International Journal of Cardiology xxx (xxxx) xxx

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Sudden cardiac death in childhood RASopathy-associated hypertrophic cardiomyopathy: Validation of the HCM risk-kids model and predictors of events☆

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ARTICLE INFO

Keywords:
Hypertrophic cardiomyopathy
Sudden cardiac death
RASopathies

ABSTRACT

Background: RASopathies account for nearly 20% of cases of childhood hypertrophic cardiomyopathy (HCM). Sudden cardiac death (SCD) occurs in patients with RASopathy-associated HCM, but the risk factors for SCD have not been systematically evaluated.

Abbreviations: ANOVA, analysis of variance; CCF, congestive cardiac failure; CFCS, cardiofaciocutaneous syndrome; CHD, congenital heart defects; CS, Costello syndrome; CV, cardiovascular; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; IQR, interquartile range; LA, left atrium; LP, likely pathogenic; LV, left ventricle; LVH, left ventricular hypertrophy; LVOT, left ventricular outflow tract; LVOTO, left ventricular outflow tract obstruction; MACE, major arrhythmic cardiac events; MLVWT, maximal wall thickness; NS, Noonan syndrome; NS-LAH, Noonan syndrome with loose anagen hair; NSML, Noonan syndrome with multiple lentigines; NYHA, New York heart association; P, pathogenic; RA, right atrium; RV, right ventricle; RVOT, right ventricular outflow tract; RVOTO, right ventricular outflow tract obstruction; SCD, sudden cardiac death; VT, ventricular tachycardia; NSVT, non-sustained ventricular tachycardia..

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

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https://doi.org/10.1016/j.ijcard.2023.131405
Received 12 July 2023; Received in revised form 9 September 2023; Accepted 27 September 2023
Available online 28 September 2023

Noonan syndrome
Pediatrics

Aim: To validate the HCM Risk-Kids SCD risk prediction model in children with RASopathy-associated HCM and investigate potential specific SCD predictors in this population.
Methods: Validation of HCM Risk-Kids was performed in a retrospective cohort of 169 patients with a RASopathy-associated HCM from 15 international paediatric cardiology centres. Multiple imputation by chained equations was used for missing values related to the HCM Risk-Kids parameters.
Results: Eleven patients (6.5%) experienced a SCD or equivalent event at a median age of 12.5 months (IQR 7.7–28.64). The calculated SCD/equivalent event incidence was 0.78 (95% CI 0.43–1.41) per 100 patient years. Six patients (54.54%) with an event were in the low-risk category according to the HCM Risk-Kids model. Harrell's C index was 0.60, with a sensitivity of 9.09%, specificity of 63.92%, positive predictive value of 1.72%, and negative predictive value of 91%; with a poor distinction between the different risk groups. Unexplained syncope (HR 42.17, 95% CI 10.49–169.56, $p < 0.001$) and non-sustained ventricular tachycardia (HR 5.48, 95% CI 1.58–19.03, $p < 0.007$) were predictors of SCD on univariate analysis.
Conclusion: Unexplained syncope and the presence of NSVT emerge as predictors for SCD in children with RASopathy-associated HCM. The HCM Risk-Kids model may not be appropriate to use in this population, but larger multicentre collaborative studies are required to investigate this further.

1. Introduction

Sudden cardiac death (SCD) is the most common cause of death in childhood hypertrophic cardiomyopathy (HCM) after the first year of life [1–3], with higher annual rates compared to adults with HCM [4,5]. The identification of children at high risk of SCD, who would benefit most from the implantation of a primary prevention implantable cardioverter defibrillator (ICD), is a cornerstone of HCM management in childhood. We have recently published a validated model, HCM Risk-Kids, which provides an estimated 5-year risk for SCD in children with HCM based on clinical parameters: maximal left ventricular wall thickness (MLVWT), left atrial diameter (LAd), left ventricular outflow tract (LVOT) gradient, unexplained syncope, and non-sustained ventricular tachycardia (NSVT) [6,7]. However, this model has not been validated in children with syndromic HCM.

RASopathies are the most common cause of syndromic HCM, representing up to 18% of HCM cases presenting in childhood [1,3,8]. Although traditionally the risk of SCD in children with RASopathy-associated HCM has been considered to be low, recent data suggest a prevalence of SCD of up to 4% [3,8], with a a recent, large, international, multicentre study showing comparable rates to children with non-syndromic HCM [9]. Despite this, there are no published risk factors for SCD in this patient cohort. The primary aim of this study was to determine whether the HCM Risk-Kids model is an accurate tool for predicting SCD in children with RASopathy-associated HCM, with a secondary aim to investigate predictors of SCD in this population.

2. Methods

2.1. Patient population

The study cohort consisted of patients aged 18 years or younger with HCM and a clinical and/or genetic diagnosis of a RASopathy syndrome [Noonan syndrome (NS); Noonan syndrome with multiple lentigines (NSML); Costello syndrome (CS); Cardiofaciocutaneous syndrome (CFCS); Noonan syndrome with loose anagen hair (NS-LAH); and Noonan-like syndrome]. These patients were consecutively evaluated between January 1, 1985, and December 31, 2020, in 13 paediatric cardiology centres in the United Kingdom (see Supplemental Table 1), Our Lady's Children's Hospital in Dublin, Ireland, and the German Heart Centre in Munich. A diagnosis of HCM was defined as a MLVWT >2 standard deviations above the body surface area–corrected population mean (z score $\geq$ 2) that could not be solely explained by abnormal loading conditions [10]. Noonan-like syndrome was defined as a clinical diagnosis of a RASopathy syndrome without an identified pathogenic/likely pathogenic variant in RAS-MAPK. Eligible patients were identified by the principal investigator at each collaborating site. Data were collected independently at participating centres, and data integrity was ensured by each participating author.

2.2. Genetics

Patients were diagnosed with a RASopathy syndrome following systematic assessment of phenotype, and genetic testing that was performed at the treating clinician's discretion. In patients in whom genetic testing had been performed, the following data were collected: date of testing; size of gene panel; and variant(s) identified (gene and protein change). The pathogenicity of reported variants was reclassified by the authors according to the American College of Medical Genetics and Genomics (ACMG) classification [11].

2.3. Patient assessment and data collection

Anonymized clinical data from baseline evaluation were collected retrospectively, including demographics; clinical syndrome diagnosis; genetic variant identified; heart failure symptoms [New York Heart Association (NYHA) [12] or Ross [13] functional classification]; resting and ambulatory electrocardiogram (ECG); and two-dimensional (2D), Doppler and colour transthoracic echocardiogram. HCM Risk-Kids [6] predictor variables were recorded at the time of baseline evaluation: specifically, unexplained syncope (defined as a transient loss of consciousness with no identifiable cause), NSVT (defined as $\geq$3 consecutive ventricular beats at a rate of $\geq$120 beats per minute lasting $\leq$30 s on ambulatory ECG monitoring), MLVWT *Z* score [14], LAd *Z* score [15], and maximal LVOT gradient (defined as the maximal LVOT gradient at rest or with Valsalva provocation using continuous wave Doppler from apical three- or five-chamber views). LVOT obstruction (LVOTO) was defined as a peak instantaneous gradient $\geq$30 mmHg[10].

2.4. Clinical outcomes

The primary study endpoint was SCD or an equivalent event (aborted cardiac arrest, appropriate ICD therapy for a ventricular tachyarrhythmia, or sustained VT associated with haemodynamic compromise), as previously described [6]. SCD was defined as a witnessed sudden death with or without documented cardiac failure, death within 1 h of new symptoms, or a nocturnal death with no history of worsening symptoms [10]. Outcomes were determined by the treating cardiologist at each centre without knowledge of the HCM Risk-Kids estimates.

2.5. Statistical methods

Body surface area was calculated from weight [16]. Maximal left ventricular wall thickness and LAd measurements are expressed in millimetres and as body surface area-corrected z-scores. Cardiac dimensions were corrected for body size using previously published normative data [14,15]. All z-scores were recalculated using the absolute values provided by the individual centres. Follow-up time was calculated from the time of baseline evaluation to the date of reaching

the study end-point, death from another cause, or the date of the most recent evaluation. Continuous variables are described using mean [standard deviation (SD)] or median (25th, 75th percentiles), as appropriate. Categorical variables were described using frequencies and percentages. In order to compare participants' characteristics, as assessed in the baseline evaluation, the chi-square test for categorical data, *t*-test for normally distributed continuous data, or Mann–Whitney *U* test for non-normally distributed continuous data were used. A significance level of 0.05 was used for all comparisons. The Kaplan–Meier method was used to estimate the incidence of reaching the study endpoint. Univariable Cox regression models were used to investigate the association of clinical variables with the study endpoint. All statistical analyses were performed with STATA (Stata statistical software release 18; StataCorp LP, College Station, TX).

2.6. Missing data

Patients with more than three missing values in predictor variables used in HCM Risk-Kids were excluded from the validation cohort. Logistic regression was used to identify predictors of missingness and the data were found to be missing at random. We used multiple imputation by chained equations to impute (100 times) missing values of baseline variables and clinical parameters. The imputation model included all predictors of missingness, the outcome, all prespecified predictors of the HCM Risk-Kids model, and the estimate of the cumulative hazard function. The number of iterations in each imputation was set at 500. The imputation model included potential predictors of missingness, the outcome, and SCD risk predictor variables. A total of 100 imputed data sets were created and estimates from imputed data sets were combined using Rubin's rule. Trace plots and Kernel density for observed and imputed data are available in figs. S1 and S2, respectively.

2.7. HCM Risk-Kids model validation

The estimated 5-year risk of SCD was calculated for each individual patient using the HCM Risk-Kids model [6]:

$$P(\text{SCD at 5 - years}) = 1 - 0.949437808^{(\text{prognostic index})},$$

where prognostic index $= 0.2171364 \times (\text{MWT z score} - 11.09) - 0.0047562 \times (\text{MWT z score}^2 - 174.12) + 0.130365 \times (\text{LA diameter z score} - 1.92) + 0.429624 \times \text{unexplained syncope} + 0.1861694 \times \text{NSVT} - 0.0065555 \times (\text{maximal LVOT gradient} - 21.8)$.

In order to evaluate the predictive performance of the SCD risk score, discrimination and calibration measures were used. Discrimination (i.e., the ability of a model to differentiate between high-risk and low-risk patients) was assessed using Harrell's overall concordance C-statistic [17], which ranges from 0.5 (no predictive discrimination) to 1.0 (perfect discrimination). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were estimated for various cut-offs of the risk score. To graphically assess the agreement between the 5-year probability of SCD, we used a calibration plot (i.e., agreement between the predicted probability by the HCM Risk-Kids score and observed risk of SCD). To evaluate the calibration accuracy, two optimal cutoff values (0.04 and 0.06) were used to divide patients into low-risk, medium-risk, and high-risk groups [7].

2.8. Ethics

This study complies with the Declaration of Helsinki. Local ethical approval was obtained for each collaborating centre with a waiver of informed consent for retrospective, anonymized data.

3. Results

3.1. Baseline clinical characteristics

The study cohort included 169 patients of whom 8 (4.7%) were first assessed between 1981 and 1990, 24 (14.2%) between 1991 and 2000, 60 (35.5%) between 2001 and 2010, and 77 (45.6%) between 2011 and 2020. Sixteen patients (13.7%) of the 117 that this information was available for were diagnosed antenatally; in the remainder, the median (25th–75th percentile) age at diagnosis was 0.3 (0–10.3) months. The median (25th–75th percentile) age at first assessment at a paediatric cardiology centre of 18.7 (3.9–76.6) months. Seventy-eight patients (52%) were referred for routine cardiac screening following a diagnosis of a RASopathy syndrome, 62 (41.3%) due to congestive heart failure (CHF) symptoms and 10 (6.7%) due to a murmur on auscultation. Eighteen patients (10.7%) had a family history of HCM, 2 (1.2%) of SCD and 8 patients (8% of 100 patients in whom these data were available) had a family history of a RASopathy syndrome, of whom 3 also had a family history of HCM. Table 1 details baseline demographic, clinical and echocardiographic characteristics in the whole cohort and separately in patients with and without a SCD equivalent event.

3.2. Genetics

One-hundred and three patients (60.9%) had a gene variant in the RAS-MAPK pathway identified, of which 61 (59.2%) were pathogenic, 5 (4.9%) were likely pathogenic, and 5 (4.9%) were classified as a variant of uncertain significance (VUS). Thirty-nine patients (37.9%) had a variant in *PTPN11*, 26 (25.2%) had a variant in *RAF1* and 11 (10.7%) in *RIT1*. A detailed table of genetic variants with nucleotide and protein changes identified for each syndrome can be found in Table 2. Five patients had an additional variant in a cardiomyopathy gene of interest

Table 1
Demographic and clinical characteristics of patients based on sudden cardiac death endpoints.

Variable	Whole cohort *N* = 169	Patients With SCD-equivalent *n* = 11	Patients without SCD-equivalent *n* = 158	*p-value*
Gender (Male), n (%)	104 (61.5)	6 (54.5)	98 (62.0)	0.751[1]
Family history, n (%)	18 (10.7)	0 (0.0)	18 (11.4)	0.609[1]
Age at diagnosis (months), median (25th–75th)	0.0 (0.0–8.5)	3.8 (0.0–31.4)	0.0 (0.0–8.1)	0.422[2]
Unexplained syncope, n (%)	5 (3.0)	4 (36.4)	1 (0.6)	<0.001[1]
NSVT, n (%)	11 (6.5)	4 (36.4)	7 (4.4)	0.003[1]
NYHA/Ross classification, n (%)				1.000[1]
1	100 (61.0)	7 (63.6)	93 (60.8)	
≥2	64 (39.0)	4 (36.4)	60 (39.2)	
Maximal wall thickness (mm), median (25th–75th)	10.5 (8.0–13.5)	7.0 (6.0–8.0)	11.0 (8.0–14.0)	0.012[2]
Maximal wall thickness z-score, median (25th–75th)	9.2 (5.6–15.8)	6.6 (3.9–8.1)	9.2 (5.7–15.8)	0.245[2]
LA diameter (mm), median (25th–75th)	25.0 (20.0–29.0)	22.0 (18.0–28.0)	26.0 (20.0–30.0)	0.260[2]
LA diameter z-score, median (25th–75th)	2.0 (1.0–2.9)	1.4 (0.5–2.2)	2.0 (1.0–3.0)	0.239[2]
LV outflow tract peak gradient, median (25th–75th)	28.5 (10.0–61.5)	9.0 (4.0–100.0)	30.0 (10.0–60.0)	0.360[2]
LV outflow tract obstruction, n (%)	63 (37.5)	6 (54.5)	57 (36.3)	0.334[1]

Abbreviations: NYHA, New York Heart Association; LA: left atrium; LV, left ventricular.
[1] Fisher's exact test.
[2] Mann–Whitney *U* test.

Table 2
Clinical syndrome by gene affected, nucleotide and protein change.

Clinical syndrome	Affected Gene	N (%)	Nucleotide	Protein	N (%)	Significance
NS	PTPN11	27 (21.1)	c.923 A > G	p.Asn308Ser	4 (14.8)	P
			c.922 A > G	p.Asn308Asp	3 (11.1)	P
			c.836 A > G	p.Tyr279Cys	3 (11.1)	P
			c.1528C > G	p.Gln510Glu	2 (7.4)	P
			c.124 A > G	p.Thr42Ala	1 (3.7)	P
			c.1391G > C	p.Gly464Ala	1 (3.7)	P
			c.1403C > T	p.Thr468Met	1 (3.7)	P
			c.188 A > G	p.Tyr63Cys	1 (3.7)	P
			c.218C > T	p.Thr73Ile	1 (3.7)	P
			c.236 A > G	p.Glu79Arg	1 (3.7)	P
			c.317 A > C	p.Asp106Ala	1 (3.7)	P
			c.417G > C	p.Glu139Asp	1 (3.7)	P
			c.846C > G	p.Ile282Met	1 (3.7)	P
			c.854 T > C	p.Phe285Ser	1 (3.7)	P
			c.923 A > C	p.Asn308Thr	1 (3.7)	P
	RAF1	26 (20.3)	c.770C > T	p.Ser257Thr	5 (19.2)	P
				p.Ser257Leu	2 (7.7)	P
				p.Ser257Gly	1 (3.9)	P
			c.775 T > A	p.Ser259Thr	4 (15.4)	P
			c.781C > T	p.Pro261Ser	3 (11.5)	P
			c.768G > T	p.Arg256Ser	2 (7.7)	P
			c.779C > T	p.Thr260Ile	1 (3.9)	LP
			c.776C > T	p.Ser259Phe	1 (3.9)	P
			c.1082G > C	p.Gly361Ala	1 (3.9)	P
			c.766 A > G	p.Arg256Gly	1 (3.9)	LP
	RIT1	11 (8.6)	c.170C > G	p.Ala57Gly	2 (18.2)	P
			c.244 T > C	p.Phe82Leu	2 (18.2)	P
			c.151G > T	p.Asp51Tyr	1 (9.9)	VUS
			c.284G > C	p.Gly95Ala	1 (9.9)	P
			c.229G > A	p.Ala77Thr	1 (9.9)	P
			c.244 T > A	p.Phe82Ile	1 (9.9)	P
	LZTR1	4 (3.1)	c.1234C > T	p.Arg412Cys	1 (3.7)	VUS*
			c.290G > T	p.Arg97Leu	1 (3.7)	VUS
	KRAS	2 (1.6)	c.179G > T	p.Gly60Val	1 (50.0)	P
			c.346 A > C	p.Asn116His	1 (50.0)	LP
	MAP2K2	1 (0.8)	N/A	N/A		
	SHOC2	1 (0.8)	N/A	N/A		
	Not tested	32 (25.0)				
	Variant undentified	24 (18.8)				
NSML	PTPN11	12 (63.2)	c.836 A > G	p.Tyr279Cys	4 (33.3)	P
			c.1528C > G	p.Gln510Glu	2 (16.7)	P
	KRAS**	1 (5.3)	c.173C > T	p.Thr58Ile	1 (100.0)	P
	Variant undentified	6 (31.6)				
CS	HRAS	9 (90.0)	c.34G > A	p.Gly12Ser	6 (66.7)	P
			c.34G > T	p.Gly12Cys	1 (11.1)	P
			c.466 T > C	p.Phe156Leu	1 (11.1)	P
			c.64C > A	p.Gln22Lys	1 (11.1)	LP
	Variant undentified	1 (10.0)				
CFCS	BRAF	3 (50.0)	c.1782 T > G	p.Asp594Glu	1 (33.3)	LP
	MAP2K2	1 (16.7)	c.619G > A	p.Glu207Lys	1 (100.0)	LP
	KRAS	1 (16.7)	N/A	N/A		
	Variant undentified	1 (16.7)				
NS_LAH	SHOC2	3 (50.0)	c.4 A > G	p.Ser2Gly	1 (33.3)	P
	KRAS	1 (16.7)	c.179G > T	p.Gly60Val	1 (100.0)	P
Noonan-like syndrome	Variant not identified	2 (33.3)				

Abbreviations: NS, Noonan syndrome; NSML, Noonan syndrome with multiple lentigines; CS, Costello syndrome; CFCS, Cardiofaciocutaneous syndrome; NS_LAH, Noonan syndrome with loose anagen hair; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance; VUS (+), hot VUS.

* Conflicting evidence according to ClinVar suggesting this genetic variant might also be considered likely pathogenic.

** Although KRAS is not considered a classical NSML gene, the clinical phenotype was felt to be consistent with a diagnosis of NSML by the referring clinician.

identified (LP in *MAP2K2* with P in *MYH7* (familial), LP in *RAF1* with VUS in *MYH7*, P in *PTPN11* with VUS in *MYH7*, unknown RAS-MAPK variant with VUS in *FLH1*, P in *KRAS* with additional VUS in *MEK1*).

3.3. Clinical outcomes and sudden cardiac death end-points

Twenty-eight patients (16.6%) died [8 (28.6%) CHF; 3 (10.7%) SCD; 6 (21.4%) non-cardiac cause; and 11 (39.3%) unknown] at a median (25th–75th percentile) age of 105 (12.8–191.1) months. Thirty-one patients (18.6%) underwent myectomy, and 9 (5.4%) had a primary prevention ICD implanted. No patient underwent cardiac transplantation or secondary prevention ICD implantation during the follow-up period.

Eleven patients (6.5%) experienced a SCD equivalent event [3 (27.3%) SCD; 5 (45.5%) aborted cardiac arrest; 1 (9.1) appropriate ICD shock; and 2 (18.2%) sustained VT] at a median (25th–75th percentile) age of 12.5 (2.9–44.8) months, of whom 9 had a diagnosis of NS, 1 NSML and 1 CS. Four patients did not have a gene variant identified, 2 had a pathogenic variant in *RAF1*, 2 in *PTPN11* and 1 each in *RIT1*, *LZTR1* and *HRAS*. The calculated SCD equivalent event incidence was 0.86 (95% CI

0.48–1.56) per 100 person-years (see Table 3).

3.4. Missing data

Eighty-four patients (49.7%) had one or more missing data from the HCM Risk-Kids model predictor variables: 30 (17.8%) had one missing variable, 29 (17.2%) had two missing variables, and 25 (14.8%) had three missing variables. Supplemental Table 2 contains further information on missing data per variable.

Table 3
Sudden Cardiac Death (SCD) incidence in children with RASopathy-associated hypertrophic cardiomyopathy (HCM) from a Cox proportional hazards model.

Variable	N (%)	Events	PYs	Incidence per 100 PYs (95% CI)
All participants	169 (100.0)	11	1277.1	0.86 (0.48–1.56)
Gender				
Male	104 (61.5)	6	792.5	0.76 (0.34–1.69)
Female	65 (38.5)	5	484.6	1.03 (0.43–2.48)
Family history				
No	151 (89.3)	11	1085.2	1.01 (0.56–1.83)
Yes	18 (10.7)	0	191.9	
NYHA/Ross classification				
1	100 (61.0)	7	695.3	1.01 (0.48–2.11)
≥ 2	64 (39.0)	4	537.0	0.74 (0.28–1.98)
Clinical syndrome				
Noonan syndrome	128 (75.7)	9	1021.2	0.88 (0.46–1.69)
Noonan syndrome with multiple lentigines (NSML)	19 (11.2)	1	137.6	0.73 (0.10–5.16)
Costello syndrome	10 (5.9)	1	48.7	2.05 (0.29–14.58)
Cardiofaciocutaneous syndrome (CFCS)	6 (3.6)	0	52.3	
Noonan syndrome with loose anagen hair (NS_LAH)	3 (1.8)	0	8.4	
Noonan-like syndrome	3 (1.8)	0	9.0	
Gene				
RIT1	11 (6.5)	1	75.4	1.33 (0.19–9.41)
RAF1	26 (15.4)	2	197.2	1.01 (0.25–4.05)
PTPN11	39 (23.1)	2	265.2	0.75 (0.19–3.02)
HRAS	9 (5.3)	1	47.0	2.13 (0.30–15.10)
Unknown	66 (39.1)	4	548.1	0.73 (0.27–1.94)
Unexplained syncope				
No	164 (97.0)	7	1253.3	0.56 (0.27–1.17)
Yes	5 (3.0)	4	23.8	16.84 (6.32–44.87)
NSVT				
No	158 (93.5)	7	1169.0	0.60 (0.29–1.26)
Yes	11 (6.5)	4	108.1	3.70 (1.39–9.86)
LV outflow tract obstruction				
No	105 (62.5)	5	727.5	0.69 (0.29–1.65)
Yes	63 (37.5)	6	532.0	1.13 (0.51–2.51)

Abbreviations: NYHA, New York Heart Association; LA, left atrium; NSVT, non-sustained ventricular tachycardia; LVOTO, left ventricular outflow tract obstruction.

3.5. HCM Risk-Kids validation

The performance of the HCM Risk-Kids model in predicting risk at 5 years in this cohort was assessed. Harrell's C index was 0.60 (95% CI 0.5–1). In investigating the ability of the risk score to differentiate between high and low risk, using a cut-off of 6%, the sensitivity was 9.4%, specificity 63.9%, positive predictive value 1.7%, and negative predictive value 91%. Fig. 1 (A,B) describes the survival in the whole group and by risk category (low, medium, high) as defined by the HCM Risk-Kids score, showing overlap between different risk categories. The clinical syndrome, genetics, HCM Risk-Kids risk score parameters of individuals with SCD equivalent event are detailed in Table 4, showing that 6 out of 11 (54.5%) patients who had an event were in the low-risk category.

3.6. Predictors of SCD equivalent events

Unexplained syncope (HR 42.17, 95% CI 10.49–169.56, $p < 0.001$) and the presence of NSVT on Holter monitoring (HR 5.48, 95% CI 1.58–19.03, $p < 0.007$) were found to be predictors of SCD or equivalent event on univariate analysis (see Table 5). Fig. 1 (C, D) demonstrates the event free survival with and without unexplained syncope and with and without NSVT in this cohort.

4. Discussion

To our knowledge, this is the first study to validate a paediatric SCD risk prediction model, HCM Risk-Kids, in children with RASopathy syndromes and HCM. The findings suggest that HCM Risk-Kids does not have good discriminatory ability in this population, although this may be related to sample size and a relatively low event rate. Unexplained syncope and NSVT appear to be predictors of SCD risk in children with RASopathy-associated HCM.

5. Clinical significance

5.1. Prevalence of SCD

The reported prevalence of SCD in children with RASopathy-associated HCM has been estimated at 4% [3,8]. Although the prevalence of SCD and equivalent events in this study was relatively high at 6.5%, the annual incidence is lower than that seen in paediatric non-syndromic populations [1–3]. It is possible that this may be overestimated in our study as the cohort consists of patients referred to a paediatric cardiology centre, and may therefore have a more severe phenotype. This may also explain the findings of a recent study suggesting a similar cumulative incidence of SCD in children with RASopathy-associated HCM and those with non-syndromic disease [9]. Nevertheless, the findings highlight the fact that SCD can occur in patients with RASopathy-associated HCM, emphasizing the importance of SCD risk prediction in this group of patients.

5.2. Validation of HCM Risk-Kids model in patients with RASopathy-associated HCM

The findings of this study suggest that the HCM Risk-Kids model may not have good discriminatory ability between low, medium, and high risk categories of patients in children with RASopathy-associated HCM, and has a very low specificity and positive predictive value. This is supported by the fact that the majority of patients who had a SCD equivalent event had a low estimated 5-year SCD risk. Individuals with RASopathy-associated HCM have a distinct phenotype compared to patients with sarcomeric gene variants [1–3]. Despite a comparable prevalence of SCD equivalent events compared to the original HCM Risk-Kids cohort [6], our group was more symptomatic at baseline evaluation, had unexplained syncope less frequently, and were more

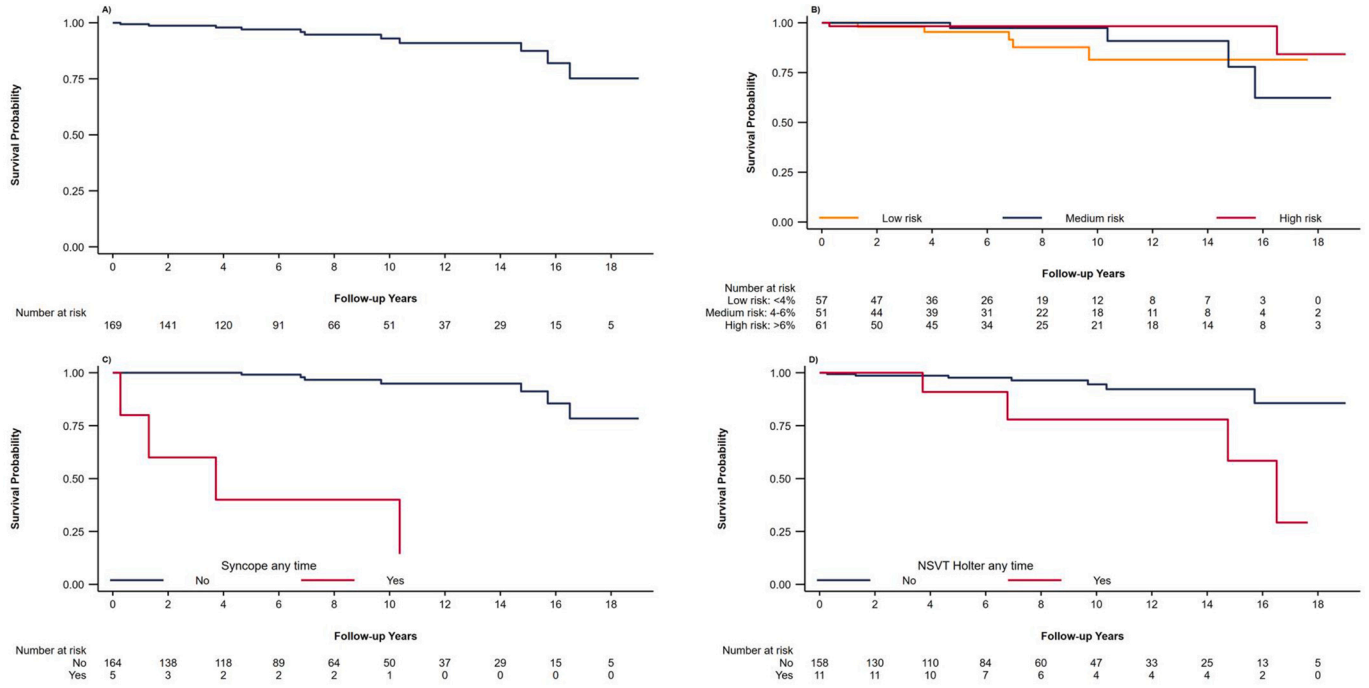

Fig. 1. Kaplan-Meier survival curves for sudden cardiac death equivalent for whole cohort (A), by risk category (B), in patients with and without a history of syncope (C) and in patients with and without evidence of NSVT on holter monitoring (D) in follow up time (years).

Table 4
Clinical diagnosis, genetics, HCM Risk-Kids score parameters of patients with sudden cardiac death (SCD) equivalent event.

Clinical diagnosis	Affected Gene	Risk category	5-year risk (%)	MLVWT z score	LA diameter z score	History of syncope	NSVT on Holter	LVOT gradient (mmHg)
Noonan syndrome	Unknown	Low risk	2.31	+3.4	+1.4	No	No	4
Noonan syndrome	Unknown	Low risk	2.74	+2.4	+1.4	Yes	No	14
Noonan syndrome	Unknown	Low risk	3.42	+8.2	−0.5	No	No	16
Noonan syndrome	RAF1	Low risk	3.56	+6.2	+1	No	No	10
Noonan syndrome	RIT1	Low risk	3.60	+6.5	+1.8	Yes	Yes	110
Costello syndrome	HRAS	Low risk	1.56	+1.4	+0.5	No	Yes	8
Noonan syndrome	PTPN11	Medium risk	4.21	+6.7	+2.2	No	No	4
Noonan syndrome	LZTR1	Medium risk	4.37	+3.9	+2.6	Yes	No	2
Noonan syndrome	Unknown	High risk	6.12	+8.1	+2	No	Yes	10
Noonan syndrome	RAF1	High risk	7.43	+11.6	+1.3	No	Yes	27
NSML	PTPN11	High risk	8.63	+28.5	+1.1	Yes	No	100

Abbreviations: NSML, Noonan syndrome with multiple lentigines; MLVWT, maximal left ventricular wall thickness; LA, left atrium; NSVT, non-sustained ventricular tachycardia; LVOT, left ventricular outflow tract.

likely to have LVOTO. It is possible that the poor performance of the HCM-Risk-Kids model in children with RASopathy-associated HCM may be related to the relatively small numbers of patients included in this study, supported by the finding that 2 of the variables included in the model (NSVT and syncope) appear to be predictors of SCD for this population as well. Nonetheless, the findings suggest that the use of the HCM Risk-Kids model for the 5-year SCD prediction may not be appropriate in this population on current evidence, and larger multi-centre studies are required to explore this further.

5.3. Predictors of SCD

Unexplained syncope and the presence of NSVT were shown to be predictors for SCD on univariate analysis in this study, in line with adult and paediatric risk prediction models for nonsyndromic HCM [6,18,19]. Syncope in patients with HCM may be related to arrhythmic causes, haemodynamic abnormalities such as LVOTO, or abnormal vascular responses [20]; our findings suggest that these mechanisms may also be important in patients with RASopathy-associated HCM. Similarly, NSVT is an established risk factor for SCD in patients with nonsyndromeic HCM, particularly in young individuals [21–23] and the findings in the present study suggest that this may also be the case in children with RASopathy-associated HCM. In contrast, MLVWT and LAd did not emerge as predictors of SCD in this cohort [24]. These findings highlight the need to identify specific risk factors in RASopathy-associated HCM and explore independent predictors in this population.

6. Limitations

This study is limited by the fact that it is a retrospective study and inherently has missing or incomplete data. To ensure our imputation of missing data was robust, we incorporated all predictors in the imputation model that we considered to be important to explain missingness. The proportion of missing data was similar to the HCM Risk-Kids cohort. Imputation diagnostics by a comparison of means and distribution of predictors before and after imputation confirmed that the data had not been distorted. Moreover, we are investigating a rare condition with a low number of events, lower than the paediatric sarcomeric cohort that this model was developed in. Variations in clinical assessment and patient management are inevitable as patients were recruited from

Table 5
Sudden Cardiac Death (SCD) predictors from a univariate analysis (Cox proportional hazards model).

Variable	HR (95%CI)	p-value
Gender		
Male	1	
Female	1.33 (0.41–4.36)	0.638
Family history		
No	1	
Yes	0	1.000
Age at diagnosis	1.01 (0.99–1.02)	0.298
Age at first assessment	0.99 (0.84–1.18)	0.932
NYHA/Ross classification		
1	1	
≥ 2	0.78 (0.23–2.69)	0.695
Clinical syndrome		
Noonan syndrome	1	
Noonan syndrome with multiple lentigines	0.79 (0.10–6.29)	0.637
Costello syndrome	3.43 (0.41–28.69)	0.265
Cardiofaciocutaneous syndrome	0	1.000
Noonan syndrome with loose anagen hair	0	1.000
Noonan-like syndrome	0	1.000
Gene		
PTPN11	0.58 (0.05–6.45)	0.659
RAF1	0.85 (0.08–9.42)	0.893
RIT1	1	
HRAS	2.38 (0.14–39.89)	0.547
Unknown	0.57 (0.06–5.14)	0.616
Unexplained syncope		
No	1.00	
Yes	42.17 (10.49–169.56)	<0.001
NSVT	5.48 (1.58–19.03)	0.007
Maximal wall thickness z score	0.90 (0.78–1.03)	0.134
LA diameter z score	0.73 (0.45–1.16)	0.177
LV outflow tract peak gradient	0.99 (0.97–1.01)	0.323
LV outflow tract obstruction		
No	1	
Yes	1.49 (0.45–4.91)	0.513

Abbreviations: NYHA, New York Heart Association; NSVT, non-sustained ventricular tachycardia; LA, left atrium; LV, left ventricular.

multiple centres and across different eras. Genetic testing was performed at the participating clinicians' discretion. Although a high proportion of patients with a RASopathy syndrome had a disease-causing variant identified on genetic testing, it is not known whether genetic testing results altered the final diagnosis or confirmed previous clinical suspicions. Variations in echocardiographic protocols and availability of images for retrospective assessment in different centres and eras resulted in missing variables. Data collection for this cohort relied on patients being referred to collaborating paediatric cardiology centres. The true incidence of SCD events occurring in RASopathy-associated HCM is unknown and while this study provides an event rate, this may not be representative of the true population prevalence. The small population sample and low event rate resulted in wide CIs for the C-index values, which represents uncertainty in the estimates. This also prevented investigation of independent predictors of SCD using a multivariate analysis. Further, this study represents a childhood cohort, and the findings may not necessarily be applicable to older adolescents and young adults. The limitations of the study design could be addressed with future prospective, large multicentre studies investigating predictors of SCD in patients with RASopathy syndromes and HCM. Further studies to assess the potential role of additional imaging (including echocardiography and cardiac MRI), electrocardiographic, and circulating biomarkers in SCD risk prediction in children with RASopathy-associated HCM may provide additional insights into risk assessment in this population.

7. Conclusion

This study shows that SCD and malignant ventricular arrhythmias can occur in children with RASopathy-associated HCM. The HCM Risk-Kids SCD risk prediction model does not appear to have good discriminatory and calibration power for children with RASopathy-associated HCM, and its use to predict SCD risk may therefore not be appropriate in this population. Unexplained syncope and the presence of NSVT appear to be predictors for SCD in children with RASopathy-associated HCM, but large multicentre studies are needed to investigate this further.

CRediT authorship contribution statement

Olga D. Boleti: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Sotirios Roussos:** Formal analysis, Methodology, Software, Visualization, Validation, Writing – original draft. **Gabrielle Norrish:** Data curation, Methodology, Writing – review & editing. **Ella Field:** Data curation, Writing – review & editing. **Jennifer Tollit:** Data curation, Writing – review & editing. **Stephanie Oates:** Methodology, Writing – review & editing. **Gauri Nepali:** Data curation, Project administration, Writing – review & editing. **Vinay Bhole:** Data curation, Project administration, Writing – review & editing. **Orhan Uzun:** Data curation, Project administration, Writing – review & editing. **Piers E.F. Daubeney:** Data curation, Project administration, Writing – review & editing. **Graham A. Stuart:** Data curation, Project administration, Writing – review & editing. **Precylia Fernandes:** Data curation, Project administration, Writing – review & editing. **Karen McLeod:** Data curation, Project administration, Writing – review & editing. **Maria Ilina:** Data curation, Project administration, Writing – review & editing. **Muhammad Najih Ali Liaqath:** Data curation, Project administration, Writing – review & editing. **Tara Bharucha:** Data curation, Project administration, Writing – review & editing. **Grazia Delle Donne:** Data curation, Project administration, Writing – review & editing. **Elspeth Brown:** Data curation, Project administration, Writing – review & editing. **Katie Linter:** Data curation, Project administration, Writing – review & editing. **Bernadette Khodaghalian:** Data curation, Project administration, Writing – review & editing. **Caroline Jones:** Data curation, Project administration, Writing – review & editing. **Jonathan Searle:** Data curation, Project administration, Writing – review & editing. **Sujeev Mathur:** Data curation, Project administration, Writing – review & editing. **Nicola Boyd:** Data curation, Project administration, Writing – review & editing. **Zdenka Reindhardt:** Data curation, Project administration, Writing – review & editing. **Sophie Duignan:** Data curation, Project administration, Writing – review & editing. **Terence Prendiville:** Data curation, Project administration, Writing – review & editing. **Satish Adwani:** Data curation, Project administration, Writing – review & editing. **Martin Zenker:** Investigation, methodology, Writing – review & editing. **Cordula Maria Wolf:** Data curation, Investigation, Methodology, Writing – review & editing. **Juan Pablo Kaski:** Conseptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Funding support

This work was supported by the Onassis Foundation. EF and JPK are supported by Max's Foundation and Great Ormond Street Hospital Children's Charity. JPK is supported by a Medical Research Council (MRC) Clinical Academic Research Partnership (CARP) award (MR/T024062/1). CMW was supported by the Deutsche Herzstiftung e.V., Gerd-Killian Award. MZ received funding from the German Ministry of Education and Research (BMBF), project titles GeNeRARe (FKZ 01GM1902A) and NSEuroNet (FKZ 01GM1921A).

Declaration of Competing Interest

Wolf CM: consultancy with Day One Biopharmaceuticals, Inc., BioMarin Pharmaceuticals, Adrenomed AG, and Pliant Therapeutics;

ownership interest: Preventage Therapeutics. Zenker M: consultancy with Day One Biopharmaceuticals, Inc. and Novo Nordisk.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2023.131405.

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