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ORIGINAL ARTICLE

Utility of genetics for risk stratification in pediatric hypertrophic cardiomyopathy

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Children with hypertrophic cardiomyopathy (HCM) experience sudden cardiac death (SCD) and other life-threatening events. We assessed if affected gene and variant burden predict outcomes. Patients <18 years old with primary HCM with a pathogenic variant or variant of uncertain significance in cardiomyopathy genes were included. Association of gene and variant number and type with freedom from major adverse cardiac events (MACE), that is, ICD insertion, myectomy, aborted SCD, transplantation or death, was assessed by Cox regression. A total of 98 of 155 gene-tested patients carried a non-benign variant. The primary affected gene was *MYH7* in 35% (*MYH7*+) and *MYBPC3* in 49% (*MYBPC3*+). *MYH7*+ patients had earlier disease onset and higher risk of MACE (hazard ratio 2.7, 95% CI 1.3-5.7). Risk of MACE was also higher in patients with multiple variants (n = 16) (HR 2.5, CI: 1.1-5.9) compared to a propensity score-matched single variant subset, after adjustment for primary gene, and in patients with de novo (*n* = 18) vs inherited variants (HR 5.7, CI: 2.6-12.7). Affected gene (eg, *MYH7*), higher variant burden and de novo variant status are independently associated with earlier onset and higher frequency of adverse outcomes in pediatric HCM, highlighting the importance of genetic risk stratification in HCM.

KEYWORDS

genetics, hypertrophic cardiomyopathy, myectomy, myosin, pediatrics, sudden death

1 | INTRODUCTION

The description of familial hypertrophic cardiomyopathy (HCM) due to a missense mutation in the β-myosin heavy chain in 1990 marked the initial association of sarcomeric mutations with HCM.[1] Ever-broadening gene panels can now identify a genetic cause in up to 63% of familial HCM cases[2–4] and 50% to 60% of non-familial cases.[5,6] At least 18 genes have been associated with HCM, with varying levels of evidence for pathogenicity.[7]

Hitherto, such genetic data have been used primarily for cascade or predictive screening of at-risk relatives after the causative lesion is identified in a HCM proband, rather than to inform prognosis or guide management in the affected individual.[8,9] There is a high incidence of sudden cardiac death (SCD) and other complications in HCM. Decision making regarding prevention of SCD is driven entirely by clinical and echocardiographic risk factors rather than genetic factors, as encapsulated in the recent HCM SCD risk calculator in adults published by the European Society of Cardiology in 2014.[10] There are scant data describing the association between genetic etiology and outcomes in HCM, especially in children. Adult studies have reported a more severe phenotype and earlier onset of ventricular hypertrophy in patients with *MYH7* mutations compared to those with other mutations albeit the data are inconsistent.[3,4,8,11–14] Adult studies have also reported earlier presentation, more severe hypertrophy and higher rates of myectomy and ICD implantation in those with multiple mutations.[9,15,16] Pediatric studies have been limited to case reports[17,18] with no systematic evaluation of influence of gene type and mutation number on outcomes. Due to perceived greater risk of adverse outcomes in children with early onset HCM, knowledge of genetic predictors is critical to facilitate timely interventions before the onset of these adverse events.

This study aimed to define the association of (1) the gene involved, and (2) the number of variants with clinical outcomes in a longitudinally followed pediatric HCM cohort.

2 | METHODS

2.1 | Study cohort

This retrospective single center study longitudinally evaluated outcomes in variant-positive pediatric HCM patients (age < 18 years at diagnosis) following institutional ethics board approval. Waiver of consent was obtained. Clinical, genetic and echocardiographic data were collected by retrospective review of patient records. All patients who were seen between January 2005 and October 2014 with a primary diagnosis of HCM or referred for HCM screening were reviewed. Patients with 1 or more non-benign variants, that is, pathogenic or variant of uncertain significance (VUS) on clinical genetic testing in the following HCM-associated genes were included: *MYH7*, *MYL2*, *MYL3*, *TNNT2*, *TNNI3*, *TNNC1*, *TPM1*, *ACTC*, *MYBPC3*, *ACTN2*, *MYOZ2*, *MYH6*, *TTN*, *CSRP3*, *TCAP*, *VCL*, *CASQ2* and *JPH2*.[7] Patients with an end-diastolic inter-ventricular septal (IVSD) or LV posterior wall diameter (LVPWD) *z*-score of ≥ +2.0, per published standards,[19] or end-diastolic IVSD:LVPWD ratio greater than 1.5 were considered phenotype positive. Patients with secondary causes of hypertrophy including hypertension, endocrine conditions (eg, infants of diabetic mothers, exogenous corticosteroid exposure), malformation syndromes, metabolic conditions, neuromuscular conditions and other HCM phenocopies (eg, Danon disease, *PRKAG2* variants) were excluded. The first member of each kindred who presented to our service with a diagnosis of HCM was deemed the proband for the purpose of this analysis. (This study was approved by the Hospital for Sick Children's institutional research ethics board.)

2.2 | Classification of variants identified on clinical genetic testing

Per institutional practice, if a disease-causing variant had been identified in a previous affected family member, then only cascade testing for that particular variant was performed in our pediatric patient. If however, a previous affected family member had not undergone genetic testing or if our pediatric patient was the presenting proband, then HCM panel testing (typically 5-11 genes) was performed as first line testing followed by expanded or panCMP panel testing (typically 47-62 genes) as secondary testing in those who were HCM panel negative. All variants in HCM-associated genes were reclassified per the recently updated American College of Medical Genetics (ACMG) guidelines.[20] In applying these guidelines, the following definitions and tools were used. ***Prior report*** of variants was determined by review of institutional experience, testing company report, the literature, ClinVar and Human Gene Mutation databases.[21–23] ***Rare*** variants were those with a prevalence of ≤0.1% in all reference populations (Exome Variant Server, Exome Aggregation Consortium). Variants were considered ***previously reported*** if reported ≥2 times and were considered to ***co-segregate*** with HCM if they did so with ≥3 phenotype-positive individuals in ≥2 affected kindreds in 1 or more of these sources.

Missense variants were assessed using the following *in-silico* tools: SiFT (deleterious if score < .05),[24] Polyphen-2 (HumVar probability >.95),[25] Provean (score < −2.5),[26] MutationTaster (probability >.95)[27] and Align-GVGD (class c65).[28] Align-GVGD, SiFT, Polyphen-2 and MutationTaster scores were determined using Alamut v2.1 (Interactive Biosoftware, Rouen, France). Align-GVGD and SiFT analysis employed the orthologue alignments provided by Alamut for the respective genes. For these missense variants, such *in-silico* evidence was considered to support pathogenicity if ≥4 out of 5 tools predicted a deleterious variant. Nonsense and frame-shift variants were considered ***null variants*** if they occurred proximal to the last 50 bases of the penultimate exon.

Variants were classified as ***pathogenic*** (including ACMG class II, probably pathogenic), ***variant of uncertain significance*** (***VUS***), or ***benign*** (including ACMG class IV, probably benign). Patients harboring pathogenic variants or VUS were considered ***variant positive*** for the purposes of this study. Those with ≥2 non-benign variants including homozygous variants were considered ***multiple-variant-positive.*** For such patients, the variant with a higher class of pathogenicity was considered the ***primary variant***, while other variants were labeled ***secondary variants***. Variants were further classified by inheritance status as de novo or inherited.

2.3 | Statistical analysis

The composite primary endpoint was freedom from major adverse cardiac events (MACE) defined as implantable defibrillator-cardioverter (ICD) implantation, surgical myectomy, resuscitated cardiac arrest, transplantation or death. Secondary endpoints included freedom from each of the constituent MACE endpoints, freedom from ***severe*** (IVSD or LVPWD z-score ≥ 5) or ***massive hypertrophy*** (IVSD or LVPWD *z*-score ≥ 10), freedom from occurrence of any ***LV outflow tract obstruction*** (LVOTO; LVOT peak gradient ≥50 mmHg), as well as freedom from ***severe LVOTO*** (LVOT peak gradient ≥100 mmHg).

Data were reported as medians with interquartile ranges (IQR) or frequencies with percentages as appropriate. Intergroup differences were assessed with the Kruskal-Wallis test and Fisher exact tests. Timed endpoints were modeled using the Kaplan-Meier method using univariable Cox regression to compare cumulative event rates between groups. The proportional hazards assumption was verified by applying a test based on Schoenfeld-residuals and by visual inspection of Schoenfeld-residual plots. Patients with multiple variants were propensity score-matched to those with single variants by family history, maximal wall-dimension *z*-score at presentation and use of panel testing (to adjust for otherwise unmeasured confounders), in 2:1 ratio without replacement, using an optimal matching algorithm (minimizing the sum of pair-wise distances). Multivariable analysis was performed on this matched subset by Cox proportional hazards modeling with use of a robust variance estimator to account for matching.[29] Statistical tests were 2-tailed and *P* values of less than .05 were considered statistically significant. Analysis employed R software v3.1.3,[30] extended with the *MatchIt*,[31] *survival*,[32] and *rms* packages.[33]

3 | RESULTS

3.1 | Patient characteristics

During the study period, 507 patients were seen in our institutional cardiomyopathy clinic for screening due to a positive family history of HCM (n = 423; 83%), or symptoms or findings in the patient leading to a diagnosis of HCM (n = 84, 17%). The breakdown of this cohort is depicted in Figure S1 (Supporting information). Genetic testing was performed in 155 patients, yielding 98 patients from 76 kindreds who had 1 or more non-benign variants, and who constitute the study cohort. Patient characteristics are shown in Table S1. The following gene panels were used: HCM 5 gene (n = 22), HCM 11 gene (n = 32), HCM 18 gene (n = 8) and expanded panels with 46 to 62 genes (n = 8). Panel-tested patients were less likely to have a family history of HCM and were more frequently phenotype positive at initial encounter, with greater LV wall and LA dimensions than those who underwent variant-specific testing.

3.2 | Variant spectrum

Figure 1 describes the genes involved and the variant spectrum. Of 81 patients with a primary variant initially classified as pathogenic, 2 were reclassified as VUS. Of 31 variants initially classified as VUS by vendor, 6 were reclassified as pathogenic, while 1 was reclassified as benign. Following reclassification, 89 variants were considered pathogenic, 18 were de novo. Sixteen patients had multiple non-benign variants. *MYBPC3* (n = 48, 49%) and *MYH7* (n = 34, 35%) accounted for the majority of primary variants. *MYBPC3* also accounted for the majority (63%) of secondary variants.

3.3 | Association of genotype with phenotype

Patients in the overall cohort became phenotype positive at a median age of 12.4 years (IQR 5.7; 15.2). On subgroup analysis, *MYH7*+ patients were phenotype positive earlier at a median age of 9.0 years (IQR 5.2; 14.1) compared to 13 years (IQR 7.0; 15.8) for those with variants in other genes (see Table 1), and were more likely to have a missense variant than those with primary variants in other genes. Table 1 describes baseline clinical characteristics by the primary gene involved and by single vs multiple non-benign variants. The latter were more likely to be phenotype positive at presentation, to have greater wall thickness z-scores at presentation and to have undergone panel genetic testing. Patients with multiple variants were phenotype positive at a younger median age of 8.9 years (IQR 3.1; 11.2) compared to 13.1 years (IQR 6.9; 15.8) years in single variant patients. Patients with multiple variants were less likely to have presented for screening due to a family history (38% vs 70%, P = .009). There was no difference in age of presentation in those with de novo vs inherited variants. Median (IQR) age at presentation in those with de novo variants was 5.9 (1.4-13.1) and 6.3 (3.0-10.5) in those with inherited variants (P = .75).

3.4 | Clinical outcomes

Twenty-nine of 98 variant-positive patients (30%) suffered a MACE at a median age of 10.3 years (IQR 6.5; 13.2), a median of 1.4 years (IQR 0.4; 3.0) after their first visit. Freedom from MACE at 5, 10 and 15 years of age was 94.7%, 85.8% and 61.6%, respectively. The initial MACE event was ICD implantation in 14 (48%), myectomy in 8 (28%), resuscitated cardiac arrest in 3 (10%), death in 3 (10%), and transplantation in 1 (3%). Two deaths were due to heart failure, and 1 patient experienced sudden death.

3.4.1 | ICD

Nineteen (19%) patients received an ICD. Median age at implantation was 11.7 years (IQR 9.7; 13.1), a median of 1.5 years (IQR 0.3; 5.9) after presentation. Three were for secondary prevention whilst 16 were for primary prevention. Of the latter, median septal thickness at implant was 25 mm (IQR 14; 30), with a median z-score of 15 (IQR 8.2; 21.5), 4 (25%) had a family history of sudden death prior to age 40, 6 (32%) had an abnormal blood pressure response on exercise testing, and 2 (11%) had a history of non-sustained ventricular tachycardia. Over a

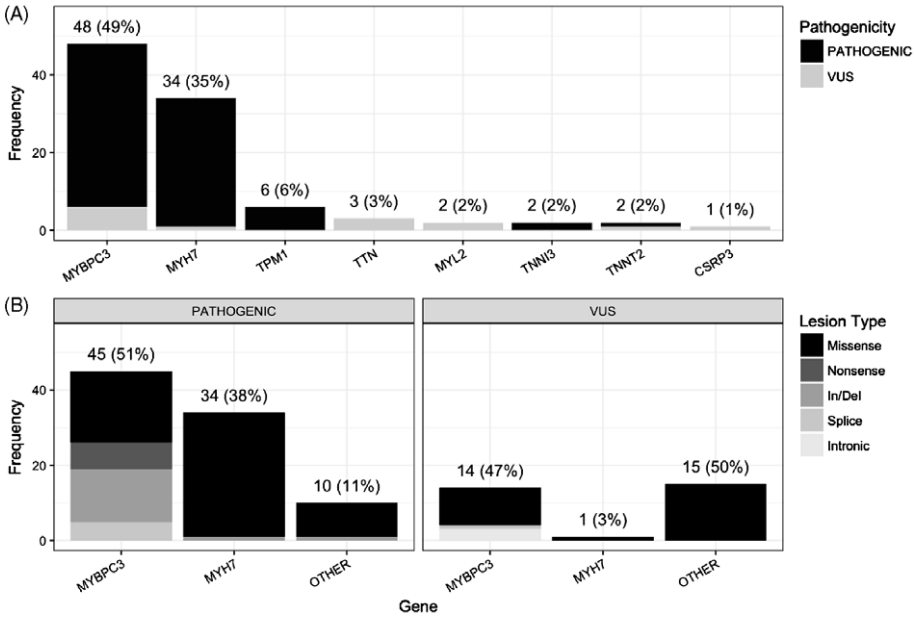


FIGURE 1 **Variant types**. A, Distribution of pathogenic variants and variants of uncertain significance (VUS) by affected gene. B, Variant types in most commonly affected genes

TABLE 1 Characteristics of the study cohort stratified by the primary affected gene and by patient variant burden

	MYH7 (*N* = 34)	Other gene (*N* = 64)	*P*	Multiple (*N* = 16)	Single (*N* = 82)	*P*
Demographics						
Gender: male (%)	24 (71)	41 (64)	.654	12 (75)	53 (65)	.567
Age, first encounter: years: median [IQR]	6.8 [2.4, 10.4]	6.0 [3.0, 11.6]	.654	8.6 [1.2, 10.2]	6.2 [3.0, 11.5]	.679
Age, last follow-up: years: median [IQR]	15.3 [9.8, 17.4]	13.3 [7.8, 17.2]	.3	15.6 [12.9, 17.5]	13.6 [8.0, 16.9]	.332
Phenotype						
Mode of presentation						
Screening	19 (56)	44 (69)	.505	6 (38)	57 (70)	.009
Murmur	6 (18)	6 (9)		2 (12)	10 (12)	
Heart failure	2 (6)	4 (6)		3 (19)	3 (4)	
Chest pain	1 (3)	0 (0)		0 (0)	1 (1)	
Syncope	0 (0)	2 (3)		0 (0)	2 (2)	
Arrhythmia	0 (0)	0 (0)		0 (0)	0 (0)	
Arrest	1 (3)	1 (2)		2 (12)	0 (0)	
Other/unknown	5 (15)	7 (11)		3 (19)	9 (11)	
Phenotype positive at first encounter: *n* (%)	21 (62)	29 (45)	.141	13 (81)	37 (45)	.012
Phenotype positive at last follow-up: *n* (%)	26 (76)	34 (53)	**.03**	15 (94)	45 (55)	.004
Phenotype positive at age: years, median [IQR]	9.0 [5.2, 14.1]	13.0 [7.0, 15.8]	**.045**	8.9 [3.1, 11.2]	13.1 [6.9, 15.8]	.014
HCM morphology						
Asymmetrical septal hypertrophy	22 (85)	29 (85)	.686	11 (73)	40 (89)	.11
Concentric	3 (12)	5 (15)		3 (20)	5 (11)	
Apical	0 (0)	0 (0)		0 (0)	0 (0)	
Biventricular hypertrophy	1 (4)	0 (0)		1 (7)	0 (0)	
Other	0 (0)	0 (0)		0 (0)	0 (0)	
Family history						
Family history of HCM: *n* (%)	29 (85)	56 (88)	.762	11 (69)	74 (90)	.035
Degree of closest family member						
1st degree	24 (83)	54 (98)	**.023**	10 (91)	68 (93)	.581
2nd degree	3 (10)	1 (2)		1 (9)	3 (4)	
3rd degree	2 (7)	0 (0)		0 (0)	2 (3)	
Family history of sudden death: *n* (%)						
Any: *n* (%)	15 (44)	22 (34)	.386	7 (44)	30 (37)	.587
< 40 y: *n* (%)	10 (30)	10 (16)	.12	4 (25)	16 (20)	.738
Genetics						
Age at genetic testing: years: median [IQR]	10.8 [6.0, 14.1]	9.3 [5.8, 14.6]	.844	10.4 [8.1, 15.7]	9.9 [5.8, 14.3]	.417
Patient had gene panel testing: *n* (%)	17 (50)	23 (36)	.2	12 (75)	28 (34)	.004
Primary variant gene						
MYH7	34 (100)	0 (0)	**<.001**	5 (31)	29 (35)	.406
MYBPC3	0 (0)	48 (75)		10 (62)	38 (46)	
Other	0 (0)	16 (25)		1 (6)	15 (18)	
Primary variant type						
Missense	33 (97)	38 (59)	**.001**	10 (62)	61 (74)	.196
In/Del	1 (3)	12 (19)		4 (25)	9 (11)	
Nonsense	0 (0)	7 (11)		2 (12)	5 (6)	
Intronic	0 (0)	7 (11)		0 (0)	7 (9)	
First echocardiogram						
Septal *z*-score: median [IQR]	4.4 [0.2, 12.2]	1.3 [0.2, 5.2]	.202	8.6 [1.3, 12.2]	1.3 [0.0, 5.2]	.014
Posterior wall *z*-score: median [IQR]	0.2 [−0.9, 1.2]	−0.1 [−0.7, 0.8]	.862	1.7 [0.2, 3.4]	−0.1 [−0.7, 0.6]	.005
Max. wall thickness *z*-score: median [IQR]	4.4 [0.3, 12.2]	1.5 [0.3, 5.5]	.206	8.6 [1.6, 12.2]	1.5 [0.2, 5.2]	.01
Wall thickness ratio: cm, median [IQR]	1.5 [1.2, 2.4]	1.2 [1.0, 1.8]	**.018**	1.5 [1.2, 2.1]	1.2 [1.0, 1.8]	.139
LA diameter *z*-score: cm, median [IQR]	1.0 [−0.0, 2.9]	0.5 [−0.0, 1.5]	.125	0.9 [−0.1, 3.0]	0.5 [−0.0, 1.6]	.455

(*Continues*)

TABLE 1 (Continued)

	MYH7 (*N* = 34)	Other gene (*N* = 64)	*P*	Multiple (*N* = 16)	Single (*N* = 82)	*P*
LVOT gradient: mmHg, median [IQR]	28.5 [8.5, 49.5]	15.5 [7.0, 21.5]	.277	19.5 [15.2, 26.5]	15.0 [7.0, 47.8]	.823
Last echocardiogram						
Septal *z*-score: median [IQR]	8.0 [1.8, 14.2]	1.1 [−0.1, 5.0]	**.004**	8.0 [4.8, 16.5]	1.6 [−0.0, 7.1]	.005
Posterior wall *z*-score: median [IQR]	0.1 [−0.6, 1.6]	−0.1 [−0.7, 0.7]	.188	1.3 [−0.2, 2.7]	−0.1 [−0.7, 0.7]	.011
Max. wall thickness *z*-score: median [IQR]	8.0 [1.8, 14.2]	1.1 [0.0, 5.0]	**.004**	8.0 [4.8, 16.5]	1.6 [0.0, 7.1]	.005
Wall thickness ratio: cm, median [IQR]	1.9 [1.1, 2.8]	1.2 [1.1, 1.6]	**.029**	1.7 [1.1, 2.7]	1.2 [1.1, 2.0]	.204
LA diameter *z*-score: cm, median [IQR]	1.5 [−0.1, 2.6]	0.5 [−0.1, 1.6]	.11	0.7 [0.3, 2.2]	0.6 [−0.1, 1.9]	.475
LVOT gradient: mmHg, median [IQR]	16.0 [8.0, 17.5]	8.0 [5.0, 18.0]	.486	7.5 [6.2, 14.0]	15.5 [7.2, 22.0]	.316

Abbreviations: HCM, hypertrophic cardiomyopathy; IQR, interquartile range; LA, left atrium; LVOT, left ventricular outflow tract; SCD, sudden cardiac death.

median follow-up of 2.8 years (IQR 1.2; 4.8) after ICD insertion, 3 (19%) of the 16 primary prevention patients received an appropriate ICD shock, whilst all 3 (100%) of the secondary prevention patients received appropriate ICD shocks, at a median frequency of 4 each (range 3-4).

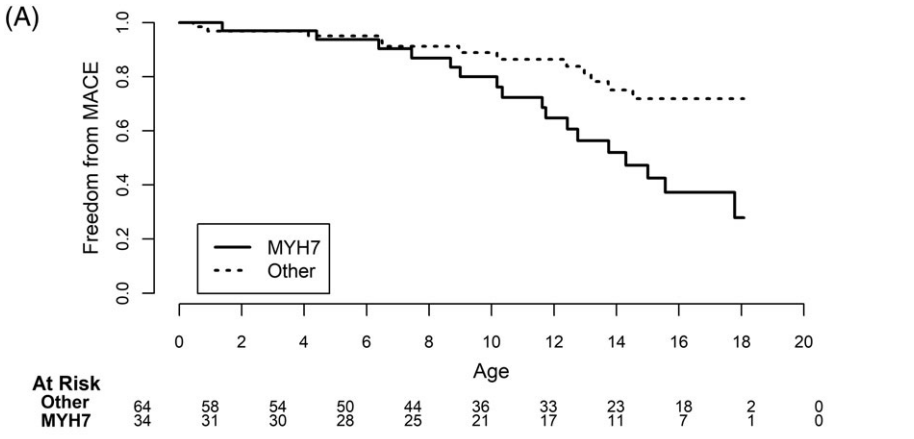
3.4.2 | Myectomy

Eight patients underwent myectomy at a median age of 8.3 years (IQR 5.9, 12.9), being a median of 2.9 years (IQR: 1.5; 3.0) after first encounter. Median septal thickness at the time of myectomy was 15.3 mm (IQR 14.5, 17.6), corresponding to a *z*-score of +9.8 (IQR +6.8; +13.8). Median LVOT peak gradient by echocardiogram was 135 mmHg (IQR 103; 187). Three (38%) had an abnormal blood pressure response to exercise prior to myectomy. Two patients underwent primary prevention ICD insertion after myectomy with no subsequent events. Patients were followed for a median of 5.9 years (IQR 4.0; 6.7) post myectomy with no occurrence of transplantation, resuscitated cardiac arrest or death.

3.5 | Association of genotype with clinical outcomes

On univariable analysis, *MYH7+* patients had lower freedom from MACE compared to those with variants in other genes (HR 2.7, 95% CI 1.3-5.7) (Figure 2A). This remained true if only probands with pathogenic variants were considered, excluding those with only VUS from analysis (*n* = 64, HR 2.7, 95% CI 1.3-5.9). Analyzed as individual events, *MYH7+* patients were at significantly higher risk of ICD implantation and myectomy during follow-up, and also experienced lower freedom from severe and massive hypertrophy and severe obstruction (Figure 2B). The median age at which patients suffered a first MACE was 14.3 years (IQR 10.3 to ∞) for *MYH7+* patients, and was not reached for the other gene group.

Patients with multiple variants had lower MACE-free survival than those who had a single variant (Figure 3A). Patients with multiple variants had lower freedom from the individual endpoints of ICD implantation, and severe and massive hypertrophy (Figures 3B). The median age at which patients with multiple



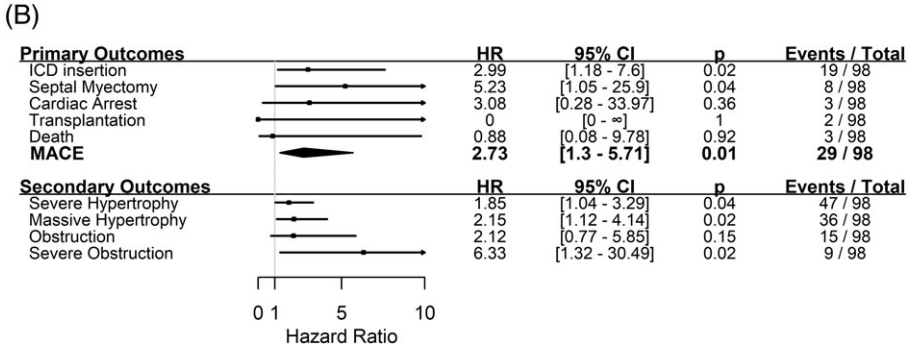


FIGURE 2 Association of affected gene with clinical outcomes. A, The Kaplan-Meier survival curve shows that patients with a primary pathogenic variant in *MYH7* gene (*MYH7+)* had a lower freedom from a MACE compared to those with primary variants in other genes (HR 2.7; CI,1.3-5.7). B, The forest plot depicts the hazard ratio for individual adverse events and secondary outcomes. *MYH7+* patients had higher risk for ICD insertion, septal myectomy, severe LV hypertrophy (*z*-score > +5), massive LV hypertrophy (*z*-score > +10) and severe obstruction (LVOT gradient >100 mmHg). MACE, major adverse cardiac event; HR, hazard ratio; ICD, implantable cardioverter defibrillator

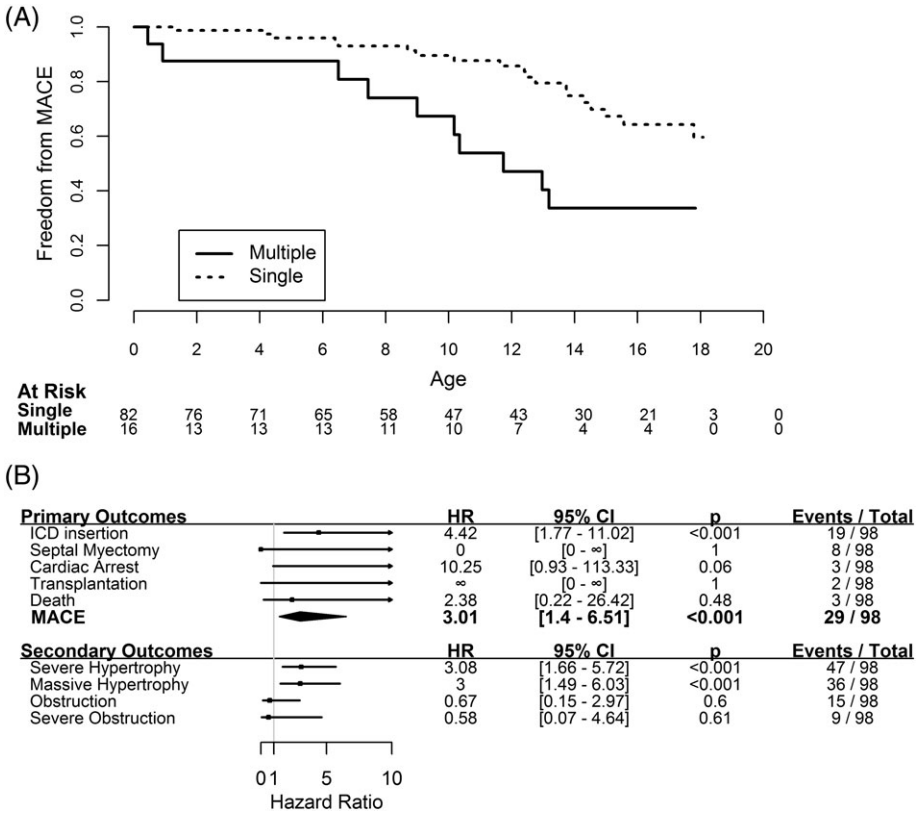


FIGURE 3 Association of variant burden with clinical outcomes. A, The Kaplan-Meier survival curve shows that patients with multiple variants in HCM-associated genes had lower freedom from a MACE (HR 3.01; CI, 1.4-6.51). B, The forest plot depicts the hazard ratio for individual adverse events and secondary outcomes. Patients with multiple variants had higher hazard of ICD insertion, severe LV hypertrophy (*z*-score > +5), and massive LV hypertrophy (*z*-score > +10). MACE, major adverse cardiac event; HR, hazard ratio; ICD, implantable cardioverter defibrillator

variants suffered a first MACE was 11.7 years (lower limit 9.0 years, upper limit not estimatable), and was not reached for those with single variants.

Detection of multiple variants is only possible in patients who undergo gene panel testing which in turn is more likely to be used in phenotype-positive patients vs variant-specific testing which is used predominantly for screening at-risk family members who may or may not be phenotype-positive. These differences in baseline phenotype and difference in family history may contribute to differences in outcomes independent of genotype. To adjust for this, patients with multiple variants (n = 16) were propensity score-matched in a 2:1 ratio to those with single variants (n = 32) on the basis of family history, wall thickness *z*-score at presentation and use of panel genetic testing. Good covariate balance was achieved between the matched patient groups (Table 2). Subsequent multivariable Cox regression analysis confirmed that both the primary affected gene and presence of multiple variants were independently associated with MACE. Patients with primary pathogenic variants in *MYH7* had a higher risk of MACE compared to those with a primary pathogenic variant in another gene (HR 2.8, CI 1.4-5.8; P = .005). Similarly, patients with multiple variants had a higher risk of MACE compared to those with single variants (HR 2.5, CI 1.1-5.9; P = .037).

Patients with de novo variants had a significantly higher hazard of a MACE compared to those with inherited variants both on univariable and on multivariable Cox regression analysis (HR 5.74, CI 2.6-12.7, P < .001 on multivariable Cox regression) (Figure 4). This association remained significant when analysis was limited to the 48 propensity score-matched subset described above (Table 3).

4 | DISCUSSION

Predicting sudden cardiac death and other adverse outcomes in HCM patients remains challenging. The recent HCM SCD risk calculator for adults developed by the European Society of Cardiology incorporates many clinical, familial and echocardiographic factors into the risk prediction score.[10] However, there remains a major gap in our understanding of predictors of outcomes in pediatric HCM including the genetic contribution to adverse outcomes. Our study showed that pediatric HCM patients carrying a primary variant in *MYH7* and/or harboring multiple non-benign variants develop an earlier and more severe HCM phenotype, and have earlier and more frequent serious cardiac events, especially need for ICD. Patients with de novo variants also have a lower freedom from MACE independent of affected gene and variant burden. These findings highlight the importance of knowledge of genetic etiology in risk stratification of pediatric HCM patients.

4.1 | Genotype-phenotype association in HCM

Attempts at genotype-phenotype correlation in HCM have suffered from tremendous genetic heterogeneity, with most kindreds harboring private variants[34] and with few reported founder variants.[35,36] Uncontrolled early series emphasized individual variants and noted earlier presentation and a high incidence of early mortality in kindreds with lesions such as R719W,[37] G716R,[38] R403Q,[39] and R453C[40] in *MYH7* and the R92W variant in *TNNT2*.[41] Such variants were considered malignant, though most kindreds were too small to permit statistical inference, let alone document the effects of that lesion outside of the original kindred. Further, with such lesions representing fewer

TABLE 2 Patients with multiple variants were propensity matched in 1:2 ratio to single variant patients on basis of family history of HCM, maximal wall thickness *z*-score at presentation and use of panel genetic testing. The matched subset was better matched for these and other measured covariates, as shown

	Unmatched			Matched		
	Multiple	Single	SMD	Multiple	Single	SMD
Total: *n*	16	82		16	32	
Demographics						
Gender: Male (%)	12 (75)	53 (65)	0.227	12 (75)	21 (66)	0.206
Age, first encounter: years: median [IQR]	8.6 [1.2, 10.2]	6.2 [3.0, 11.5]	0.087	8.6 [1.2, 10.2]	7.6 [3.0, 12.5]	0.223
Age, last follow-up: years: median [IQR]	15.6 [12.9, 17.5]	13.6 [8.0, 16.9]	0.304	15.6 [12.9, 17.5]	15.5 [13.6, 17.8]	0.14
Phenotype						
Mode of presentation						
Screening	6 (38)	57 (70)	0.968	6 (38)	13 (41)	0.817
Murmur	2 (12)	10 (12)		2 (12)	7 (22)	
Heart failure	3 (19)	3 (4)		3 (19)	2 (6)	
Chest pain	0 (0)	1 (1)		0 (0)	1 (3)	
Syncope	0 (0)	2 (2)		0 (0)	1 (3)	
Arrhythmia	0 (0)	0 (0)		0 (0)	0 (0)	
Arrest	2 (12)	0 (0)		2 (12)	0 (0)	
Other/unknown	3 (19)	9 (11)		3 (19)	8 (25)	
Phenotype positive at 1st encounter: *n* (%)	13 (81)	37 (45)	0.808	13 (81)	26 (81)	<0.001
Family history						
Family history of HCM: *n* (%)	11 (69)	74 (90)	0.552	11 (69)	24 (75)	0.139
Family history of sudden death: *n* (%)	7 (44)	30 (37)	0.147	7 (44)	12 (38)	0.128
Genetics						
Patient had gene panel testing: *n* (%)	12 (75)	28 (34)	0.9	12 (75)	26 (81)	0.152
Primary variant gene (simplified)						
MYH7	5 (31)	29 (35)	0.423	5 (31)	14 (44)	0.476
MYBPC3	10 (62)	38 (46)		10 (62)	13 (41)	
Other	1 (6)	15 (18)		1 (6)	5 (16)	
First echocardiogram						
Max. wall thickness *z*-score: median [IQR]	8.6 [1.6, 12.2]	1.5 [0.2, 5.2]	0.628	8.6 [1.6, 12.2]	6.1 [3.6, 12.2]	0.101
LVOT gradient: mmHg, median [IQR]	19.5 [15.2, 26.5]	15.0 [7.0, 47.8]	0.388	19.5 [15.2, 26.5]	22.0 [7.0, 52.0]	0.438

Abbreviations: IQR, interquartile range; LA, left atrium; SMD, standardized mean difference.

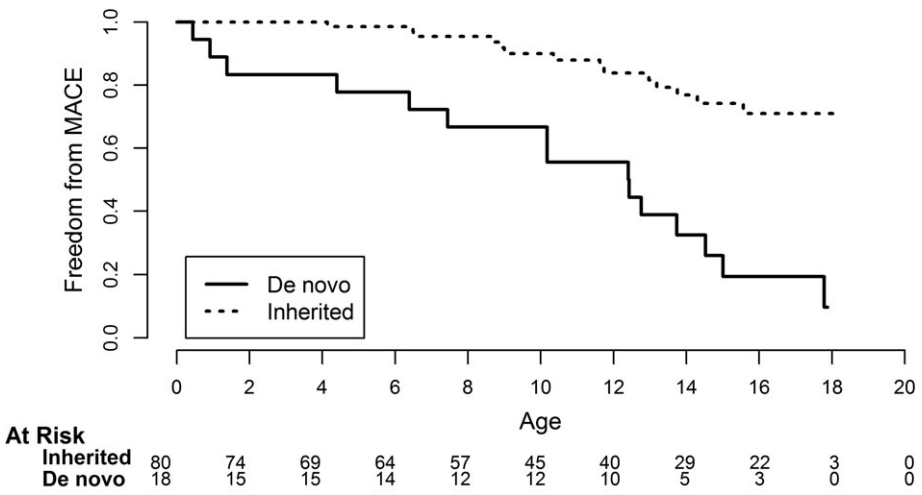


FIGURE 4 Association of de novo variant status with clinical outcomes. The Kaplan-Meier survival curve shows that patients with de novo variants (*n* = 18) had lower freedom from a MACE compared to those with inherited variants (*n* = 80) (HR 5.7; CI, 2.6-12.7, *P* < .001). MACE, major adverse cardiac event; HR, hazard ratio

than 1% of patients in some series, these findings were not broadly applicable.[34] Not surprisingly, other pedigrees harboring some of the above variants in association with a more benign clinical profile were subsequently described,[34,42] reflecting these limitations.

Later studies examined larger cohorts of HCM patients who had undergone systematic genetic screening and consistently observed a more malignant phenotype in *MYH7+* patients compared to those with variants in *MYBPC3*,[3,4,11–13] though this was not observed in the

TABLE 3 Independent genetic predictors of major adverse cardiac events on multivariable Cox regression analysis

Variable	HR [95%CI]	*P*-value
Full dataset		
MYH7	2.69 [1.20-6.04]	.016
Multiple variants	6.06 [2.52-14.56]	<.001
De novo variant	5.74 [2.60-12.67]	<.001
Propensity matched dataset		
MYH7	2.70 [1.15-6.34]	.023
Multiple variants	3.91 [1.50-10.21]	.005
De novo variant	3.79 [1.58-9.10]	.003

largest series.[43] While we did not evaluate the association with phenotype at a variant level, our findings at a gene level reinforce the differences in age of onset and phenotypic severity depending on type and number of genes involved. In the most comparable study to our own, however, the disrupted gene did not predict heart failure events, atrial fibrillation, stroke, or the occurrence of myectomy, over a mean follow-up of 6.9 years. This difference is likely related to the older age of the study cohort which included only adult HCM probands.[14] It is possible that differential phenotypic expression between gene groups is more evident in early onset HCM which may have a more aggressive natural history compared to adult onset HCM.

Studies exploring the impact of variant burden on phenotypic expression have been more limited and were generally anecdotal descriptions of aggressive, often infantile, disease expression.[18,44] A modest association with an earlier age of presentation and greater wall thickness was noted in a cross-sectional sample of Chinese probands with multiple variants, compared to those with isolated variants.[45] Another large report found multiple variants in 2.6% and were associated with a significantly earlier age at presentation, greater wall thickness during cross-sectional assessment and greater incidence of myectomy.[15] Our observation that the presence of multiple variants influences outcomes, despite most secondary variants being considered VUS, would support that variant burden matters in disease outcomes.

We believe our findings are clinically significant since they not only identify a genetically vulnerable subset within the larger cohort of childhood onset HCM but also highlight the young age at which adverse events start occurring in this high-risk subset. About 15% patients in the overall cohort had a MACE by 10 years of age. These findings have the potential to change when and how clinical and genetic surveillance is conducted in at risk individuals. Current American Heart Association clinical guidelines for HCM recommend that clinical and genetic testing be initiated at the age of 12 years in children who are deemed at risk due to family history of HCM.[46] Our findings suggest that surveillance should not be delayed till 12 years of age since many patients, especially those with a high risk genetic profile, suffer adverse events or require major cardiac interventions before the age of 12 years. This subset may therefore benefit from more frequent surveillance starting at an earlier age which may improve timeliness of interventions like myectomy or ICD insertion prior to onset of lethal or life-threatening cardiac events. It also argues for studies to specifically evaluate how genetic etiology can be incorporated into SCD risk prediction in early onset HCM.

4.2 | Study Limitations

Genetic testing panels changed during the study period which can influence genetic yield. However, with the exception of *TTN*, the most frequently represented genes were included on testing panels throughout the study period. Though we adjusted for non-random utilization of panel genetic testing using propensity score-matching, there may be unmeasured confounders that associate with outcomes. Finally, based on our sample size, we were not powered to compare the association of individual genes but compared *MYH7* with non-*MYH7* positive cases. Further studies are needed to explore the association of individual genes with phenotype and outcomes in HCM.

5 | CONCLUSION

This study finds an important contribution of the primary gene, of variant burden and of variant type (de novo) to risk of adverse events in pediatric HCM. This highlights the importance of incorporating genetic findings into decision making regarding age and frequency of clinical surveillance for HCM and timing of interventions like ICD or myectomy in genetically high risk patients.

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Conflict of interest

The authors declare no potential conflict of interests.

Author contributions

J.M. helped in the concept & study design, data collection, statistical analysis, authorship and review. L.Z. did the data collection and manuscript review. M.L.R. did data analysis, manuscript edits and review. J.W. data collection and manuscript review. K.G. data collection and manuscript review. L.B. did the study design, manuscript review. S.-B. did the study design, analysis, manuscript review. S.M. concept & study design, analysis, authorship, manuscript review.

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SUPPORTING INFORMATION

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