogenic				16	Y
5	c.1321G>T	p.(Glu441Ter)	Likely pathogenic				21	N
6	c.3413G>C	p.(Arg1138Pro)	VUS				21	N
7	c.1504C>T	p.(Arg502Trp)	Pathogenic				16	Y
8	c.2373dup	p.(Trp792ValfsTer41)	Pathogenic	*MYH7* c.3854-11T>C (Predicted abnormal splicing); *ANKRD1* c.838A>G; p.(Ile280Val)	VUS; VUS		21	Y
9	c.3697C>T	p.(Gln1233Ter)	Pathogenic				16	Y
10	c.1505G>A	p.(Arg502Gln)	Pathogenic				Unknown - at least 4	N
11	c.1504C>T	p.(Arg502Trp)	Pathogenic	*TNNT2* c.341C>T; p.(Ala114Val)	Pathogenic		Predictive test	N
12	c.1484G>A	p.(Arg495Gln)	Pathogenic				11	N
13	c.927-9G>A	Predicted abnormal splicing	Pathogenic				Predictive test	Y
14	c.2054_2067+11del	Predicted abnormal splicing	Likely pathogenic				Predictive test	N

15	c.1484G>A	p.(Arg495Gln)	Pathogenic	*TNNT2* c.853C>T; p.(Arg285Cys); *JUP* c.1039G>A; p.(Ala347Thr)	Likely pathogenic; VUS		Whole exome sequencing	Y
16	c.927-2A>G	Predicted abnormal splicing	Pathogenic	*FLNC* c.7262T>A; p.(Leu2421His)	VUS		21	N
17	c.2441_2443del	p.(Lys814del)	VUS	*GLA* c.1153A>G; p.(Thr385Ala)	VUS		22	Y
18	c.1513_1515del	p.(Lys505del)	Pathogenic				16	Y
19	c.1456T>G	p.(Trp486Gly)	VUS				4	Y
20	c.1624+4A>T	Predicted abnormal splicing	Pathogenic				21	Y
21	c.174_184del	p.(Glu60AlafsTer49)	Pathogenic				Predictive test	N
22	c.1090+1G>T	Predicted abnormal splicing	Likely pathogenic				11	N
23	c.811_817del	p.(Phe271AlafsTer27)	Likely pathogenic				11	Y
24	c.655G>C	p.(Val219Leu)	Likely pathogenic	*MYH7* c.1816G>A; p.(Val606Met)	Pathogenic		11	N
25	c.2459G>A	p.(Arg820Gln)	Likely pathogenic				41	N
26	c.1302C>A	p.(Tyr434Ter)	Likely pathogenic	*MYH6* c.622G>A; p.(Asp208Asn)	Likely benign		Unknown	Y
27	c.1224-19G>A	Predicted abnormal splicing	Likely pathogenic				12	Y
28	c.3226_3227insT	p.(Asp1076ValfsTer6)	Likely pathogenic				11	Y
29	c.1504C>T	p.(Arg502Trp)	Pathogenic				11	Y
30	c.927-9G>A	Predicted abnormal splicing	Pathogenic				Unknown	N
31	c.3747dup	p.(Ile1250HisfsTer16)	Likely pathogenic				16	N

32	c.1504C>T	p.(Arg502Trp)	Pathogenic				11	Y
33	c.2545del	p.(Val849SerfsTer30)	Likely pathogenic				Unknown	N
34	c.2459G>A	p.(Arg820Gln)	Likely pathogenic				41	Y
35	c.1505G>A	p.(Arg502Gln)	Pathogenic				3	N
36	c.2610del	p.(Ser871AlafsTer8)	Likely pathogenic				Predictive test	N
37	c.2610del	p.(Ser871AlafsTer8)	Likely pathogenic				Predictive test	N
38	c.1504C>T	p.(Arg502Trp)	Pathogenic				Predictive test with additional 16 gene panel	N
39	c.1504C>T	p.(Arg502Trp)	Pathogenic				Unknown	N
40	c.1483C>G	p.(Arg495Gly)	Pathogenic				14	Y
41	c.772G>A	p.(Glu258Lys)	Pathogenic				Predictive test	N
42	c.1504C>T	p.(Arg502Trp)	Pathogenic	*MAP2K1* c.144_145delinsA; p.(Arg49AlafsTer15)	Pathogenic		Unknown	Y
43	c.3190+5G>A	Predicted abnormal splicing	Likely pathogenic				Predictive test	N
44	c.772G>A	p.(Glu258Lys)	Pathogenic				Unknown	Y
45	c.2308G>A	p.(Asp770Asn)	Pathogenic				Predictive test	Y
46	c.1224-19G>A	Predicted abnormal splicing	Likely pathogenic				21	N
47	c.2096del	p.(Pro699GlnfsTer55)	Pathogenic				Predictive test	N

48	c.2458C>T	p.(Arg820Trp)	Likely pathogenic	*MYBPC3* c.2573G>A; p.(Ser858Asn); *MYL3* c.457del; p.(Leu153PhefsTer12)	Likely pathogenic; VUS	*trans*	104	Y
49	c.772G>A	p.(Glu258Lys)	Pathogenic	*MYBPC3* c.2429G>A; p.(Arg810His); mitochondrial m.(955A>G)	Likely pathogenic; VUS	*trans*	104	Y
50	c.1505G>A	p.(Arg502Gln)	Pathogenic	*MYBPC3* c.3763G>A; p.(Ala1255Thr)	Likely pathogenic	*trans*	16	Y
51	c.3330+5G>C	Predicted abnormal splicing	Pathogenic	*MYBPC3* c.2533C>T; p.(Arg845Cys)	Likely pathogenic	Unknown	16	Y
52	c.3330+5G>A	Predicted abnormal splicing	Pathogenic	*MYBPC3* c.495G>C; p.(Glu165Asp); *CSRP3* c.251C>T; p.(Thr84Met)	Likely pathogenic; VUS	Unknown	90	N
53	c.2373dup	p.(Trp792ValfsTer41)	Pathogenic	*MYBPC3* c.961G>A; p.(Val321Met)	VUS	Unknown	18	Y
54	c.177_187del	p.(Glu60AlafsTer49)	Pathogenic	*MYBPC3* c.1504C>T; p.(Arg502Trp)	Pathogenic	*trans*	6	N
55	c.1504C>T	p.(Arg502Trp)	Pathogenic	*MYBPC3* c.2096del; p.(Pro699GlnfsTer55)	Pathogenic	*trans*	11	Y
56	c.1483C>G	p.(Arg495Gly)	Pathogenic	*MYBPC3* c.3572C>T; p.(Ser1191Leu)	VUS	Unknown	41	Y
57	c.927-2A>G	Predicted abnormal splicing	Pathogenic	*MYBPC3* c.2870C>G; p.(Thr957Ser)	Pathogenic	Unknown	3	N
58	c.2429G>A	p.(Arg810His)	Likely pathogenic	*MYBPC3* c.3763G>A; p.(Ala1255Thr)	Likely pathogenic	Unknown	11	Y
59	c.2373dup	p.(Trp792ValfsTer41)	Pathogenic	*MYBPC3* c.1813G>A; p.(Asp605Asn)	VUS	Unknown	11	N
60	c.772G>A	p.(Glu258Lys)	Pathogenic				Predictive test	N
61	c.772G>A	p.(Glu258Lys)	Pathogenic				16	Y
62	c.305delinsTGAGG	p.(Pro102LeufsTer12)	Pathogenic				21	N

ACMG = American College of Medical Genetics

Supplemental Table 3. Comparison of probands and non-probands

	Probands	Non-probands	p-value
Diagnosed in infancy	10 (32.3%)	2 (6.5%)	0.024
Symptoms at baseline	10 (32.3%)	11 (35.4%)	>0.999
Reasons for diagnosis			
Incidental	16 (51.6%)	2 (6.5%)	
Symptoms	10 (32.6%)	1 (3.2%)	
Out-of-hospital cardiac arrest	5 (16.1%)	1 (3.2%)	
Family screening		26 (83.9%)	
Unknown		1 (3.2%)	
Baseline echocardiogram variables			
Mean MLVWT Z-score	14.5±7.9	9.9±6.1	0.02
Median lateral E/E' ratio	13.3 [IQR: 9-20.3]	6.9 [IQR 6-8]	0.008
Median septal E/E' ratio	16.2 [IQR: 13.3-20.7]	12.3 [IQR: 8.4-14.8]	0.026
Baseline MRI variables			
Mean indexed LV mass	142.6±46.4g/m^2	73.4±25.5g/m^2	0.025
Mean LV end-systolic volume Z-score	0.3±0.48	-1.1±1.34	0.04
Mean LV end-diastolic volume Z-score	0.9±0.98	-0.7±1.27	0.035

LV = left ventricular; MLVWT = maximal left ventricular wall thickness

SUPPLEMENTARY MATERIAL – REFERENCES:

1. Richards SR, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a join consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 2015;17(5):405-424.