Deep phenotyping of paediatric Rasopathy-associated hypertrophic cardiomyopathy… Natural history and outcomes

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[redacted]

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Declaration

I, Olga Boleti, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Background:

Hypertrophic cardiomyopathy (HCM) associated with Rasopathy syndromes is the second most common cause of HCM in childhood and represents a unique clinical entity characterized by early-onset disease, variable phenotypic expression, and increased risk of morbidity and mortality in childhood.

Objectives:

To characterize the phenotypic spectrum, natural history, electrocardiographic (ECG) and imaging features, and risk predictors for major adverse cardiovascular events (MACE) and sudden cardiac death (SCD) in a large, multicentre cohort of children with RAS-HCM (RAS-HCM).

Methods:

This retrospective cohort study included data from paediatric patients with genetically or clinically confirmed Rasopathy syndromes and HCM (RAS-HCM), recruited across multiple international centres. Longitudinal data on clinical course, cardiac imaging, ECG and ambulatory monitoring were analysed.

Results:

RAS-HCM presents with a heterogeneous phenotype, with marked differences in severity and outcomes based on specific syndromes and genotypes. Key findings included a more severe cardiac phenotype in patients with a *RAF1* and *RIT1* gene variant, and in the whole cohort the finding of progressive left atrial dilation, diastolic dysfunction, and the emergence of complex atrial arrhythmias in early adulthood. Functional status (NYHA/Ross class > I), presence of NSVT, unexplained syncope, and elevated LVOT gradient were independently associated with adverse outcomes. The risk model currently used to predict sudden cardiac death (SCD) in children with non-syndromic HCM, HCM Risk-Kids, underperformed in risk stratification for this population.

Conclusions:

RAS-HCM is a distinct clinical entity requiring tailored approaches to diagnosis, monitoring, and risk stratification. Early identification of high-risk patients is essential. Multimodal longitudinal assessment should be considered to guide therapy and surveillance.

Impact statement

Rasopathy-associated hypertrophic cardiomyopathy (RAS-HCM) represents a disproportionately understudied yet clinically high-impact subgroup of paediatric cardiomyopathy. Despite accounting for a significant proportion of infantile-onset HCM, RAS-HCM has historically been grouped with other syndromic forms or analysed through the lens of sarcomeric disease, thereby obscuring its distinct natural history, risk factors, and therapeutic considerations. The paucity of longitudinal, genotype-informed, and multimodal phenotyping data has hindered the development of tailored surveillance protocols and risk prediction models. Moreover, existing risk stratification tools—validated exclusively in non-syndromic cohorts—may not adequately capture the arrhythmogenic or haemodynamic complexities of RAS-HCM.

This thesis delivers the largest and most comprehensive deep phenotyping analysis of paediatric RAS-HCM conducted to date. Drawing upon a uniquely assembled international multicentre cohort, it directly addresses these gaps by providing a detailed and structured analysis of RAS-HCM across clinical, imaging, electrocardiographic, and functional domains, while simultaneously identifying limitations in current predictive models and proposing syndrome-specific risk determinants. In doing so, it lays essential groundwork for the redefinition of RAS-HCM as a discrete clinical entity deserving of bespoke diagnostic algorithms, therapeutic approaches, and future trial frameworks.

A major clinical impact of this work lies in its direct relevance to precision cardiology. The thesis demonstrates that RAS-HCM is not merely a syndromic variant of sarcomeric HCM but represents a phenotypically and prognostically distinct disease with unique progression patterns, genotype–phenotype correlations, and risk profiles. This is of vital importance for cardiologists, as it challenges the conventional reliance on non-syndromic HCM paradigms and urges a departure from the 'one-size-fits-all' model in paediatric cardiomyopathy management. By identifying independent predictors of adverse outcomes—particularly functional status, non-sustained ventricular tachycardia (NSVT), and left atrial dilation—this

study provides actionable metrics that can be integrated into routine surveillance and early intervention pathways.

Another key implication is the evaluation of the HCM Risk-Kids prediction tool in the RAS-HCM population. The study illustrates its limited predictive utility in this subgroup, underscoring the urgent need for syndrome-specific risk stratification models. These insights hold the potential to shape forthcoming clinical guidelines by the European Society of Cardiology (ESC) and American Heart Association (AHA), ensuring they provide more detailed recommendations for syndromic HCM subtypes. As clinical risk stratification increasingly informs decisions regarding implantable cardioverter-defibrillators (ICDs), transplant referral, and advanced therapies, this thesis provides further evidence for children with RAS-HCM.

This thesis bridges molecular genetics with clinical cardiology by demonstrating that genotype can inform phenotype, not only in terms of cardiac morphology but also electrophysiological behaviour and functional decline. This integration paves the way for biologically-informed disease modelling. In particular, the findings offer a framework for future mechanistic studies exploring Ras/MAPK pathway dysregulation and its direct impact on myocardial architecture, arrhythmogenesis, and fibrosis. Such a framework is essential for translational research efforts targeting disease-modifying therapies.

Furthermore, this thesis lays the groundwork for therapeutic innovation. As MEK inhibitors and other targeted molecular therapies emerge from oncology and rare disease research into the cardiogenetics space, this thesis provides the clinical phenotype and natural history data needed to design and power interventional trials in RAS-HCM. Moreover, it identifies which patients might benefit from early pharmacological intervention, potentially modifying disease trajectory before irreversible remodelling or arrhythmic events occur.

Finally, the multidisciplinary nature of this work, spanning genetics, paediatric cardiology, imaging, electrophysiology, and clinical epidemiology, is an example of the current approach required to tackle rare cardiovascular diseases. Its findings are relevant not only to

paediatric cardiologists, but also to geneticists, electrophysiologists, imaging specialists, and clinical trialists, fostering collaborative networks essential for rare disease research.

In summary, this thesis significantly contributes to the understanding of a rare disease entity, with implications for risk prediction, guideline development therapeutic targeting.

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List of abbreviations

2D: 2 dimensional

ACMG: American College of Medical Genetics

ANOVA: analysis of variance

ASH: asymmetric septal hypertrophy

BP: blood pressure

BSA: body surface area

BVH: biventricular hypertrophy

CFCS: cardio-facio-cutaneous syndrome

CHD: congenital heart defects

CHF: congestive cardiac failure

CMR: cardiac magnetic resonance imaging

CS: Costello syndrome

CV: cardiovascular

ECG: electrocardiogram

EF: ejection fraction

FHx: family history

FS: fractional shortening

FU: follow-up

HCM: hypertrophic cardiomyopathy

HR: heart rate

ICD: implantable cardioverter defibrillator

IVRT: isovolumetric relaxation time

IVS: intraventricular septum

IQR: interquartile range

LA: left atrium

LBBB: left bundle branch block

LGE: late gadolinium enhancement

LP: likely pathogenic

LV: left ventricle

LVCO: left ventricular cardiac output

LVEDD: left ventricular end diastolic diameter

LVESD: left ventricular end systolic diameter

LVEDV: Left ventricular end diastolic volume

LVESV: Left ventricular end systolic volume

LVH: left ventricular hypertrophy

LVMi: Left ventricular mass index

LVPWT: Left ventricular posterior wall thickness

LVOT: left ventricular outflow tract

LVOTO: left ventricular outflow tract obstruction

MACE: major adverse cardiac events

MAPK: mitogen-activated protein kinase

MLVWT: maximal wall thickness

NS: Noonan syndrome

NS-LAH: Noonan syndrome with loose anagen hair

NSML: Noonan syndrome with multiple lentigines

NSVT: non-sustained ventricular tachycardia

NYHA: New York heart association

OR: Odds ratio

P: pathogenic

RA: right atrium

RAS-HCM: Rasopathy-associated hypertrophic cardiomyopathy

RBBB: right bundle branch block

RV: right ventricle

RVH: right ventricular hypertrophy

RVOT: right ventricular outflow tract

RVOTO: right ventricular outflow tract obstruction

SCD: sudden cardiac death

SD: standard deviation

s-HCM: sarcomeric hypertrophic cardiomyopathy

TTE: transthoracic echocardiogram

VF: ventricular fibrillation

VT: ventricular tachycardia

VUS: variant of uncertain significance

QTc: QT corrected

Personal contributions

This project was conceptualised by Professor Juan Pablo Kaski. The international cohort of paediatric Rasopathy-associated hypertrophic cardiomyopathy was a continuation of the United Kingdom paediatric hypertrophic cardiomyopathy cohort established by prof. Juan Pablo Kaski and further expanded upon by Dr Gabrielle Norrish during her PhD work. Data collection was performed by local investigators in each participating site. I was responsible for the subsequent conduct of all aspects of the study including obtaining ethical approval, data collection locally at Great Ormond Street Hospital and data analysis.

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Aims of thesis

This work comprises the largest comprehensive and systematic investigation of paediatric Rasopathy-associated hypertrophic cardiomyopathy. The specific aims were to:

- Develop an international multi-centre cohort of children (presenting under the age of 18 years) with hypertrophic cardiomyopathy and an underlying diagnosis of a Rasopathy syndrome to allow the description of the natural history of this disease and investigate potential predictors of major adverse cardiac events
- Describe the risk of sudden cardiac death in this population and seek to validate the existing risk model for sudden cardiac death in childhood hypertrophic cardiomyopathy (HCM Risk-Kids)
- Characterise the long-term phenotypic progression of this disease using serial data and investigate for any independent-of-time risk factors for major adverse cardiac events
- Explore for any population-specific markers in second line and advanced cardiac investigations

Chapter 1 - Introduction

1.1 Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is the most common form of cardiomyopathy, affecting approximately 1 in 500 individuals, and is known to be leading cause of sudden cardiac death (SCD)[1]. It was first described in 1958 by an English pathologist, Dr Donald Teare, as 'a tumour of the heart'[2]. He noted the 'disordered arrangement of muscle bundles' in the myocardium, now known as the hallmark of HCM, myocyte disarray. This disease was later discovered to be familial and linked to sudden death, even in younger individuals[3 4]. Now we know that HCM is a clinically and genetically heterogenous condition characterised by left ventricular hypertrophy (LVH), unexplained by abnormal loading conditions[5]. The first discovery of a molecular basis of HCM, linked to a missense variant in the beta cardiac myosin heavy chain (MYH7) was made in 1990[6]. Since then, through research, several gene variants have been identified and thought to have a causal link with HCM and are most often mutations in sarcomere genes or mutation in sarcomere-related proteins[7-9]. Causes also include inborn errors of metabolism (IEM), Rasopathy syndromes, neuromuscular disease[5]. This is condition has a uniqueness in that it can present at any age, from infancy to older individuals[1,5,10].

1.1.1 Hypertrophic cardiomyopathy in children

HCM is the most common cardiomyopathy in the paediatric population in Europe[11], second most common in North America[12] and Australia[13], and a leading cause of sudden cardiac death (SCD) in childhood[14], with aetiological and clinical heterogeneity[15-18]. This may present at any age, with the highest peak being in infancy, represented primarily by patients with no family history of HCM, and a second peak in adolescence, with a higher proportion being patients with familial HCM[12,17,19].

1.1.1.1 Epidemiology

HCM is rarer in the paediatric population compared to adults[20], with an estimated prevalence of less than 3:100,000[13,16]. There is a reported male predominance[17-19], which has been hypothesised to be secondary to sex hormones[21]. However, since this difference exists in the pre-adolescent population as well[22], the aetiology might be multifactorial and include epigenetic factors.

1.1.1.2 Aetiology

Most cases in children, similarly to the adult population[23], are caused by mutations in the genes encoding the sarcomeric units of the cardiac muscle fibres[24] (see Figure 1-1), inherited as an autosomal dominant trait. Syndromic and metabolic aetiologies nevertheless account for a significant minority of cases, particularly in infancy and early childhood[17].

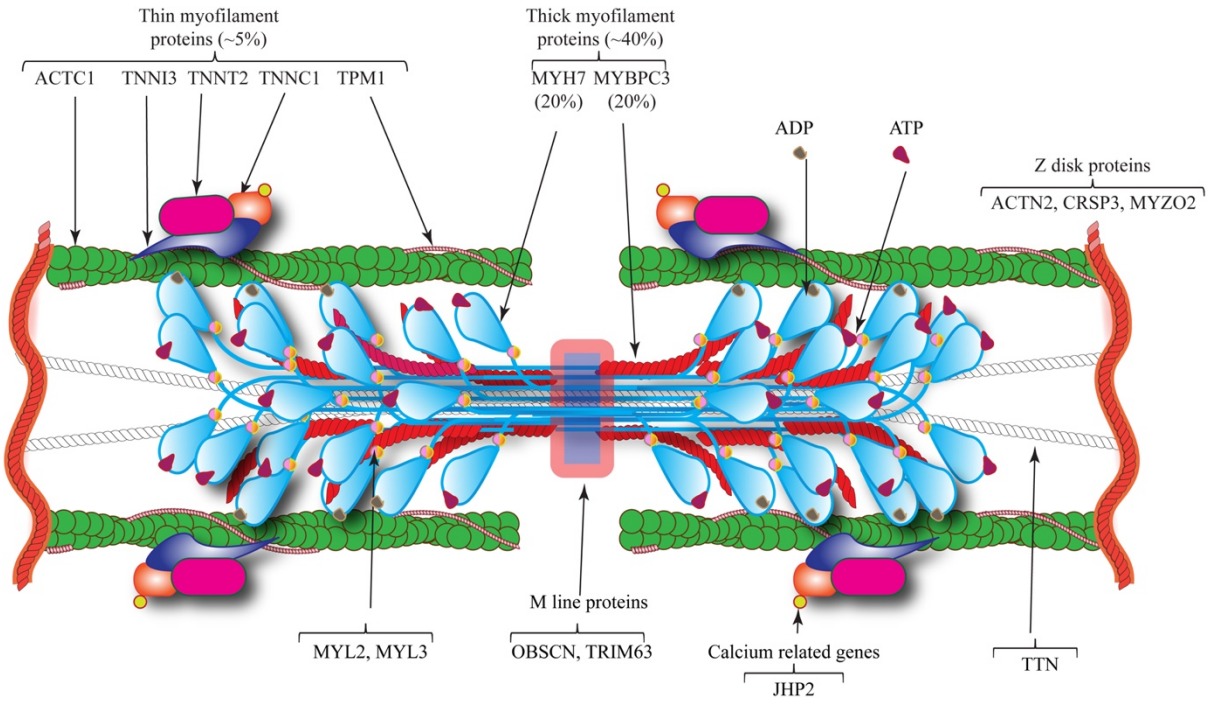


Figure 1-1: Cardiac sarcomere unit demonstrating the proteins of which gene variants cause HCM[25]

In a microscopic level, mutations in sarcomeric proteins increase myofilament activation resulting in cardiomyocyte hypercontractility, increased energy demand and usage[26-28]. Changes in the energy status of the cardiomyocytes are also known to be a result of mutations affecting primary energy generation in the cells, such as in the mitochondrial RNA or in variants of the AMP-activated protein kinase (AMPK)[26]. Any such changes result in impaired myocyte relaxation and promote myocyte growth accompanied by disarray and fibrosis (see Figure 1-2). Additional disease mechanisms involve impaired Ca2+ regulation, resulting in incomplete relaxation and impaired diastolic function, further increasing the energy expenditure[29,30].

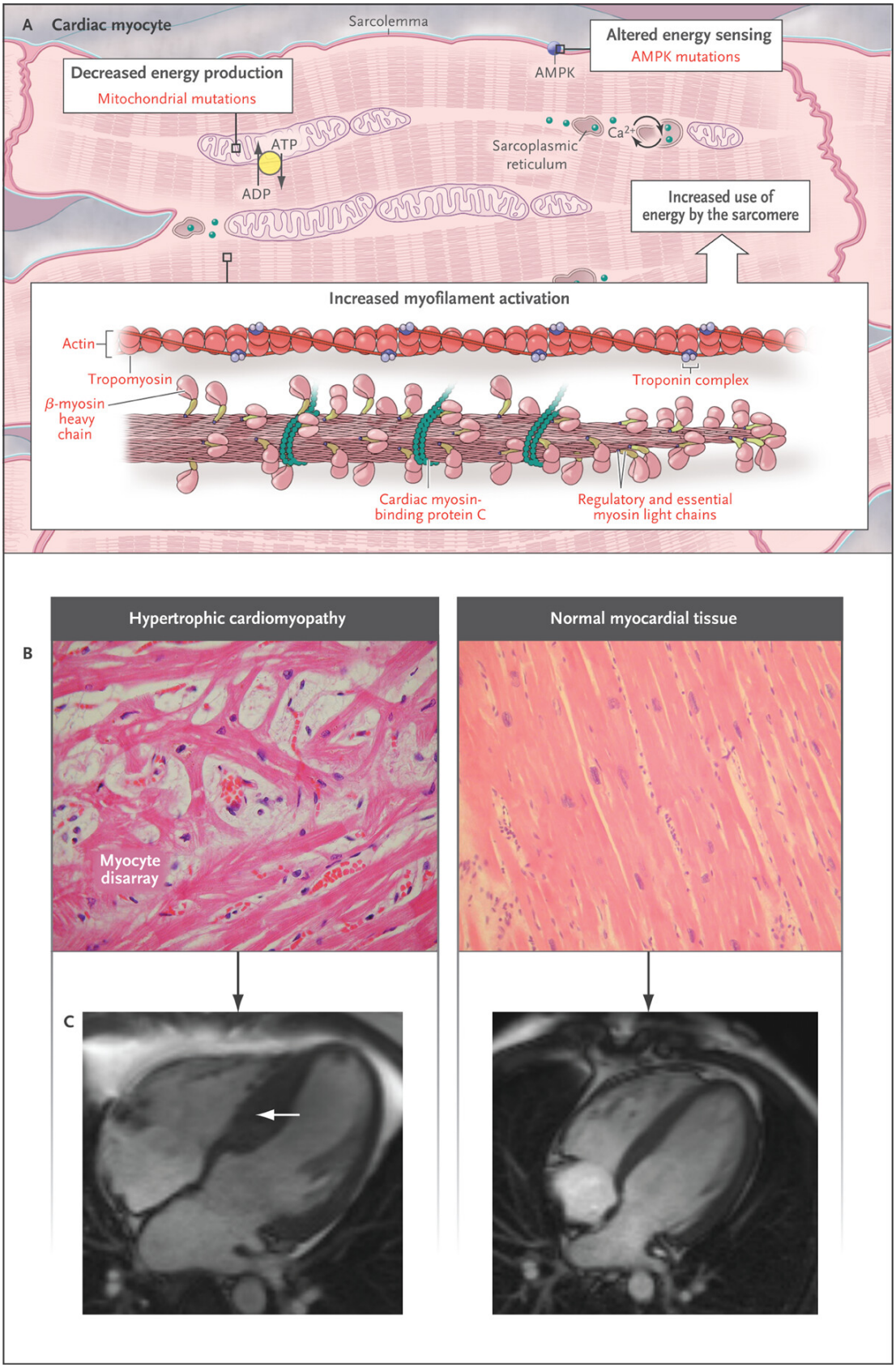


Figure 1-2: (a) cardiomyocyte with common causes of HCM, zoom into the structure of the sarcomeric unit. (b) histopathology samples from cardiac tissue with HCM vs normal and (c) macroscopic changes in cardiac magnetic resonance imaging[31]

Following sarcomeric gene variants, Rasopathy syndromes are the second most common cause of childhood HCM, accounting for up to 18% of paediatric HCM cases[17,18,32,33] and up to 42% of cases in infancy[34]. This will be discussed in further detail in chapter 1.2.2.2.1.
Inborn errors of metabolism (IEM) account for 8-10% of cases of paediatric HCM[12,17,19]. The majority of those are secondary to glycogen storage diseases[12] such as Pompe disease, Danon disease and AMPK disease. Other causes are disorders of fatty acid metabolism, lysosomal storage disorders and cardiomyopathies secondary to mitochondrial syndromes. They have an overall poor survival, especially in the early neonatal period, with a reported 1-year survival of 82% and a 10-year survival of 66%[17,32].
Neuromuscular disorders account for a similar percentage to IEMs[12,17,19]. The most common such disorder associated with HCM is Friedreich's ataxia (FA)[35], with HCM being a feature in up to 85% of cases[36] with a 10-year survival reported as 80%[37].
A non-genetic condition that is responsible for LVH in the paediatric population is that of an infant of a diabetic mother. This is thought to be due to increased maternal levels of insulin-like growth factor[38] and is usually asymptomatic and transient in nature[39].

1.1.1.3 Clinical presentation

The presentation of HCM in children can be variable[11], but is most commonly diagnosed secondary to referral to a paediatric cardiology centre for family screening, followed by an incidental diagnosis during testing for another reason. Children are also being referred with symptoms such as chest pain, exertional syncope to receive a diagnosis of HCM. Out-of-hospital cardiac arrest (OOHCA) and SCD remain a rare, about 2-3%[11,20], but clinically important proportion of first presentation and diagnosis of HCM. As in some cases HCM in children might be secondary to an underlying syndrome, clinical presentation may vary according to the underlying cause.

1.1.1.4 Evaluation of phenotype

According to recent guidelines[40,41], the cardiac phenotype in paediatric HCM is evaluated serially, through a constellation of investigations, each aimed to assess a different aspect of the condition.

1.1.1.4.1 Echocardiogram

1.1.1.4.1.1 Left ventricular hypertrophy

To reach a diagnosis of HCM in children, we must take into account somatic growth and correct left ventricular (LV) wall thickness with normal values according to body surface area (BSA). The definition of HCM in the paediatric population is therefore: a maximal left ventricular wall thickness (MLVWT) greater than 2 standard deviations (>2 Z scores) above the population mean[5,10].

The distribution of LVH may vary and present as asymmetric septal hypertrophy (ASH), which is overall the most common distribution of LVH[12,17,19], concentric, which is commonest in syndromic aetiologies, such as Rasopathy-associated HCM (RAS-HCM), IEM or FA[12,17,19,34,37,42,43], but may more rarely present in other patterns[44].

The progression of LVH during childhood is incompletely understood. Initial studies from 1986 reported progression of LVH more frequently during adolescence[45], and to date there have not been any large studies investigating LVH progression serially in paediatric HCM. However, there are more recent studies suggesting that earlier disease onset is an important reality[22] and along with studies reporting on regular screening of first-degree relatives[46,47] and gene carriers[48] have helped shift the paradigm[5] and current guidelines recommend regular screening in gene-carriers and first-degree relatives from neonatal age onwards[40,41]. The only cause of HCM in children whose progression has been most characterised is Danon disease, where LVH is known to progress rapidly in men[49] and less so in women[50].

Concomitant right ventricular hypertrophy (RVH) may co-exist in around 15% of cases, and has been associated with worse LV function and major adverse cardiac events (MACE)[51] in the adult population. In children, co-existing RVH is a red flag for an underlying diagnosis of a Rasopathy syndrome[17,34,43].

1.1.1.4.1.2 Left ventricular outflow tract obstruction

Left ventricular outflow tract obstruction (LVOTO) is a common finding in HCM, with varied prevalence in childhood of 22-60%[17,32,52], likely reflecting the variance in underlying aetiologies of HCM in children. It is defined as a maximal LVOT gradient, as measured using Doppler echocardiography, above 30mmHg at rest or during provoking manoeuvres that alter LV loading conditions (such as Valsalva or exercise)[5], with haemodynamic effects

typically being present at a gradient of 50mmHg or above[53]. Exercise stress-echocardiogram is recommended in symptomatic patients to elicit exercise-induced LVOTO[5], which may be unveiled in up to 70% of patients[54]. The mechanisms of LVOT obstruction are complex and include a narrowed LVOT, basal anteroseptal hypertrophy and systolic anterior motion (SAM) of the mitral valve[45].

1.1.1.4.1.3 Left ventricular function

Systolic function in paediatric HCM is typically described as hyper-dynamic with preserved global measures of LV function[17,18]. In a minority of patients, typically with syndromic disease, this can progress to a dilated phase with systolic dysfunction and LV thinning[55]. In those cases, heart transplant remains a viable long-term treatment option[56]. Diastolic dysfunction, although challenging to assess, has been observed in paediatric HCM, often preceding the development of LVH[57].

1.1.1.4.1.4 Left atrial dilatation

Left atrial (LA) dilatation is a well-recognised feature of HCM and the mechanism behind this is is likely due to a combination of SAM related mitral valve regurgitation and secondary to diastolic impairment leading to increased atrial pressures. Another possible mechanism is this of primary atrial myopathy component[58]. LA enlargement is known to be a risk factor for adverse outcomes in HCM[59] and for the development of complex atrial arrhythmias, specifically atrial fibrillation(AF)[60], which in turn may lead to stroke in adults[61] and much more rarely in the paediatric population[11].

1.1.1.4.2 Electrocardiogram

The standard 12 lead electrocardiogram (ECG) is recommended in screening and surveillance as it may show features of the disease such as Q waves[62], a feature associated with septal hypertrophy[63], voltage criteria for LVH, ST segment and T wave abnormalities[64], which is associated explained by asymmetric hypertrophy or myocardial scarring[62,63]. ECG changes may precede echocardiographic evidence of the condition[65] and a normal ECG is present in less that 3% of children with HCM[66].

The 12-lead ECG may also be suggestive of a specific underlying diagnosis based on certain features[67]. Ventricular pre-excitation with a short PR interval and a delta wave is a common feature of several storage (Pompe[68], Danon[50]) and mitochondrial disorders[69] whereas AV block is more prevalent in mitochondrial aetiologies [70] and Anderson-Fabry disease[71].

1.1.1.4.3 Ambulatory monitoring

Hypertrophic cardiomyopathy is associated with both atrial and ventricular arrhythmias. Supraventricular tachycardias, which may be related to symptoms, occur in up to 37% of patients[72]. Complex atrial arrhythmias, in particular AF, while common in adults, are rare in children. Nevertheless, AF, as previously explained, is associated with risk for stroke and therefore its detection is important. Non-sustained ventricular tachycardia (NSVT), defined as three or more consecutive ventricular beats occurring at a rate of 120 bpm or above[5] and lasting <30 seconds, is a common finding in up to 25% of adults with HCM[72-74], and in children it has been reported in up to 27% of ambulatory ECG monitors[52,75,76], although this is much lower in larger cohorts[12,17,19]. It is a widely recognised risk factor SCD in patients with HCM[77], including children[14]. Sustained, asymptomatic ventricular tachycardia (VT) has also been described, although it is not considered to contribute more to SCD risk than NSVT[78]. Ambulatory ECG monitoring is therefore recommended in patients in HCM to help unveil these arrhythmias and risk-stratify patients[40].

1.1.1.4.4 Cardiopulmonary exercise testing (CPET)

CPET encompasses conventional exercise evaluation parameters, including blood pressure, electrocardiography, and symptom monitoring, in conjunction with ventilatory gas exchange analysis. It provides objective quantification of cardiorespiratory fitness, delineates mechanisms of exercise intolerance, and enables function-based prognostic stratification[79,80]. In adults with HCM, CPET is being used[40,41] to delineate disease pathophysiology[81], assess symptom aetiology[82] as a parameter of risk stratification for sudden cardiac death and heart failure progression[83], and to inform decision-making for therapies[84,85].

There is limited evidence in childhood HCM of the usefulness of CPET in predicting outcomes[86,87], but this is still used in clinical practice primarily for symptom assessment[88] and to evaluate the presence of ventricular ectopy[86].

1.1.1.4.5 Cardiac magnetic resonance imaging (CMR)

CMR plays a key role in assessing HCM, providing important data on cardiac morphology, function and tissue characterisation in patients with HCM [40,89]. In particular, it can identify and quantify areas of myocardial fibrosis with late-gadolinium enhancement (LGE)[90], which has been shown to be progressive[91] and present in ~33% of children with sarcomeric HCM[92]. In adults with HCM, LGE on CMR has been associated with adverse events including sudden

cardiac death (SCD)[93,94]. Similar findings have recently been reported in childhood HCM, although the role of LGE in SCD risk stratification in children remains unclear[91,95,92].

1.1.1.5 Symptoms and treatment

In children, symptoms of HCM can be due to variable underlying mechanisms and may be challenging to assess and treat. Chest pain, palpitations, dyspnoea, fatigue, presyncope and syncope are the most common symptoms described[76].

Chest pain in HCM is typically multifactorial, due to LVOTO, diastolic dysfunction, or myocardial ischaemia secondary to increased LV mass[96]. Heart failure symptoms such as dyspnoea and fatigue are usually caused by diastolic function impairment, since systolic impairment is rarer in the childhood setting[17,18]. Syncope can be due to haemodynamic, primarily secondary to LVOTO, or arrhythmic in nature, which is important to distinguish from a risk stratification and management point of view.

Treatment focuses on symptomatic relief. In the presence of LVOTO, first line treatment is beta-blockers[97]. Additional options include disopyramide[98] and calcium channel blockers[99,100], which can be used in combination[40]. In adults, there has been recent introduction of myosin inhibitors (macavamten[101,102] and aficamten[103]) in the management options, with ongoing trials for the paediatric population. Surgical myectomy is reserved for those with refractory symptoms or fixed obstruction, with low peri-operative mortality or morbidity in experienced centres[104,105]. In the absence of LVOTO on standard echocardiography, stress echocardiography can be useful to reveal exercise-induced LVOTO[106]. If this is not present, symptoms could likely be attributable to diastolic impairment or myocardial ischaemia. Treatment is aimed at reducing LV diastolic pressures thus improving filling. Options include b-blockers and verapamil, with a cautious use of loop diuretics to avoid dehydration[40]. Ranolazine has also been proven to improve chest pain symptoms in the absence of LVOTO[107,108].

Transplantation is a viable treatment strategy reserved in those patients developing heart failure related symptoms not responding to maximal medical therapy, or, more rarely, refractory arrhythmia. This has been reported to be the case in 1.5-2.1% of the paediatric population[17], with limited data showing worse early survival post heart transplant than their counterparts with dilated cardiomyopathy (DCM), but similar long term survival[109].

1.1.1.6 Mortality

Initial publications of paediatric HCM populations were of small sample size and portrayed a poor prognosis with annual mortality rates up to 7%. In more recent years, larger population studies have provided us with an updated annual mortality of around 3%. However, there is great variability depending on the underlying aetiology, and even further dependent of age at presentation. Patients with non-syndromic disease have an overall higher survival, approximately 83% at 5 years and 76% at 10 years. Conversely, survival is worse in children with HCM due to an underlying IEM where survival is reported at around 54% at 1 year and 42% at 5 years[12]. In cases where children present with infantile HCM, survival is reported to be 85% at 1 year[12,110], likely reflecting the higher proportion of syndromic cases in this population. However, in children surviving beyond the age of 1 year, mortality reaches a plateau, with annual mortality rates of 1-2%[12], and is similar to this of children diagnosed at a later stage in life, and comparable to the adult population.

Cause of death also varies depending on underlying aetiology and age at presentation. Overall, the most common cause has been reported as SCD in about 3% of children with HCM[17]. Congestive heart failure is the most common cause of cardiovascular death in the infantile population, where CHF represents up to 5% of deaths[110], once again likely representing the higher percentage of syndromic cases. It is important to note the multifactorial cause of death in the syndromic population, reflecting the multi-system involvement, which is not the case in children with familial disease[17,110].

An important cause of death linked to HCM is sudden cardiac death (SCD), which has an overall estimated incidence in childhood of 1.3-8.5 per 100,000 patient years, representing the most common cause of death in children outside of infancy, and is more frequent than in the adult population. The mechanism of SCD is poorly understood, but likely occurs due to a combination of inherent myocardial disarray and fibrosis, which disrupt normal architecture, leading to abnormal conduction, as well as myocardial ischaemia and strain, that potentially lead to arrhythmogenesis due to depolarisation abnormalities. Animal studies in HCM models have shown an altered homeostasis of calcium, reducing the refractory period in cardiomyocytes, causing transmural dispersion of repolarisation and thus predisposing to ventricular arrhythmias.

Stroke is a cause of morbidity and mortality in HCM patients with a reported incidence of 1% per year in the adult population but is much rarer in the paediatric population. This occurs most likely as a result of left atrial dilatation, leading to stasis and atrial arrhythmias.

1.1.1.6.1 Prediction of mortality

Prediction of mortality in the paediatric population is a challenge due to the heterogeneity in age at presentation, aetiology. Additional risk factors have been identified such as presentation with CHF symptoms, concentric LVH, severe LVH and concomitant RVH.

1.1.1.6.1.1 Risk prediction of SCD and management

SCD is a devastating outcome in patients with HCM, and therefore its prediction and prevention remain a cornerstone for the management of this group. Patients at an estimated high risk of SCD are offered primary prevention implantable cardioverter-defibrillator (ICD) implantation, while survivors of a significant event, such as aborted cardiac arrest or ventricular tachycardia (VT)/ventricular fibrillation (VF) with haemodynamic compromise, are offered secondary prevention ICD[40,41].

Several studies identified isolated risk factors of SCD in childhood HCM – malignant arrhythmias, namely VT with haemodynamic compromise and VF[52,75], a history of non-sustained VT (NSVT) (defined as $\geq$ 3 consecutive ventricular beats at a rate of $\geq$ 100 bpm)[76,111], unexplained syncope[75,112,113] and extreme ventricular hypertrophy (MLVWT $\geq$ 30mm / z-score $\geq$ 6)[111]. These parameters were included in the joint American College of Cardiology Foundation (ACCF) and American Heart Association (AHA) task force guidelines for HCM in 2011[114] and the European Society of Cardiology (ESC) guidelines from 2014[5] as major risk factors for SCD in children. However, this approach was shown to have limited discriminatory power and a low positive-predictive value[115].

In more recent years, two models for 5-year risk prediction of SCD in non-syndromic childhood HCM have been published.

HCM Risk-Kids was published in 2019[14], using data from 1024 children aged 1-16 years with a diagnosis of non-syndromic HCM. This identified five non-invasive clinical parameters that can be used in an algorithm to estimate the 5-year risk for SCD in paediatric non-syndromic HCM – left atrial diameter (LAd), MLVWT, LVOT gradient, presence of NSVT and unexplained syncope. The C-index of the model was 0.69 (95% 0.66-0.72) with a calibration slope of 0.98 (95% CI 0.59-1.38) and risk-groups were categorised into low risk ($\leq$4% 5-year estimate risk),

intermediate risk (4-6%) and high risk (≥6%). The model was found to out-perform its adult equivalent in a childhood population. These findings were validated in an external, independent cohort of 421 patients in 2021[116] and two further smaller studies[117,118]. HCM Risk-Kids has been recommended in the 2023 ESC guidelines for the management of cardiomyopathies[40].

A more recent model, PriMaCy[119] was published in 2020, using data from 572 patients < 18 years of age with a diagnosis of HCM due to a non-syndromic cause. This model uses age at diagnosis, intraventricular septal thickness (IVST) z-score, left ventricular posterior wall thickness (LVPWT) z-score, LAd, LVOT gradient, the presence of NSVT and a history of syncope as its parameters, with an alternate model using genetic data also, giving a C-index of 0.75 and 0.76 respectively. Similarly, the patients were split in 3 risk categories, low (<4.7%), medium (4.7-8.3%) and high risk (>8.3%). These findings were, in the same study, validated with an independent cohort of 285 patients. An independent study in 2023[120] confirmed that the discrimination between low and high risk groups were similar between HCM Risk-Kids and PriMaCy, but the latter overestimates risk for some patients, potentially leading to more patients being offered preventative ICD implantation.

1.2 The Rasopathies

The Rasopathies are a group of genetic syndromes caused by germline mutations in genes that encode components or regulators of the Ras/mitogen-activated protein kinase (MAPK) pathway, with a cumulative incidence of approximately 1 in 1000-2000 live births[121]. The Ras/MAPK pathway plays an essential role in regulating the cell cycle and cellular growth, differentiation, and senescence, all of which are critical to normal development[122]. Collectively known as the Rasopathy syndromes, these disorders include neurofibromatosis type 1, Noonan Syndrome (NS), Noonan Syndrome with multiple lentigines (NSML; previously known as LEOPARD syndrome – lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary valve stenosis, abnormalities of the genitals, retardation of growth, deafness), capillary malformation–arteriovenous malformation syndrome, Costello Syndrome (CS), cardiofaciocutaneous syndrome (CFCS), NS with loose anagen hair (NS-LAH), and Legius syndrome[121]. Of these, NS, NSML, CS, CFCS and NS-LAH share a number of distinct features, including distinct dysmorphic features,

propensity for tumours, short stature/growth delay, variable degree of developmental delay, and cardiovascular involvement[123].

1.2.1 Genetics and molecular pathogenesis

The Ras/MAPK pathway, also known as the Ras-Raf-MEK-ERK pathway, is a signal transduction pathway that transmits signals from the cell surface to the nucleus, where gene expression is regulated[124]. This pathway consists of multiple protein kinases arranged in a cascade, with each kinase activating the next one in the sequence (Figure 1-3).

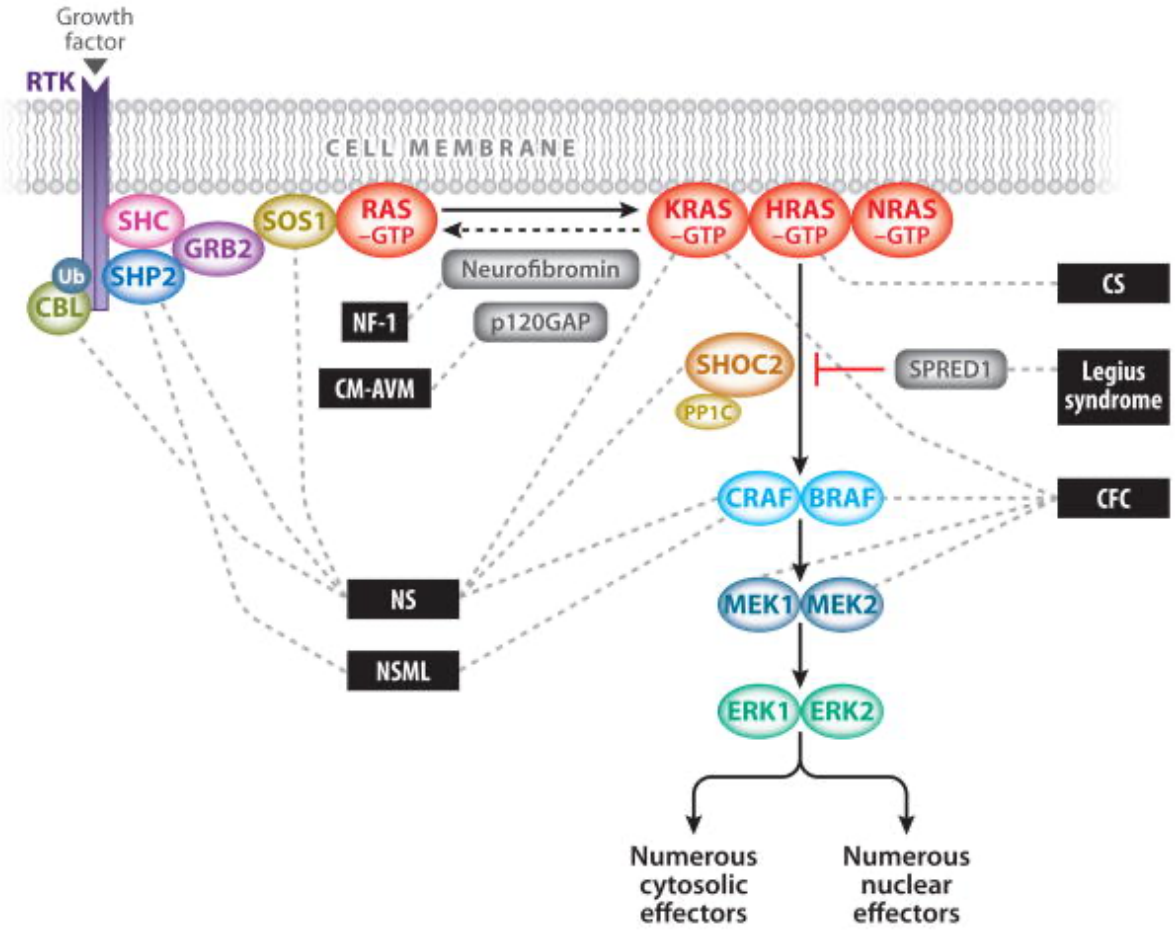


Figure 1-3: The Ras/MAPK signal transduction pathway[121]

The major components of the Ras/MAPK pathway can be broken down into receptors, GTPases, kinases, and transcription factors. The cascade can be broken down as follows[125]:

(a) Growth factor receptor activation

(b) Activation of RAS GTPase

(c) Raf activation

(d) MEK activation

(e) ERK activation

This pathway therefore regulates a wide range of biological processes, including cell growth and proliferation, differentiation, survival, migration and metabolism[125-127].

In some genetic disorders (e.g., Rasopathies), cancer, and other diseases, components of the Ras/MAPK pathway can become mutated or dysregulated, resulting in uncontrolled cell growth and survival, leading to pathological consequences[121,126,128].

Several genes that regulate the Ras/MAPK pathway are commonly affected in Rasopathies. Some of the most important ones include:

- KRAS, NRAS, HRAS: Mutations in these genes can cause Ras to bind on permanently, leading to overactive signalling[129-131].
- BRAF, MEK1, MEK2, RAF1: These genes are involved in the downstream part of the Ras/MAPK pathway. Mutations in these can lead to increased MAPK signalling, contributing to cell overgrowth or developmental issues[132-135].
- PTPN11: Mutations in this gene, which encodes the SHP-2 protein, lead to abnormal activation of the Ras/MAPK pathway[136-138].
- SOS1: This gene is involved in stabilising Ras in an inactive form and therefore mutations result in the increase of active Ras and hyperactivation of the Ras/MAPK pathway[121].

1.2.2 Phenotype

Because the Ras/MAPK pathway regulates key cellular functions, its dysregulation can cause a variety of symptoms and developmental issues seen in Rasopathies. In line with their shared molecular pathogenesis, there are significant similarities and overlap among NS, NSML, CS, CFCS and NS-LAH and they are sometimes referred to as NS and related syndromes. Table 1-1 details the different genetic variants and phenotypic features of each clinical syndrome.

Table 1-1: Phenotypic features of Rasopathy syndromes associated with HCM[139-141]

Syndrome	Gene	Phenotype
NS	PTPN11 SOS1 RAF1 KRAS NRAS SHOC2 CBL RIT1 LZTR1	Craniofacial dysmorphic features, CHD; short stature; undescended testicles; ophthalmologic abnormalities; bleeding disorders; normal neurocognitive function or mild impairment; predisposition to cancer
NSML	PTPN11 RAF1 RIT1	Same as NS, but with possible development of multiple skin lentigines
CS	HRAS	Coarse craniofacial dysmorphisms; CHD; FTT; short stature; ophthalmologic abnormalities; multiple skin manifestations; normal neurocognitive function or mild impairment; hypotonia; predisposition to cancer
CFCS	BRAF MAP2K1 MAP2K2 KRAS	Craniofacial dysmorphisms; CHD; FTT; short stature; ophthalmologic abnormalities; multiple skin manifestations; normal neurocognitive function or mild impairment; hypotonia
NS-LAH	SHOC2	Craniofacial dysmorphisms; darkly pigmented and hairless skin; LAH; CHD; FTT; short stature; severe GH deficiency; mild psychomotor delay with ADHD; ectodermal abnormalities

NS: Noonan syndrome; NSML: Noonan syndrome with multiple lentigines; CS: Costello syndrome; CFCS: cardio-facio-cutaneous syndrome; NS-LAH: NS with loose anagen hair; CHD: congenital heart defects; FTT: failure to thrive; GH: growth hormone; ADHD: attention deficit and hyperactivity disorder

1.2.2.1 Non-cardiac phenotype

Craniofacial

Coarse craniofacial features are a distinctive characteristic of Rasopathy syndromes. Overarching features include widely spaced eyes, downslanting palpebral fissures, ptosis, low set ears and a broad, webbed neck[142]. These may be more apparent in young children than with increasing age.

Lymphatic

Abnormalities of the lymphatic system are frequent in NS and related syndromes, but this varies according to underlying genotype and may take various forms such as congenital lymphoedema, chylothorax, pleural effusions or ascites, identified both pre and postnatally. Prenatal findings such as polyhydramnios[143], cystic hygroma, pleural effusion, ascites and non-immune hydrops can raise the suspicion for an underlying Rasopathy syndrome[144]. Patients with NS syndrome, particularly secondary to SOS1 and RIT1 variants seem to be more affected[145]. Lymphatic anomalies are often a bad prognostic sign and an impediment to cardiothoracic surgery[146].

Endocrine

Short stature is a common feature of NS, thought to be either due to complete or partial growth hormone (GH) insensitivity and reduced response to insulin-like growth factor I (IGF-I)[147]. This affects children as they grow older, while neonatal weight and height are usually in the normal range[148]. Delayed puberty is another common finding which may exacerbate the short stature, along with delayed bone maturation[149]. Short stature is more pronounced in patients with CS and patients with SOS1 and RIT1 variants are most often of normal adult stature[150]. In some patients, GH supplementation becomes necessary, and produces reassuring results[150,151]. Nevertheless, considering that GH affects other areas apart from somatic growth, including hypertrophy in cardiomyocytes[152], where studies show a strong stimulating effect of GH[153] and a resulting increase in LV mass, even in non-syndromic patients with previously normal echocardiograms[154]. Most often HCM is considered a contraindication for GH therapy[150,155], even though there have been studies demonstrating a favourable cardiovascular safety profile in children with NS[151,156], even in the presence of cardiac comorbidities, including HCM, albeit with limited cases, owing to the rarity of the condition.

Haemato-oncology

The RAS-MAPK pathway is involved, as previously detailed in chapter 1.2.1, cell growth and proliferation, differentiation, survival, migration and metabolism[125-127] and as such, somatic mutations of this pathway have been implicated in several cancers[157,158]. Similarly, patients with Rasopathy syndromes have a predisposition for malignancies and tumour-like lesions, with a higher predisposition in patients with CS, with a reported cumulative incidence of cancer of 15% by age 20 in patients with CS, compared to 4% in NS[159]. Most commonly overall malignancies include juvenile myelomonocytic leukaemia[160], myeloproliferative disorders[161], neuroblastoma and rhabdomyosarcoma[162]. Screening for such conditions is recommended in this population at regular intervals.

Moreover, there is a link between Rasopathy syndromes and bleeding disorders[163,164], particularly in NS, with 50-89% of patients affected[165]. Four aetiologies are primarily suggested in literature – thrombocytopaenia, platelet dysfunction, von Willebrand disease and specific factor deficiencies[165]. This association becomes particularly important when we consider the peri-operative risks of bleeding and as such, patients with Rasopathy syndromes should be screened for bleeding disorders before any procedures.

Genitourinary

Cryptorchidism is the most common genitourinary abnormality, reported in up to 50% of males[166]. Other abnormalities include pyelectasis, duplex collecting systems and unilateral renal agenesis[121,166].

Gastrointestinal

Feeding difficulties in neonates are very common, but severity is variable[167,168] and these are more pronounced in CS and CFCS with resulting failure to thrive. These issues most commonly resolve outside of childhood[169].

Neurological

Neurological and cognitive difficulties are reported in up to 50% of patients with NS and related disorders, with patients with PTPN11, KRAS, RAF1 and SHOC2 having a higher prevalence of cognitive impairment[170].

Musculoskeletal

Several musculoskeletal issues are commonly reported in patients with Rasopathy syndromes, most prominently pectus deformities in 70-95% of patients, both carinatum and excavatum[141,171], joint hyperextensibility and cubitus valgus[164].

1.2.2.2 Cardiac phenotype

1.2.2.2.1 Congenital heart defects (CHD)

The original description of Noonan syndrome was in 1968, as an 'Turner phenotype' associated with congenital heart disease, namely pulmonary valve stenosis[172]. In 1975, the association between Noonan syndrome and hypertrophic cardiomyopathy was made[173]. Since then, there have been multiple large studies, reporting cardiac associations in 60-90% of patients with Rasopathy syndromes[123,146,174-176]. The most common associated congenital heart defects include pulmonary valve stenosis (PS), atrial (ASD) and ventricular (VSD) septal defects[146,175,177].

PS is observed in overall 65% of patients with Rasopathy syndromes and ranges from severe, in around 30% of cases, moderate in an estimated 10% or mild in the majority of cases[175,178,179]. Severe or moderate-severe pulmonary valve stenosis may need urgent balloon valvuloplasty, with high rates of reintervention[174,179,180]. Those with mild PS are unlikely to need intervention and their long-term outcomes have been shown to be similar to those without PS[181,182].

Atypical CHD have been reported in association with Rasopathy syndromes[183] both in isolation and in combination with each other. Most noteworthy such defects are atrioventricular canal defects, in up to 15% of cases[177,184], which may explain the higher prevalence of mitral valve abnormalities[174,178,179], and coronary artery abnormalities[183], mainly aneurysms, which may contribute to myocardial ischaemia.

Table 1-2 details the most common cardiac defects associated with each clinical syndrome.

Table 1-2: Primary cardiac associations with different Rasopathy syndromes[174,181,185]

Syndrome	Cardiac involvement	Percentage
NS	Pulmonary valve stenosis	60-70%
	Hypertrophic cardiomyopathy	14-30%
	Atrial septal defect	10-30%
	Atrioventricular canal defect	5-15%
	Ventricular septal defect	5-10%
	Aortic coarctation	3-10%
NSML	Hypertrophic cardiomyopathy	20-73%
CS	Hypertrophic cardiomyopathy	70-75%
CFCS	Pulmonary valve stenosis	33-40%
	Hypertrophic cardiomyopathy	33-40%

NS: Noonan syndrome; NSML: Noonan syndrome with multiple lentigines; CS: Costello syndrome; CFCS: Cardiofaciocutaneous syndrome; HCM: hypertrophic cardiomyopathy

1.2.2.2.1 Rasopathy-associated hypertrophic cardiomyopathy

1.2.2.2.1.1 Epidemiology

The prevalence of HCM in patients with Rasopathy syndromes varies depending on the underlying gene involved. HCM is reported in 80-100% of patients with RAF1 and RIT1 variants[139,140] and in 60-70% of patients with HRAS variants[186], whereas the prevalence of HCM in patients with BRAF, SHOC2, PTPN11 and SOS1 variants is 37.5-75%, 30%, 20% and 16%, respectively[123,185,187].

1.2.2.2.1.2 Aetiology

Histologically RAS-HCM is indistinguishable from sarcomeric HCM, with myocyte disarray and fibrosis[188,189], the clinical presentation and natural history can be substantially different. The pathogenesis of HCM in Rasopathies is not fully understood but is thought to be linked to the abnormal activation of the Ras/MAPK signalling pathway, which disrupts normal cardiac muscle development and function, promoting cardiomyocyte growth, proliferation and survival[137,190,191].

1.2.2.2.1.3 Clinical presentation

Patients with RAS-HCM are generally diagnosed at an earlier age, with a peak in infancy[34,174,192], and have a smaller BSA[193] than their counterparts with sarcomeric HCM,

owing to their syndromic nature. In addition, HCM appears to often co-exist with CHD[34,174], both of which are common in patients with RAS-HCM and have been shown to be linked to worse outcomes[179].

1.2.2.2.1.4 Evaluation of cardiac phenotype

1.2.2.2.1.4.1 Echocardiography

A few distinct features of RAS-HCM have been describe, serving as 'red flags' for their diagnosis[67]. They commonly present with biventricular hypertrophy[34,174], likely in part owing to the pulmonary valve involvement. Even if there is no severe stenosis, the pulmonary valve often appears thickened and dysplastic[175,178]. To this point, there is also commonly concomitant right ventricular outflow tract obstruction (RVOTO)[34,174,192].

Patients with RAS-HCM present with a generally smaller, more hyperdynamic left ventricle with less severe LVH than sarcomeric patients[32,174]. Impaired LV relaxation has been shown to be a feature of children with RAS-HCM secondary to NSML[194].

Finally, LVOTO is more common in patients with Rasopathy syndromes[34,174,192], which is hypothesised to be, in addition to SAM, due to anomalous insertion of the mitral valve chordae or displacement of papillary muscles[146,195]. In fact, polyvalvulopathy is another feature in patients with RAS-HCM[146], as multiple valves may be dysplastic. This primarily affects the pulmonary and mitral valves as previously discussed, but the aortic and tricuspid valves have been reported to be dysplastic as well[183]. In the case of mitral valve anomalies specifically, limited data has linked them to worse long-term outcomes[196].

1.2.2.2.1.4.2 ECG

ECG abnormalities characteristic to Rasopathy syndromes, primarily Noonan syndrome, have been reported[197], even in the absence of HCM[198,199]. These include left axis deviation in up to 50% of cases, small R waves in the left precordial leads in nearly 25% of cases with no HCM. A unique ECG feature of RAS-HCM has been reported to be 'extreme northwest axis' in a small cohort of patients with Noonan syndrome and HCM[197].

Data on other Rasopathy syndromes and specific genotypes has not yet been reported in the literature.

1.2.2.2.1.4.3 Ambulatory monitoring

While serial ambulatory monitoring is key in the monitoring process of patients with HCM, both for diagnosing arrhythmias and delineating the risk of SCD, as previously discussed in

this chapter, there is no specific guidance for children with RAS-HCM. There is little known about the prevalence of ectopy in patients with RAS-HCM, except for case reports[200,201] and a small sub-cohort in a larger study[43], reporting the presence of both atrial and ventricular ectopy in this population. Therefore, currently, guidance on performing cardiac ambulatory monitoring is extrapolated from standardised practices in patients with non-syndromic HCM.

1.2.2.2.1.5 Symptoms and management

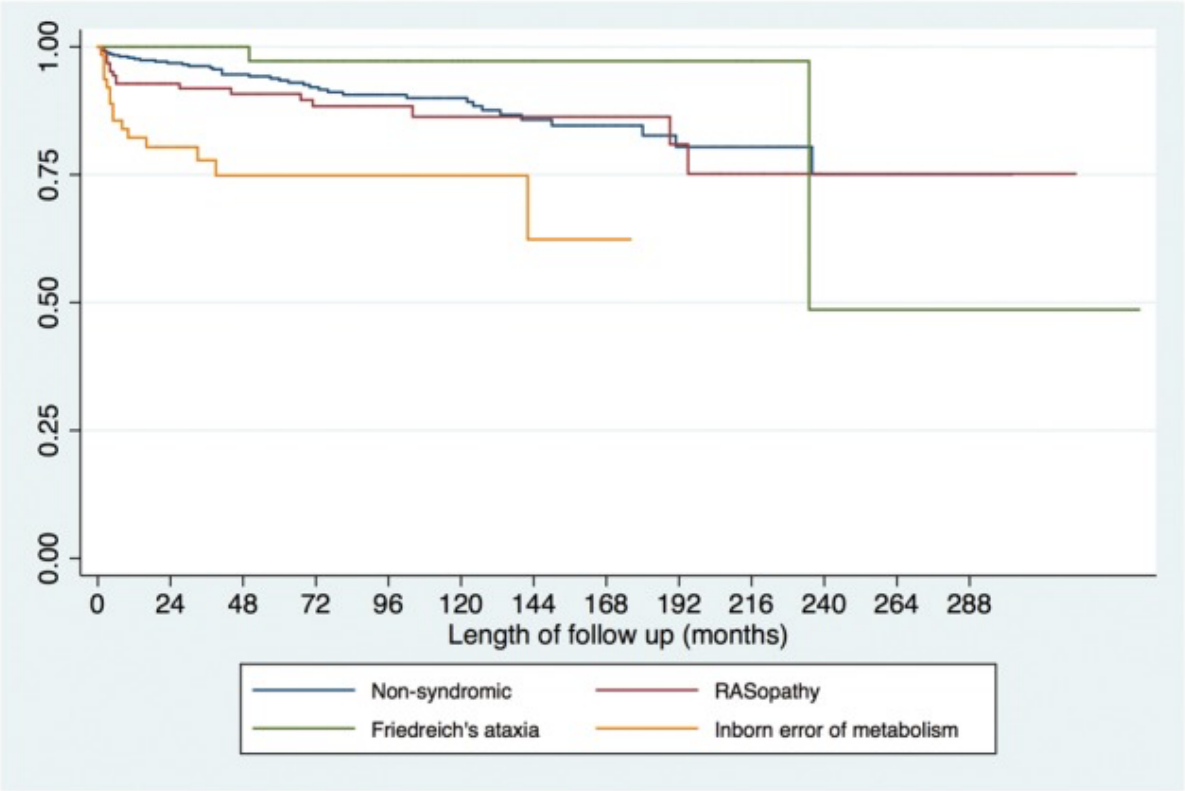
There is no specific data on symptoms in children with RAS-HCM and current data on management is extrapolated from that of non-syndromic HCM, according to the most recent European and American guidelines[40,41].

1.2.2.2.1.6 Natural history and outcomes

Large registry studies of paediatric HCM have provided valuable information regarding the long-term prognosis of patients with sarcomeric and non-syndromic HCM[17,32], but the data are more limited for non-sarcomeric aetiologies.

Population-based studies suggest that five-year survival rates for children with RAS-HCM are worse than those for children with non-syndromic HCM[17] (see Figure 1-4), primarily due to heart failure-related mortality[34,174]. However, it seems that while they have increased morbidity during the early disease course[34,43,202], they have favourable long-term outcome with lower late mortality[34,174]. It has also been suggested that patients with RAS-HCM are more likely to need early surgical septal myectomy during childhood[43] as well as catheter-based or surgical interventions to their pulmonary valves[174,181].

Disease specific risk factors are limited in literature, with early age at diagnosis and concomitant CHD requiring surgery being linked to a worse outcome[43,146], but genotypic data or population specific echocardiographic parameters have been studied.



<u>Figure 1-4:</u> Kaplan-Meier curve for survival free from all-cause mortality or cardiac transplantation, stratified by aetiology of hypertrophic cardiomyopathy[17]

1.2.2.2.1.7 Risk prediction for SCD and management

Historically, sudden cardiac death (SCD) has been thought to be extremely rare[17,32,34,174] in patients with RAS-HCM, but it has recently been shown in a UK national cohort study of childhood HCM that SCD can occur in up to 3% of children with this diagnosis[14] and perhaps even carry a risk comparable to that of sarcomeric disease[203]. Furthermore, a model for predicting the 5-year estimated risk for SCD in children with HCM has been developed and validated, but only for children with non-syndromic disease[14,116]. Further insight is needed with studies focusing specifically on SCD in this population and its predictors.

Despite differences, the clinical management and risk stratification of patients with Rasopathy-related HCM is currently extrapolated from that of sarcomeric HCM, and specific clinical evaluation and management guidelines for RAS-HCM have not been developed. An improved understanding of the relationship between aetiology, phenotype and outcomes is necessary in order to optimise clinical care in this distinct population.

1.2.2.2.1.8 Targeted therapies in RAS-HCM

Targeted treatments for Rasopathy syndromes are still an evolving area of clinical research. In the last decade, novel therapeutic approaches that target the underlying pathophysiological mechanism in the RAS/MAPK pathway have shown promise for the prevention and regression of HCM in specific patients with Rasopathy syndromes.

MEK inhibitors is the most studied drug in the context of RAS-HCM. These drugs were first used in NF-1 related plexiform neurofibromas[204], and have shown promise in benefiting patients with Rasopathy syndromes with a RIT1 and RAF1 mutation[205,206]. Since MEK is a key component of the MAPK cascade, the initial premise was that inhibiting it can block the hyperactivation of the pathway that is central to these diseases. MEKi therefore work by blocking the MEK1/MEK2 kinases, which are activated downstream of Ras and Raf in the MAPK pathway. By inhibiting MEK, these drugs can reduce the activation of ERK, the final kinase in the pathway, and thus dampen the downstream effects on gene expression, cell growth, and survival[207]. Trametinib, a highly selective reversible allosteric inhibitor of MEK1/2 activity, has been shown to alter contractility of in myocardial cells of children with RAS-HCM[208] and was used on two patients with severe early-onset HCM caused by RIT1 mutations with hypertrophy regression and obstruction improvement as well as catch up in somatic growth within 4 months of initiation of treatment[205]. An open-label study of MEK162 inhibitor in NS adults with HCM has been commenced[209]. The identification of HRAS mutations as the molecular cause of CS raised the possibility that farnesyl transferase inhibitors may provide clinical benefit to patients[121]. Low doses of dasatinib, a multitargeted inhibitor of bcr-abl and Src family kinases approved for paediatric cancers, in a mouse model of NS improved cardiac function and in NSML prevented progression of HCM[210]. A recent retrospective study comparing 30 children with RAS-HCM treated with trametinib plus standard of care treatment for cardiomyopathy versus 31 children with RAS-HCM using standard of care treatment, showed decreased mortality and morbidity, improved cardiac status and minimal, non-life threatening side effects[211]. Larger, human studies are needed to best determine which Rasopathy patients, with perhaps specific genotypes, will benefit from specific treatments and at which timepoint in their disease phenotype.

1.2.3 Unmet needs in paediatric Rasopathy-associated hypertrophic cardiomyopathy

Natural History

Large registry studies of paediatric HCM have provided valuable information regarding the long- term prognosis of patients with sarcomeric and non-syndromic HCM[17,32], but the data are more limited for non-sarcomeric aetiologies. Furthermore, despite differences between sarcomeric and RAS-HCM as previously described in this chapter, the clinical management and risk stratification of patients with RAS-HCM is currently extrapolated from that of sarcomeric HCM, and specific clinical evaluation and management guidelines for RAS-HCM have not been developed. An improved understanding of the relationship between aetiology, genotype, phenotype and outcomes is necessary in order to optimise clinical care in this distinct population.

Sudden cardiac death and its prediction

Historically, SCD has been thought to be extremely rare in patients with RAS-HCM, but it has recently been shown in a UK national cohort study of childhood HCM that SCD can occur in up to 3% of children with this diagnosis[14]. Furthermore, a model for predicting the 5-year estimated risk for SCD in children with HCM has been developed and validated, but only for children with non-syndromic disease[14,116]. Further insight is needed with studies focusing specifically on SCD in this population and its predictors.

Disease progression

Regression of infantile HCM in patients with Rasopathies has been described in up to 17% of patients[43,174]. It is not clear whether this represents true regression of LVH or relative wall thinning in relation to somatic growth of the LV cavity. However, progression of LVH is also reported in up to 34% of patients[43], as well as LVH stabilisation[174,212]. A systematic approach to reviewing disease progression and the role genotype plays in this is needed to better understand this cohort and help guide tailored management, including with novel therapies.

Chapter 2 - General methods

2.1 Study population

An initial patient cohort was formed consisting of patients ≤ 18 years with a Rasopathy syndrome (NS, NSML, CS, CFCS, NS-LAH and Noonan-like syndrome) and HCM from all 13 UK paediatric cardiology centres and one in Dublin, Republic of Ireland, consecutively evaluated between January 1st, 1985 and December 31st, 2023.

This initial cohort was then supplemented by adding patients from the Heart Centre in Munich, Germany, University of Campania "Luigi Vanvitelli", Monaldi Hospital, Naples, Italy and Virgen de la Arrixaca Hospital, Murcia, Spain.

A diagnosis of HCM was defined as a left-ventricular wall thickness greater than 2 standard deviations above the body surface area–corrected population mean (z score ≥2) that could not be explained solely by abnormal loading conditions[5]. The investigators from each participating centre guaranteed the integrity of data from their institution. Eligible patients were identified by the principal investigator at each collaborating site. Data were collected independently at each participating centre.

The aspects of the methodology common to all the chapters in this thesis are detailed below. Additional methodological details specific to each chapter, including contributing centres and corresponding numbers of patients, are detailed in the relevant chapters.

2.2 Diagnosis of Rasopathy syndrome & 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. The genetic panel used for these patients changed according to guidance from Genomics England, or relevant local authorities for other centres. Before 2011, targeted testing for Rasopathy syndromes was available with Sanger sequencing using a panel of 1-3 genes. After this, next generation sequencing became available on an expanded panel which included testing for variants in the following genes: *PTPN11, RAF1, BRAF, SOS1, KRAS, HRAS, NRAS, SHOC2, CBL, SPRED1, MAP2K1, MAP2K2*. Patients with a primary diagnosis of HCM were tested on a paediatric cardiomyopathy panel (R135) according to guidance from Genomics England, which includes the Rasopathy genes, after which a diagnosis of RAS-HCM arose. In patients in whom genetic testing had been performed, the following data were

collected: date of testing; size of gene panel; and variants identified (gene and protein change). The pathogenicity of reported variants was reclassified according to the American College of Medical Genetics and Genomics (ACMG) classification[213] by Ms Stephanie Oates, cardiac genetic counsellor at Great Ormond Street Hospital. Variants were described as pathogenic (P), likely pathogenic (LP) and variants of unknown significance (VUS).

2.3 Patient assessment and data collection

Anonymized, non-invasive clinical data were collected retrospectively, including demographics; family history of HCM/SCD; co-morbidities; syndrome; genetic analysis results; heart failure symptoms (New York Heart Association (NYHA)/Ross functional classification[214,215]); medication; resting and ambulatory 12-lead electrocardiogram; and 2-dimensional Doppler and colour transthoracic echocardiogram (from contemporaneously written reports). Age at diagnosis was defined as the age at which HCM was first diagnosed, which may have been prior to the patient(s) being seen for the first time in a paediatric cardiology service. Data were collected at first assessment and at last clinical follow up in a paediatric cardiology centre. End of follow-up was defined as last clinical follow up or transition to adult services, whichever came first, with the exception of the disease progression arm of the study where end of follow-up was defined as last clinical follow up, including data from adult services, where available. Data was entered by myself or collaborators into a RedCap research database designed originally by Dr Gabrielle Norrish as part of her PhD and expanded by myself to include data relevant to my study.

2.4 Clinical investigations

2.4.1 Echocardiogram

Echocardiographic analysis was performed in line with the American Society of Echocardiography guidelines[216] and measurements were taken according to current guidelines[5]. Maximal left ventricular wall thickness (MLVWT) was defined as the maximal myocardial thickness as measured by echocardiography in any of the LV segments[5]. Left ventricular outflow tract (LVOT) obstruction (LVOTO) was defined as a peak instantaneous gradient ≥ 30 mmHg[5]. Right ventricular outflow tract (RVOT) obstruction (RVOTO) was defined as a peak instantaneous gradient ≥ 36 mmHg[217]. These were both calculated at rest or with Valsalva manoeuvres using peak doppler velocity and applying the Bernoulli

equation (gradient = $4V^2$, where V represents the peak outflow velocity). Impaired left ventricular (LV) systolic function was defined as a fractional shortening (FS) ≤ 28% or ejection fraction ≤ 55% [217]. Diastolic impairment was defined as presence of any of the following: mitral valve (MV) E/A ratio < 0.75, MV E wave deceleration time > 240ms and average of lateral and septal E/e' ratios > 14[218].

2.4.1.1 Z scores

Echocardiographic dimensional data are expressed in millimetres and as z-scores corrected for body surface area according to the population corrected mean[219,220]. There are no published z-scores for MLVWT and so pragmatically IVST z-scores were used to correct MLVWT. The equations used to calculate LAd and MLVWT z-scores are detailed below:

- LAd[220]:

  Males:

  $(((LAd\ (mm)/10.665) \times bodyweight(kg)^{0.225})-1)/0.118$

  Females:

  $(((LAd\ (mm)/10.74) \times bodyweight(kg)^{0.465})-1)/0.124$

- MLVWT[219]:

  $((MLVWT(cm)/BSA^{0.4})-0.58)/0.09$

2.4.2 Resting and ambulatory ECG

Previously published normal values for age were employed for QRS axis and electrocardiographic intervals[221]. The following parameters were measured: PR interval (ms), QRS axis (°), QRS duration (ms), QRS amplitude (mV), QT interval (ms), corrected QT interval (ms) using the Bazzett formula. Electrocardiographic criteria for LVH were based on the Sokolow-Lyon criteria[222]. The following parameters were evaluated and described: presence of atrial or ventricular ectopic beats, left or right atrial enlargement, left or right bundle branch block (LBBB/RBBB), pathological Q waves, pathological T wave inversion (>1mm beyond V1 in children over 14 years or beyond V3 in under 14 years), giant T waves (>10mm), ST segment depression or elevation (>2mm).

NSVT was defined as three or more consecutive ventricular beats > 120 beats per minute lasting less than 30 seconds on ambulatory ECG monitoring.

2.5 Outcomes

Clinical outcomes were determined by the treating cardiologist at each site and included: all-cause mortality (congestive heart failure (CHF), sudden cardiac death (SCD), other cardiovascular (CV) death, and non-CV death], the composite outcome of SCD and equivalent events [appropriate implantable cardioverter defibrillator (ICD) therapy, aborted cardiac arrest, or sustained ventricular tachycardia (VT) with haemodynamic compromise], CHF admissions to hospital, the composite outcome of major adverse cardiac events (MACE) comprising of cardiac mortality, SCD and equivalent events and CHF admissions to hospital, as well as atrial arrhythmias, ICD implantation, cardiac transplantation and surgical/catheter-based interventions.

2.6 General statistical methods

Body surface area was calculated from weight[223]. 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[219,220]. 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 17 or 18; StataCorp LP, College Station, TX).

2.7 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. Integrated research application system (IRAS) approval was sought under project number 182354.

Chapter 3 - Natural history of Rasopathy-associated hypertrophic cardiomyopathy

3.1 Introduction

Despite differences in pathophysiological mechanism, clinical presentation and outcomes, as detailed in chapter 1, the clinical management and risk stratification of patients with Rasopathy-related HCM is currently extrapolated from that of sarcomeric HCM, and specific clinical evaluation and management guidelines for RAS-HCM have not been developed. An improved understanding of the relationship between genotype, phenotype and outcomes is necessary in order to optimise clinical care in this distinct population.

3.2 Aim

The aim of this chapter is to describe the clinical features, outcomes and predictors of all-cause mortality and SCD or equivalent events in a large, multi-centre national cohort of patients with RAS-HCM diagnosed in childhood.

3.3 Methods

3.3.1 Patient cohort

The study cohort consisted of patients ≤ 18 years with HCM and a clinical and/or genetic diagnosis of a Rasopathy syndrome (NS, NSML, CS, CFCS, NS-LAH), consecutively evaluated between January 1, 1985, and December 31, 2020, in all 14 paediatric cardiology centres in the United Kingdom (Table 3-1).

Table 3-1: Collaborating centres with corresponding patient numbers

Centre	Number of patients*
Great Ormond Street Hospital, London	102
Bristol Royal Hospital for Children	15 (7 & 8)
Birmingham Children's Hospital	12 (9 & 3)
University Hospital of Wales, Cardiff	12 (8 & 4)
Royal Brompton Hospital, London	11 (6 & 5)
Glenfield Hospital, Leicester	8 (2 & 6)
Royal Hospital for Children, Glasgow	8 (2 & 6)
Evelina Children's Hospital, London	6 (2 & 4)
Southampton General Hospital	5 (2 & 3)
Alder Hey, Liverpool	3 (2 & 1)
Freeman's Hospital, Newcastle	2 (2 & 0)
Leeds General Infirmary	2 (2 & 0)
Our Lady's Children's Hospital, Dublin	2 (2 & 0)
John Radcliffe Hospital, Oxford	1 (1 & 0)

**The numbers add up to more than the total number of patients in this study – this is because some patients were seen in the local paediatric cardiology centre as well as Great Ormond Street Hospital as a national reference centre and were not included twice in the study numbers. In the parenthesis there is the breakdown of numbers, first number is patients only seen at the local centre, second number is patients seen in both the local and reference centre*

Patients with clinical features of a Rasopathy syndrome not fulfilling diagnostic criteria for one of the previously-described syndromes and without a pathogenic/likely pathogenic variant, were labelled "Noonan-like syndrome".

Patients were diagnosed with a Rasopathy syndrome clinically and/or after genetic testing. Genetic testing 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 variants identified (gene and protein change).

3.3.2 Outcomes

The follow-up time for all patients was calculated from the date of their first evaluation to the date of reaching the study end point, death from another cause, or the date of their most recent evaluation prior to the end of the study period. Age at first assessment was

categorised for analysis purposes: <6 months, 6-12 months, 12 months-5 years, >5 years. Era of presentation was categorised for analysis purposes: 1985-1999, 2000-2010, 2010-2020. Percentages expressed are based on available values.

3.3.3 Statistical methods

Estimates of survival were obtained using the Kaplan–Meier product limit method. The association of clinical variables with the outcome of interest was assessed in a univariate Cox proportional hazard model. Mortality and cardiac transplantation were censoring events for survival analyses in this study. All statistical analyses were performed with STATA (Stata statistical software release 17; StataCorp LP, College Station, TX).

3.4 Results

3.4.1 Demographics and Presentation

A total of 149 patients with a Rasopathy syndrome and hypertrophic cardiomyopathy (HCM) were identified, of which 92 (61.7%) were male. Among these, 111 patients (74.5%) were diagnosed with Noonan syndrome (NS), 12 patients (8.1%) with Noonan syndrome with multiple lentigines (NSML), 6 patients (4%) with Costello syndrome (CS), 6 patients (4%) with CFC syndrome, 11 patients (7.4%) with Noonan-like syndrome, and 3 patients (2%) with Noonan syndrome with loose anagen hair (NS-LAH). Sixty-nine patients (65.1%) had one or more extra-cardiac manifestations, as shown in Figure 3-1.

Seventeen (11.5%) had a family history of HCM. Sixty-seven patients (60.9%) had concomitant congenital heart defects (CHD), of whom 32 (29.1%) had more than one CHD (see Table 3-2).

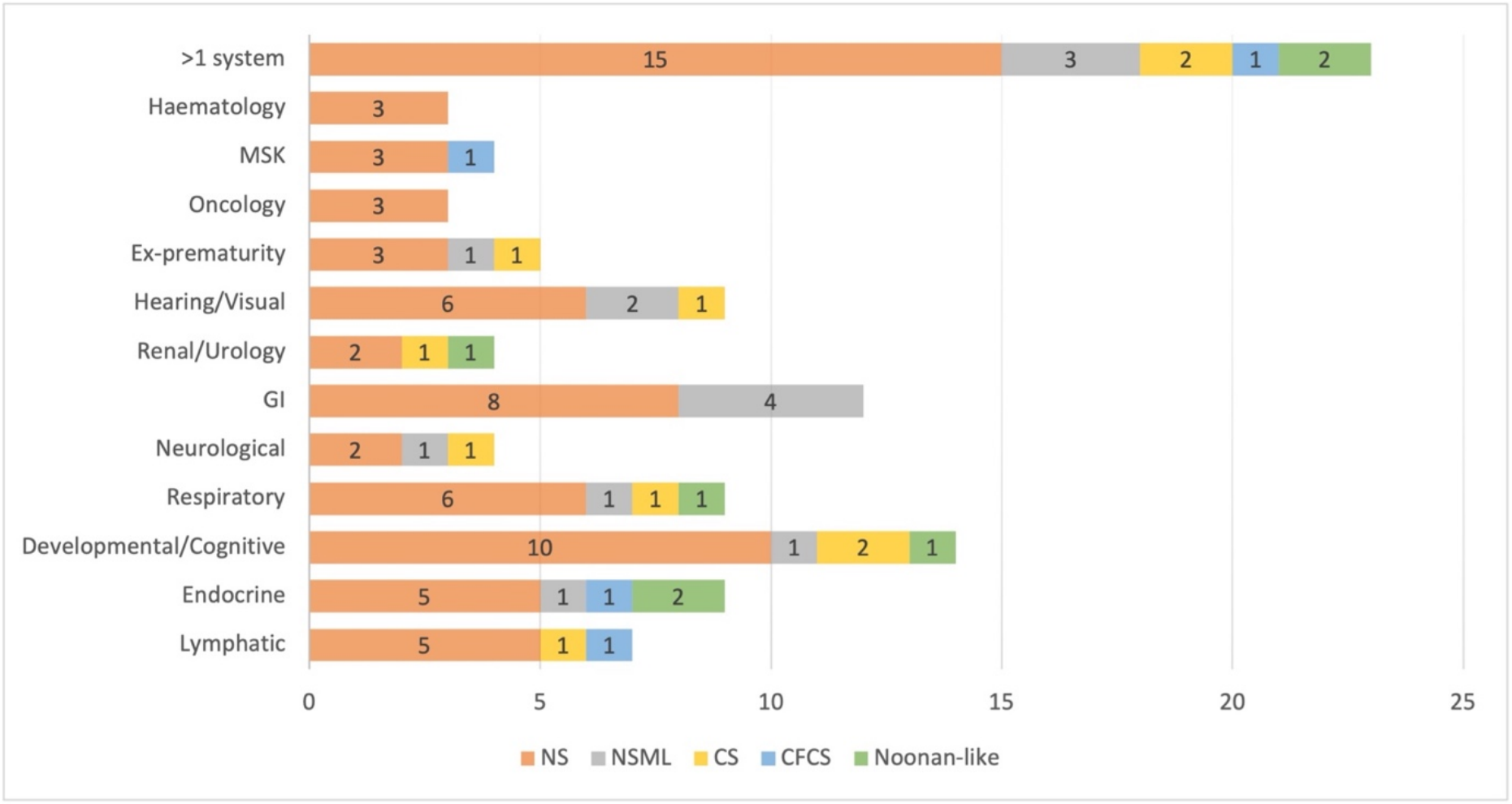


Figure 3-1: Extra-cardiac manifestations by Rasopathy syndrome

Table 3-2: Congenital heart defects by Rasopathy syndrome

	Total	NS	NSML	CS	CFCS	Noonan-like
ASD	16 (14.6)	13 (17.8)	-	-	1 (16.7)	2 (14.3)
VSD	8 (7.3)	5 (6.9)	-	-	1 (16.7)	-
PVS	33 (30)	22 (30.1)	2 (16.7)	2 (33.3)	4 (66.7)	3 (21.4)
PDA	5 (4.6)	3 (4.1)	1 (8.3)	-	-	1 (7.1)
Dysplastic valve	19 (17.3)	14 (19.2)	-	-	1 (16.7)	2 (14.3)
Polyvalvulopathy	21 (19.1)	16 (21.9)	1 (8.3)	1 (16.7)	1 (16.7)	2 (14.3)
AS	4 (3.6)	3 (4.1)	-	-	1 (16.7)	-
Other	6 (5.5)	5 (6.9)	-	-	-	-
>1	32 (29.1)	25 (34.3)	-	1 (16.7)	3 (50)	3 (21.4)
None	45 (40.9)	24 (32.9)	8 (66.7)	-	2 (33.3)	8 (57.1)
Unknown	39 (26.2)	38 (34.2)	-	1 (16.7)	-	-

NS: Noonan syndrome, NSML: Noonan syndrome with multiple lentigines, CS: Costello syndrome, CFCS: cardiofaciocutaneous syndrome, ASD: atrial septal defect, VSD: ventricular septal defect, PVS: pulmonary valve stenosis, PDA: patent ductus arteriosus, AS: aortic valve stenosis

The median age of diagnosis of HCM was 1.38 (IQR 0-10.28) months, while the median age at first assessment was 22.46 (IQR 5.67-82.89) months. The age category according to Rasopathy syndrome is shown in Figure 3-2.

Demographic and baseline clinical characteristics are summarized in Table 3-3. The clinical features of the 11 patients with Noonan-like syndrome are presented individually in Table 3-4, while the details for the 3 patients with NS-LAH are provided in Table 3-5. Patients with variants in PTPN11 and RIT1 exhibited a higher incidence of congenital heart disease (CHD) and were diagnosed at a younger age, as shown in Table 3-6.

There were no significant differences in clinical parameters across the different time periods, as outlined in Table 3-7.

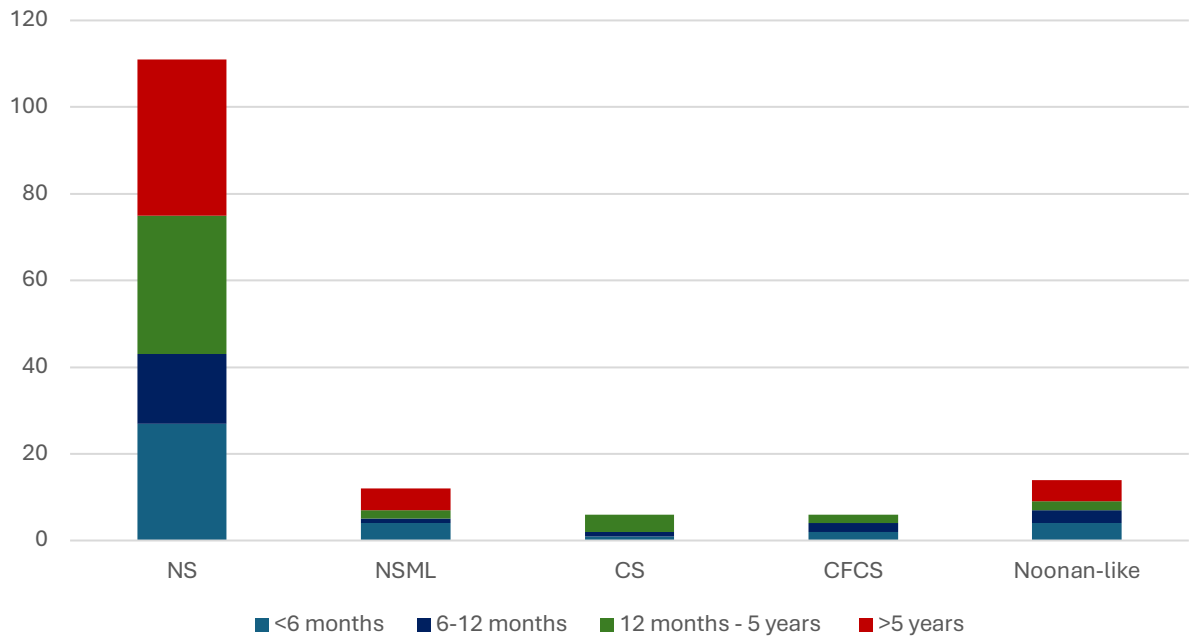


Figure 3-2: Age category by Rasopathy Syndrome

Table 3-3: Demographics and baseline characteristics

	Total N=149	NS N=111	NSML N=12	CS N=6	CFCS N=6	NLS N=11	p value
Gender (Male)	92 (61.7%)	70 (60.1%)	9 (75%)	3 (50%)	1 (16.7%)	6 (54.5%)	0.163
Age at diagnosis (months)	1.4 (0 - 10.3)	1.28 (0 - 8.7)	0 (0 - 11)	3.3 (2.4 - 71.2)	-0.16 (-0.3 - 6.7)	4.9 (-1.2 - 121.9)	0.401
Age at baseline (months)	22.5 (5.7 - 82.9)	26.4 (6.4 - 83.7)	37.7 (3 - 129.6)	13.6 (9.6 - 27.1)	8.11 (0.9 - 15.4)	14.1 (1.2 - 64)	0.563
Proband	121 (90.3%)	91 (82%)	9 (75%)	6 (100%)	5 (83.3%)	10 (90.1%)	0.269
FHx HCM	17 (11.4%)	12.6 (14%)	3 (25%)	6 (100%)	-	-	0.223
PMHx CHF	23 (22.2%)	16 (14.4%)	5 (41.7%)	-	-	2 (18.2%)	0.104
PMHx arrhythmia	7 (7.1%)	6 (5.4%)	-	-	-	1 (9.1%)	0.729
CHD	51 (46.4%)	38 (34.2%)	4 (33.3%)	-	3 (50%)	4 (36.4%)	0.174
Extra-cardiac manifestations	69 (65.1%)	54 (48.6%)	5 (41.7%)	3 (50%)	3 (50%)	4 (36.4%)	**0.001**
Symptoms	61 (57.3%)	50 (45.1%)	7 (58.3%)	1 (16.7%)	1 (16.7%)	2 (18.2%)	**0.073**
Medications	69 (47.9%)	50 (45.1%)	9 (75%)	1 (16.7%)	3 (50%)	5 (45.5%)	0.198
b-blockers	56 (81.2%)	42 (84%)	8 (88.9%)	1 (100%)	1 (33.3%)	4 (66.7%)	0.134
Diuretics	12 (17.4%)	9 (18%)	-	-	2 (66.7%)	1 (16.7%)	0.151
Disopyramide	4 (5.8%)	3 (6%)	1 (11.1%)	-	-	-	
Ca channel blockers	3 (4.3%)	1 (2%)	1 (11.1%)	-	-	1 (16.7%)	
Amiodarone	1 (1.4%)	1 (2%)	-	-	-	-	

n: number of patients, NS: Noonan syndrome, NSML: Noonan syndrome with multiple lentigines, CS: Costello syndrome, CFCS: cardiofaciocutaneous syndrome, NLS: Noonan-like syndrome, FHx: family history, HCM: hypertrophic cardiomyopathy, SCD: sudden cardiac death, PMHx: past medical history, CCF: congestive cardiac failure, CHD: congenital heart defects

Table 3-4: Patients with Noonan-like syndrome

Patient number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	8	9	10	11
Baseline demographics and clinical characteristics											
Gender	M	F	F	M	M	F	M	F	M	F	M
Proband?	Yes	Yes	-	-	-	Yes	Yes	Yes	Yes	Yes	-
Age at diagnosis (months)	4.9	121.9	-	133.4	135.2	0.4	-	2.6	1.3	1.2	-
Age at baseline (months)	29.4	63.8	7.7	133.4	135.2	0.43	0.53	1.15	0.8	11.9	16.3
PMHx CHD	No	Yes	No	No	No	Yes	Yes	Yes	No	No	No
Extra-cardiac manifestations	Yes	Yes	No	No	No	No	Yes	Yes	No	No	-
Symptoms	No	No	No	No	No	No	No	Yes	Yes	No	No
Medications	No	Yes	No	No	No	Yes	No	Yes	Yes	Yes	No
Outcomes											
Follow up (months)	216	206.7	216	159.33	216	100.1	102.8	11.4	18.6	94.3	144.9
Death	No	Yes	No	Yes	No	No	No	Yes	No	No	No
Cause of death	-	Non	-	U/K	-	-	-	U/K	-	-	-
Age at death (months)	-	12	-	191.2	-	-	-	2.4	-	-	-
SCD or equivalent event	No	Yes	No	No	No	No	No	No	Yes	No	No
CHF Admission	No	No	No	No	No	No	No	No	No	No	No
Myectomy	No	No	No	No	No	No	No	No	No	No	No
Echocardiographic parameters											
LVEDD (mm)	-	25.7	-	-	36.1	26.6	32.2	14	-	17.2	-
LVEDD z score	-	-2.4	-	-	2.6	1.9	5.5	-4.3	-	-3.2	-
LA diameter (mm)	-	-	-	-	29	-	25.6	-	-	19.6	-
LA diameter z score	-	-	-	-	-	-	20.6	-	-	15	-
MLVWT (mm)	-	6	-	10	9	6	7	-	15	-	-
MLVWT z score	-	3.4	-	-	3.2	4.3	6.5	-	-	-	-
LVOT gradient (mmHg)	-	-	-	4	-	5	10	10	117	-	-
LVOTO	-	-	-	No	-	No	No	No	Yes	No	-
Mid cavity obstruction	-	No	-	No	No	No	No	No	Yes	No	-
RVH	-	No	-	No	No	Yes	Yes	-	Yes	Yes	-
RVOT gradient (mmHg)	-	-	-	-	1	-	4	-	30	-	-
RVOTO	-	-	-	-	No	-	No	-	Yes	-	-
EF (%)	-	-	-	-	-	79	80	-	-	74	-

PMHx: past medical history, CCF: congestive heart failure, CHD: congenital heart defects, ICD: implantable cardiac defibrillator, SCD: sudden cardiac death, LVEDD: left ventricular end diastolic diameter, MLVWT: maximal wall thickness, LAd: left atrial diameter, LVOT: left ventricular outflow tract, LVOTO: LVOT obstruction, SAM: systolic anterior motion of the mitral valve, RVH: right ventricular hypertrophy, RVOT: right ventricular outflow tract, RVOTO: RVOT obstruction, EF: ejection fraction, U/K: unknown

Table 3-5: Patients with Noonan like syndrome with loose anagen hair

	Patient 1	Patient 2	Patient 3
Baseline demographics and clinical characteristics			
Gender	Male	Male	Male
Proband?	Yes	Yes	Yes
Age at diagnosis (months)	81.3	-	-
Age at baseline (months)	67.7	64	6.5
PMHx CHD	No	Yes	No
Extra-cardiac manifestations	No	No	No
Symptoms	No	No	No
Medication	No	b-blockers	No
Outcomes			
Follow up (months)	198.9	9.8	16.6
Death	No	Yes	Yes
Cause of death	-	Unknown	Unknown
Age at death (months)	-	73.8	23.1
SCD or equivalent event	No	No	No
Myectomy	No	No	No
CHF admission	No	No	No
ICD implantation	No	No	No
Heart transplant	No	No	No
Echocardiographic parameters			
LVEDD (mm)	29.7	-	-
LVEDD z score	+4.7	-	-
LA diametre (mm)	26	25	
LA diametre z score	+3.4	-	-
MLVWT (mm)	8	7	9
MLVWT z score	+9.2	-	-
LVOT gradient (mmHg)	45	16	27
LVOTO	Yes	No	No
Mid cavity obstruction	No	No	No
RVH	Yes	No	No
RVOT gradient (mmHg)	-	-	4
RVOTO	No	No	No
EF (%)	75	-	-
Systolic dysfunction	No	-	-

PMHx: past medical history, CHF: congestive heart failure, CHD: congenital heart defects, ICD: implantable cardiac defibrillator, SCD: sudden cardiac death, LVEDD: left ventricular end diastolic diameter, MLVWT: maximal wall thickness, LAd: left atrial diameter, LVOT: left ventricular outflow tract, LVOTO: LVOT obstruction, SAM: systolic anterior motion of the mitral valve, RVH: right ventricular hypertrophy, RVOT: right ventricular outflow tract, RVOTO: RVOT obstruction, EF: ejection fraction

Table 3-6: Demographics and baseline clinical characteristics by most prevalent genes

	PTPN11	RAF1	RIT1	HRAS	p value
Gender (Male), n (%)	16 (55.2)	12 (66.7)	6 (75)	3 (37.5)	0.431
Age at diagnosis (months), median (25th-75th centile)	0.4 (0 - 9)	2.7 (0.1 - 8.4)	0.23 (0 - 8.7)	2.83 (0 - 121.9)	**0.041**
Age at baseline (months), median (25th-75th centile)	11.1 (5.7 - 50.8)	37.6 (11.6 - 64.3)	2.41 (0.11 - 8.8)	12.1 (6.7 - 35.4)	0.889
Proband, n (%)	22 (75.9)	16 (88.9)	7 (87.5)	8 (100)	0.741
FHx HCM, n(%)	5 (17.2)	1 (5.6)	-	-	0.472
PMHx CHF, n(%)	10 (34.5)	2 (11.1)	-	-	0.151
PMHx arrhythmia, n (%)	3 (10.3)	1 (5.6)	1 (12.5)	1 (12.5)	1
CHD, n (%)	16 (55.2)	3 (16.7)	7 (87.5)	2 (25)	**0.002**
Extra-cardiac manifestations	10 (34.5)	10 (55.6)	2 (25)	3 (37.5)	0.531
Symptoms, n (%)	10 (34.5)	8 (44.4)	2 (25)	-	0.143
Medications, n (%)	18 (62.1)	11 (61.1)	4 (57.1)	4 (50)	0.958

n: number of patients, FHx: family history, HCM: hypertrophic cardiomyopathy, SCD: sudden cardiac death, PMHx: past medical history, CHF: congestive heart failure, CHD: congenital heart defects

Table 3-7: Clinical and genetics characteristics and outcomes by era of presentation

	1985-1999		2000-2010		2011-2020		p value (*)	p value (**)
	(n = 18)		(n = 56)		(n = 75)			
Male	10 (55.6%)		36 (64.3%)		47 (62.7%)		0.708	0.758
Age, months	89.7 (29.6 – 139.7)		323 (8.7 – 92.1)		11.9 (2.7 – 61.8)		0.003	0.063
Syndrome							0.124	0.152
NS	15 (83.3%)		43 (76.8%)		53 (70.7%)			
NSML	3 (16.7%)		2 (3.6%)		7 (9.3%)			
CS			1 (1.8%)		5 (6.7%)			
CFCS			5 (8.9%)		1 (1.4%)			
Noonan-like			4 (7.1%)		8 (10.7%)			
NS_LAH					3 (4%)			
Genetics	9 (50%)		44 (78.6%)		64 (85.3%)		**<0.001**	**<0.001**
Positive	3 (33.3%)		27 (61.4%)		50 (78.1%)		**<0.001**	**0.007**
Variant	PTPN11	2 (66.7%)	PTPN11	7 (25.9%)	PTPN11	20 (40%)	0.255	0.095
	KRAS	1 (33.3%)	RAF1	6 (22.2%)	RAF1	12 (24%)		
			RIT1	4 (14.8%)	RIT1	4 (8%)		
			HRAS	2 (7.4%)	HRAS	6 (12%)		
			KRAS	1 (3.7%)	KRAS	4 (8%)		
			LZTR1	4 (14.8%)	BRAF	1 (2%)		
			BRAF	2 (7.4%)	SHOC2	3 (6%)		
			MEK2	1 (3.7%)				
2nd variant	-		4 (7.1%)		1 (1.3%)			

Follow up, months	209.5 (167.4 – 216)	215.7 (215 – 216)	113.1 (43.9 – 182.9)	**<0.001**	**<0.001**
SCD/equivalent event	2 (11.1%)	4 (7.1%)	6 (8%)	0.457	0.959
Heart transplant	-	1 (1.8%)	2 (2.7%)		
Myectomy	3 (16.7%)	5 (8.9%)	6 (8%)	0.447	0.405
Death	3 (16.7%)	8 (14.3%)	12 (16%)	0.62	0.453

n: number of patients, IQR: interquartile range, NS: Noonan syndrome, NSML: Noonan syndrome with multiple lentigines, CS: Costello syndrome, CFCS: cardiofaciocutaneous syndrome, SCD: sudden cardiac death. (*) represents p values for whole group, (**) subgroup analysis excluding the first era

3.4.2 Genetics

Genetic testing was conducted on 117 patients (78.5%), with a pathogenic (P) or likely pathogenic (LP) variant detected in 81 patients (69.2%). The most frequently identified gene was *PTPN11* (N=28, 34.6%), followed by *RAF1* (N=18, 22.2%), *RIT1* (N=8, 9.9%), and *HRAS* (N=8, 9.9%). Five patients (4.3%) had additional variants identified, including combinations such as *RAF1* (P) & *MYH7* (VUS), *PTPN11* (P) & *MYH7* (VUS), *PTPN11* (P) & *MYH7* (LP), *KRAS* (LP) & *MEK1* (VUS), and *LZTR1* (LP) & *HRAS* (VUS). Figure 3-3 illustrates the distribution of implicated genes across different Rasopathy syndromes. Detailed information on specific nucleotide and protein alterations is provided in Table 3-8.

Over time, both the proportion of patients undergoing genetic testing and the yield of genetic findings have increased, as shown in Table 3-7.

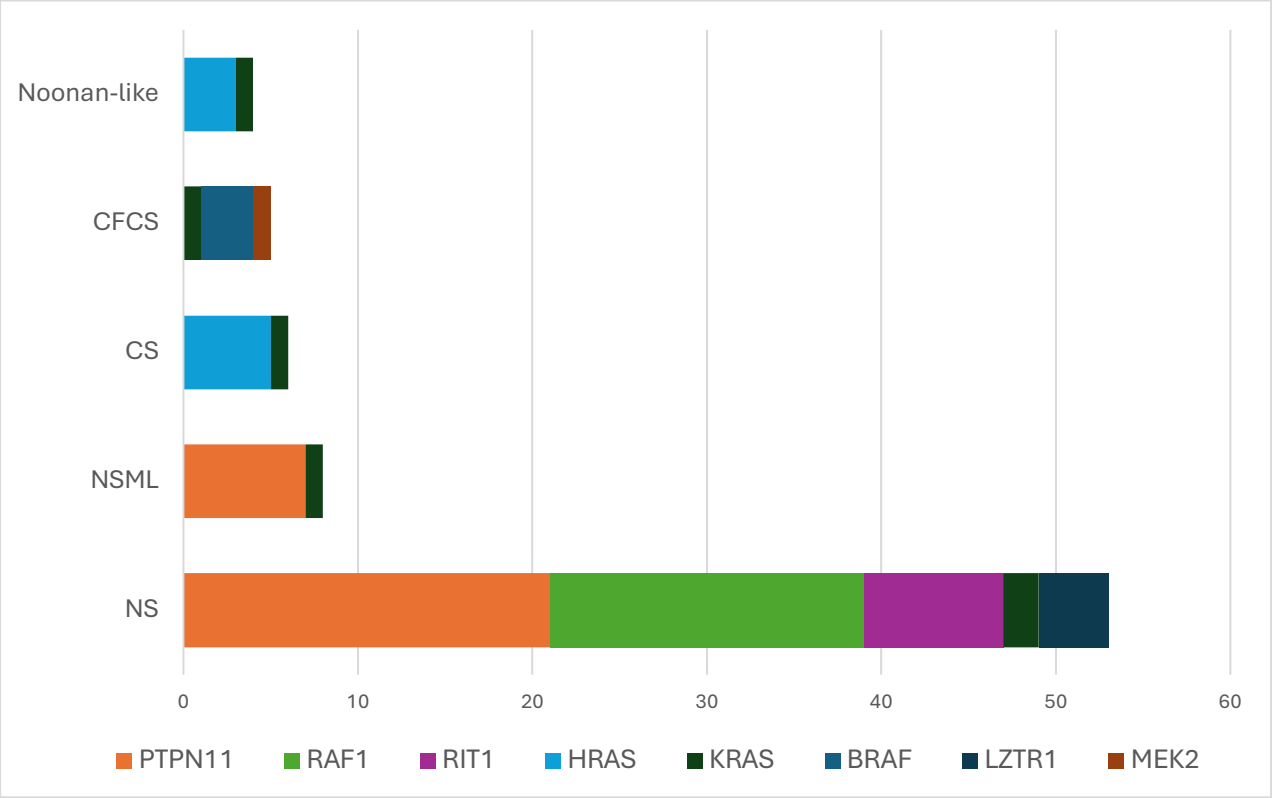


Figure 3-3: Gene mutation by Rasopathy Syndrome

Table 3-8: Gene variant nucleotide and protein changes

Affected Gene	Nucleotide code	Protein code	N
PTPN11	836A>G	Tyr279Cys	6
	1528C>G	Gln510Glu	3
	922A>G	Asn308Asp	2
	102G>T	Lys34Asn	1
	188A>G	Tyr63Cys	1
	846C>G	Ile282Met	1
	218C>T	Thr73Ile	1
	923A>G	Asn308Ser	1
	854T>C	Phe285Ser	1
	236A>G	Glu79Arg	1
	417G>C	Glu139Asp	1
	1528C>G	Gln510Glu	1
	768G>C	Asn320Ser	1
	1403C>T	Thr468Met	1
RAF1	770C>T	Ser257Leu	2
	770C>T	Ser257Gly	1
	766A>G	Arg256Gly	1
	775T>A	Ser259Thr	1
	1082G>C	Gly361Ala	1
	779c >T	Thr260Ile	1

	76BG>T	Arg256Ser	1
	781C>T	Pro261Ser	1
RIT1	244T>C	Phe82Leu	2
	151G>T	Asp51Tyr	1
	284G>C	Gly95Ala	1
	229G>A	Ala77Thr	1
	244T>A	He82Lle	1
HRAS	34G>A	Gly12Ser	5
	64C>A	Gln22Lys	1
	466T>C	Phe156Leu	1
	34G>T	Gly12Cys	1
KRAS	179G>T	Gly60Val	2
	346A>C	ASn116His	1
	173C>T	Thr58IIe	1
LZTR1	3493C>T	Lys1165Glu	1
	1234C>T	Arg412Cys	1
	290G>T	Arg97Leu	1
SHOC2	4A>G	Ser2Gly	1
BRAF	1782T>G	Asp5974Glu	1
MEK2	619G>A	Glu207Lys	1

3.4.3 Echocardiographic Characteristics

Echocardiographic data from the initial assessment at a paediatric cardiology centre were available for 116 patients (77.9%). Of these, 46 patients (48.9%) had biventricular involvement, 44 patients (45.8%) had left ventricular outflow tract obstruction (LVOTO), and 18 patients (39.1%) showed right ventricular outflow tract obstruction (RVOTO). Additionally, 9 patients (30%) had signs of diastolic dysfunction at the first assessment. The echocardiographic findings are summarized in Table 3-9, with a comparison of the echocardiographic phenotype across the most common genetic variants presented in Table 3-10.

Table 3-9: Echocardiographic features by Rasopathy syndrome

	Total	NS	NSML	CS	CFCS	Noonan-like	p value
LVEDD (mm)	23.2 (18.6 - 30.9)	23.2 (18.6 - 31)	24.9 (18.4 - 29)	20.1 (18.8 - 21)	19.1 (19 - 19.2)	26.2 (20.8 - 33.6)	0.489
LVEDD z score	-1 (0.97)	-1.57 (0.9)	-2.36 (1.2)	-3.21 (3.1)	-3.2 (0.9)	+5.5 (0.7)	**0.039**
LA diametre (mm)	25.7 (18.3 - 30.9)	23 (15.2 - 30.5)	29 (25.8 - 42)	-	-	25.6 (19.6 - 29)	0.309
LA diametre z score	+19 (3.2)	+19.9 (3.5)	-	-	-	+20.6	0.969
MLVWT (mm)	11 (8 - 14)	11 (9 - 14)	13.5 (10 - 15.5)	7.5 (7 - 8.4)	8.2 (5 - 8)	7 (6 - 12.5)	**0.004**
MLVWT z score	+9.6 (1.9)	+9.9 (2.1)	+17 (8.7)	+7 (2.1)	+6.4 (3.1)	+6.5 (5)	**0.074**
LVOT gradient (mmHg)	23 (8 - 60)	20 (9 - 60)	60 (36 - 80)	8 (4 - 45)	27 (5 - 32)	6 (4 - 10)	**0.004**
LVOTO	44 (39.1)	32 (28.9)	8 (66.7)	1 (16.7)	2 (33.3)	1 (9.1)	**0.032**
Mid cavity obstruction	36 (24.2)	28 (25.2)	6 (50)	-	1 (16.67)	1 (9.1)	**0.009**
SAM	44 (29.5)	33 (29.7)	8 (66.7)	1 (16.7)	-	2 (18.2)	**0.012**
RVH	46 (48.9)	33 (63.5)	6 (66.7)	1 (16.7)	1 (16.67)	4 (36.4)	0.287
RVOT gradient (mmHg)	10 (4 - 30)	10 (4 - 27)	5 (1 - 30)	2 (2 - 2.5)	2 (-)	4 (2.5 - 17)	**0.019**
RVOTO	18 (39.1)	14 (16.2)	3 (25)	2 (33.3)	-	1 (9.1)	0.607
EF (%)	79 (73 - 85)	77 (72 - 85)	81	83.5 (81 - 86)	89 (-)	77 (74.5 - 79.5)	0.871
Systolic dysfunction	1 (3)	1 (3)	-	-	-	-	0.631
E/E' average	10.77 (7.4 - 15.1)	10.9 (7.3 - 15.3)	10 (9.6 - 12.8)	10.2 (9.6 - 11.6)	8.6 (-)	-	0.183
Diastolic dysfunction	9 (30)	8 (7.2)	1 (8.3)	-	-	-	0.456
ASH	34 (26)	24 (21.6)	3 (25)	2 (33.3)	3 (50)	2 (16.7)	
Concentric	52 (39.7)	33 (29.7)	7 (58.3)	2 (33.3)	3 (50)	7 (58.3)	
Eccentric	4 (3.1)	4 (5.4)	-	-	-	-	

Apical	3 (2.3)	3 (4.1)	-	-	-	-
Unknown	18 (12.1)	10 (9)	2 (16.7)	2 (33.3)	-	3 (25)

NS: Noonan syndrome, NSML: Noonan syndrome with multiple lentigines, CS: Costello syndrome, CFCS: cardiofaciocutaneous syndrome, LVEDD: left ventricular end diastolic diameter, MLVWT: maximal wall thickness, LAd: left atrial diameter, LVOT: left ventricular outflow tract, LVOTO: LVOT obstruction, SAM: systolic anterior motion of the mitral valve, RVH: right ventricular hypertrophy, RVOT: right ventricular outflow tract, RVOTO: RVOT obstruction, EF: ejection fraction, ASH: asymmetric septal hypertrophy

Table 3-10: Echocardiographic data by most prevalent genes

	PTPN11	RAF1	RIT1	HRAS	p value
LVEDD (mm), median (IQR)	23.8 (20.2 - 29)	23.3 (20 - 31.5)	18.1 (17.5 - 19.6)	21 (17.2 - 26.6)	0.469
LVEDD z score, mean (SD)	+0.01 (2.6)	-0.15 (3)	-2.66 (0.9)	-	0.565
LA diametre (mm), median (IQR)	27 (25.8 - 27.9)	18.2 (15.3 - 36)	13.3 (12.6 - 26.5)	19.6 (-)	0.493
LA diametre z score, mean (SD)	+25.86 (7.3)	+28.84 (16.6)	+4.04 (1.32)	-	0.308
MLVWT (mm), median (IQR)	10.5 (8.5 - 14.5)	14 (10 - 18)	7 (6 - 10)	7 (6 - 8)	**0.002**
MLVWT z score, mean (SD)	+12.23 (6.9)	+16.65 (3.9)	+6.57 (0.4)	-	0.43
LVOT gradient (mmHg), median (IQR)	36 (17 - 60)	43 (16 - 58)	55 (7.5 - 100)	6.5 (4.5 - 26.5)	0.232
LVOTO, n (%)	14 (63.6)	8 (61.5)	2 (50)	1 (12.5)	0.338
Mid cavity obstruction, n (%)	15 (68.2)	8 (80)	2 (33.3)	-	**0.003**
SAM, n (%)	17 (65.4)	9 (64.3)	3 (42.9)	1 (12.5)	0.073
RVH, n (%)	14 (60.9)	7 (53.9)	6 (75)	3 (37.5)	0.477
RVOT gradient (mmHg), median (IQR)	18.5 (3.5 - 57.5)	21 (4 - 70.5)	16.5 (10 - 57)	2 (2 - 2.5)	0.401
RVOTO, n (%)	6 (50)	4 (57.1)	3 (50)	-	0.55
EF (%), median (IQR)	79 (77.5 - 85.5)	86 (77.5 - 92.9)	79.5 (70.5 - 87)	80 (76.5 - 83.5)	0.703
Systolic dysfunction, n (%)	-	-	-	-	
E/E' average, median (IQR)	10 (7.2 - 12.9)	10.8 (7.3 - 28.3)	15.07 (-)	10.2 (9.6 - 11.6)	0.675
Diastolic dysfunction, n (%)	2 (18.2)	2 (66.7)	1 (100)	-	0.197

LVEDD: left ventricular end diastolic diameter, MLVWT: maximal wall thickness, LAd: left atrial diameter, LVOT: left ventricular outflow tract, LVOTO: LVOT obstruction, SAM: systolic anterior motion of the mitral valve, RVH: right ventricular hypertrophy, RVOT: right ventricular outflow tract, RVOTO: RVOT obstruction, EF: ejection fraction

3.4.4 Electrocardiogram

A total of 93 patients (62.4%) had baseline electrocardiograms available. Among these, 83 patients (89.2%) exhibited one or more abnormal findings. Most patients (N=91, 97.8%) were in sinus rhythm, while one patient had atrial tachycardia and another was in junctional rhythm. Forty-seven patients (59.5%) showed QRS axis deviation, with 21 (44.7%) demonstrating a superior axis. Sixty patients (69.8%) met the criteria for left ventricular hypertrophy, and 30 patients (34.9%) presented with repolarization abnormalities, including T wave inversion in one or more leads. A summary of the electrocardiographic data is provided in Table 3-11.

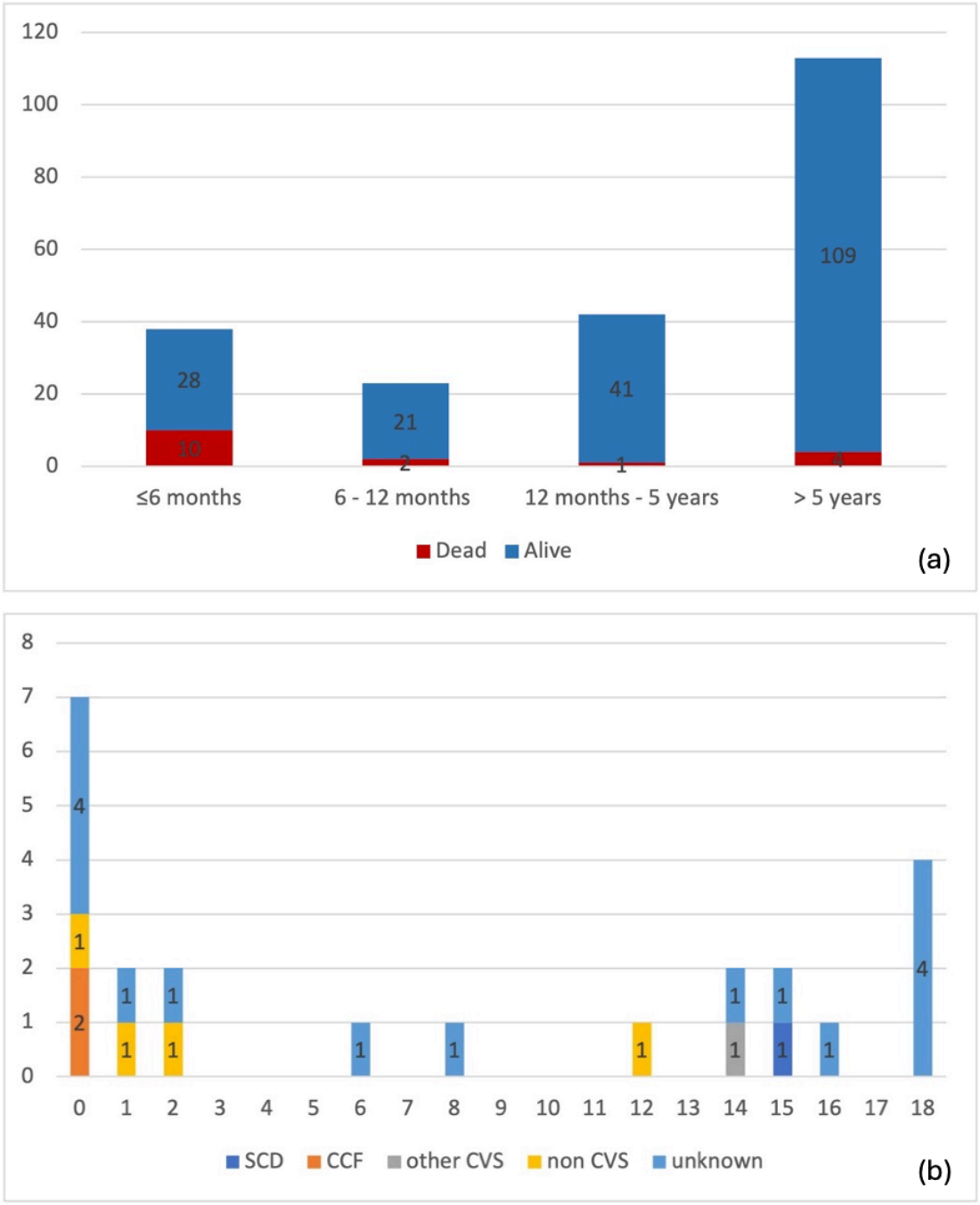
Table 3-11: Electrocardiographic data at baseline assessment

		Total	%
Sinus rhythm		91	97.8
Left axis deviation		20	25.3
Right axis deviation		27	34.2
	Superior axis	21	44.7
PR interval prolongation		5	6.4
Right atrial enlargement		17	19.8
Left atrial enlargement		18	20.9
QTc prolongation		5	6.4
Voltage criteria for LVH		60	69.8
Conduction abnormalities	Intraventricular conduction delay	43	48.9
	RBBB	2	2.3
	LBBB	4	4.6
Pathological Q waves	Inferior leads	19	21.4
	Lateral leads	10	11.2
	Anterior leads	1	1.1
	>1 location	4	4.5
T wave inversion	Inferior leads	4	4.8
	Lateral leads	13	15.5
	Anterior leads	4	4.8
	>1 location	9	10.7
ST depression (<1mm)	Inferior leads	2	2.4
	Lateral leads	2	2.4
	Anterior leads	3	3.6
	>1 location	4	4.8
ST elevation (>2mm)	Absent	86	92.9
	Present	7	7.5

LVH: left ventricular hypertrophy, RBBB: right bundle branch block, LBBB: left BBB

3.4.5 Outcomes

The median length of follow up was 197.5 (IQR 93.58-370) months, or 231.55 patient-months, with 2 patients (1.34%) lost to follow up. At the end of follow up, 126 patients (84.6%) were alive, including 14 (9.7%) who had undergone surgical myectomy (one of whom subsequently died with no documented cause of death available) and 3 (2%) who had undergone a heart transplant (of whom 1 subsequently died 14.2 years later with no documented cause of death available). Twelve patients (8.2%) had a major arrhythmic cardiac event (SCD or equivalent event) documented. A total of 23 patients (15.4%) died, at a median age of 24.1 months (IQR 5.6-175.9). The cause of death was unknown in 12 cases (52.2%). Of the known causes, 4 patients died from a non-congestive cardiac failure related CVS cause (17.4%) or from a non-CVS related cause (17.4%). Two (8.7%) patients died due to progressive congestive cardiac failure and one (4.4%) suffered a SCD (See Figure 3-4). Seven patients (31.8%) with a history of congestive heart failure (CHF) and 11 patients (29%) who were under 6 months of age at the time of their first assessment, died. A detailed breakdown of outcomes by Rasopathy syndrome is provided in Table 3-12.
There was no significant difference in survival or outcome by era of presentation or by genotype (Figure 3-5, Table 3-13).



<u>Figure 3-4:</u> (a) absolute number of deaths according to each age category (b) cause of death by age of death (years)

Table 3-12: Outcomes

	Total	NS	NSML	CS	CFCS	Noonan-like	p value
Death	21 (14.1%)	13 (11.7%)	1 (8.3%)	1 (16.7%)	1 (16.7%)	3 (27.3%)	**0.083**
SCD	1 (4.8%)	1 (7.7%)	-	-	-	-	
CHF	2 (9.5%)	1 (7.7%)	1 (8.3%)	-	-	-	
Other CVS	1 (4.8%)	1 (7.7%)	-	-	-	-	
Other	4 (19.1%)	2 (15.4%)	-	1 (100)	-	1 (33.3%)	
Unknown	12 (57.1%)	7 (53.9%)	-	-	1 (100)	2 (66.7%)	
Age at death (months)	24.1 (5.6 - 175.9)	25.9 (5.6 - 175.9)	1.7	12.9	191.1	23.1 (12 - 73.8)	0.469
Myectomy	14 (9.4%)	13 (11.7%)	1 (8.3%)	-	-	-	
ICD implantation	7 (4.7%)	7 (6.3%)	-	-	-	-	
CHF admission	10 (6.7%)	9 (8.1%)	1 (8.3%)	-	-	-	
Heart transplant	3 (2%)	3 (2.7%)	-	-	-	-	
NSVT	5 (3.4%)	3 (3%)	1 (8.3%)	-	-	1 (33.3%)	
SCD/equivalent event	12 (8.1%)	9 (8.1%)	1 (8.3%)	-	-	2 (18.2%)	

n: number of patients, N: number of values available, NS: Noonan syndrome, NSML: Noonan syndrome with multiple lentigines, CS: Costello syndrome, CFCS: cardiofaciocutaneous syndrome, CHF: congestive heart failure, ICD: implantable cardiac defibrillator, NSVT: non-sustained ventricular tachycardia.

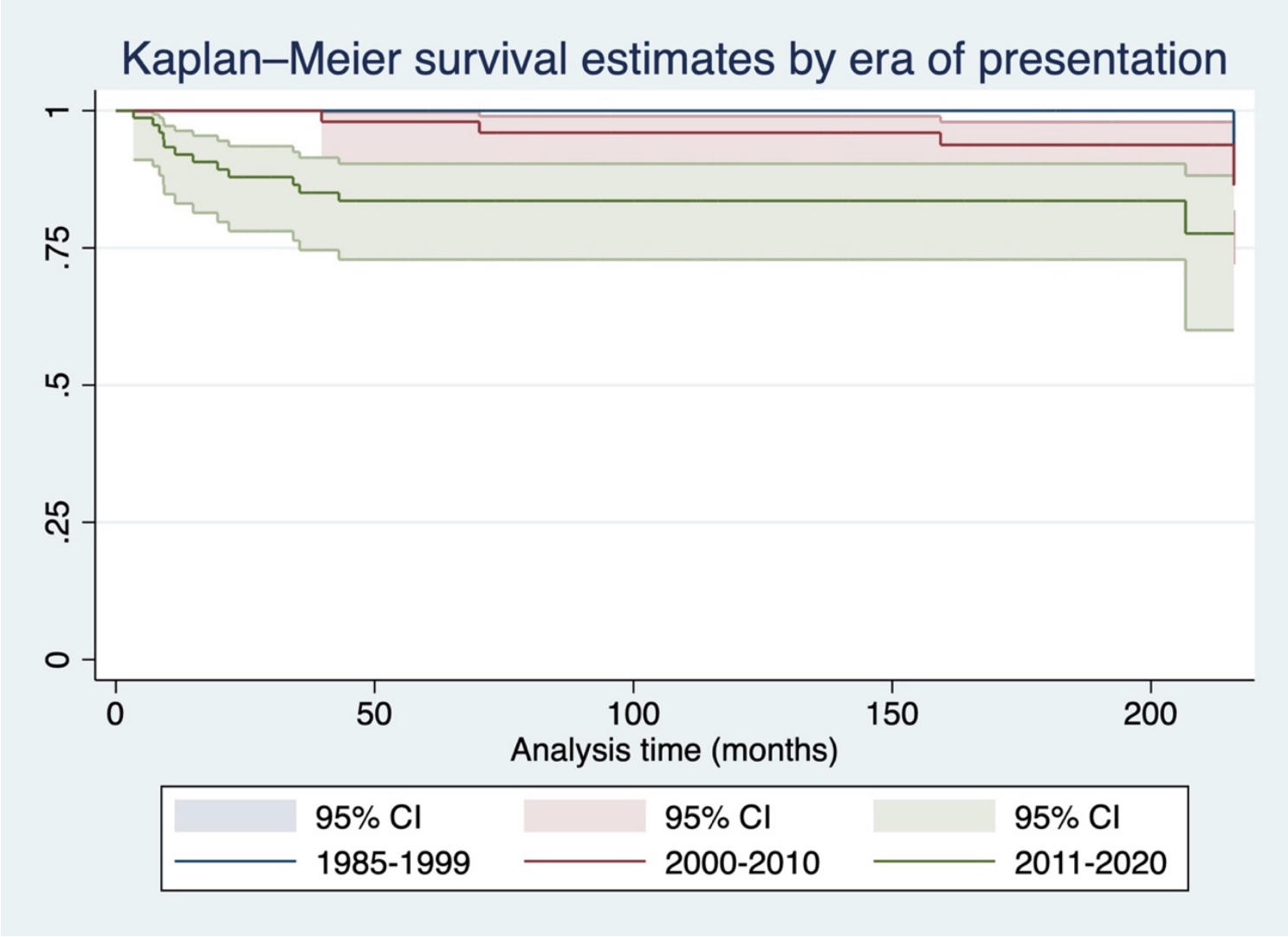


Figure 3-5: Kaplan-Meier survival estimates by era of presentation, p = 0.453

Table 3-13: Outcomes by most prevalent genes

	PTPN11	RAF1	RIT1	HRAS	p value
Death, n (%)	3 (10.3)	1 (5.6)	-	2 (25)	0.44
Age at death (months), median (25th-75th centile)	3.3 (1.7 - 24)	5.26 (-)	-	12.4 (11.9 - 12.8)	0.651
Myectomy, n (%)	3 (10.3)	3(16.7)	1 (12.5)	-	0.37
ICD implantation, n (%)	2 (6.9)	1 (5.6)	1 (12.5)	-	0.889
CCF admission, n (%)	6 (20.7)	-	2 (25)	-	0.17
Heart transplant, n (%)	2 (6.9)	-	-	-	0.733
NSVT, n (%)	1 (3.3)	1 (5.6)	-	1 (12.5)	0.523
SCD or equivalent event, n (%)	2 (6.9)	1 (5.6)	1 (12.5)	1 (12.5)	0.316

n: number of patients, CCF: congestive cardiac failure, ICD: implantable cardiac defibrillator, NSVT: non-sustained ventricular tachycardia, SCD: sudden cardiac death

3.4.6 Survival and predictors of all-cause mortality and SCD or equivalent event

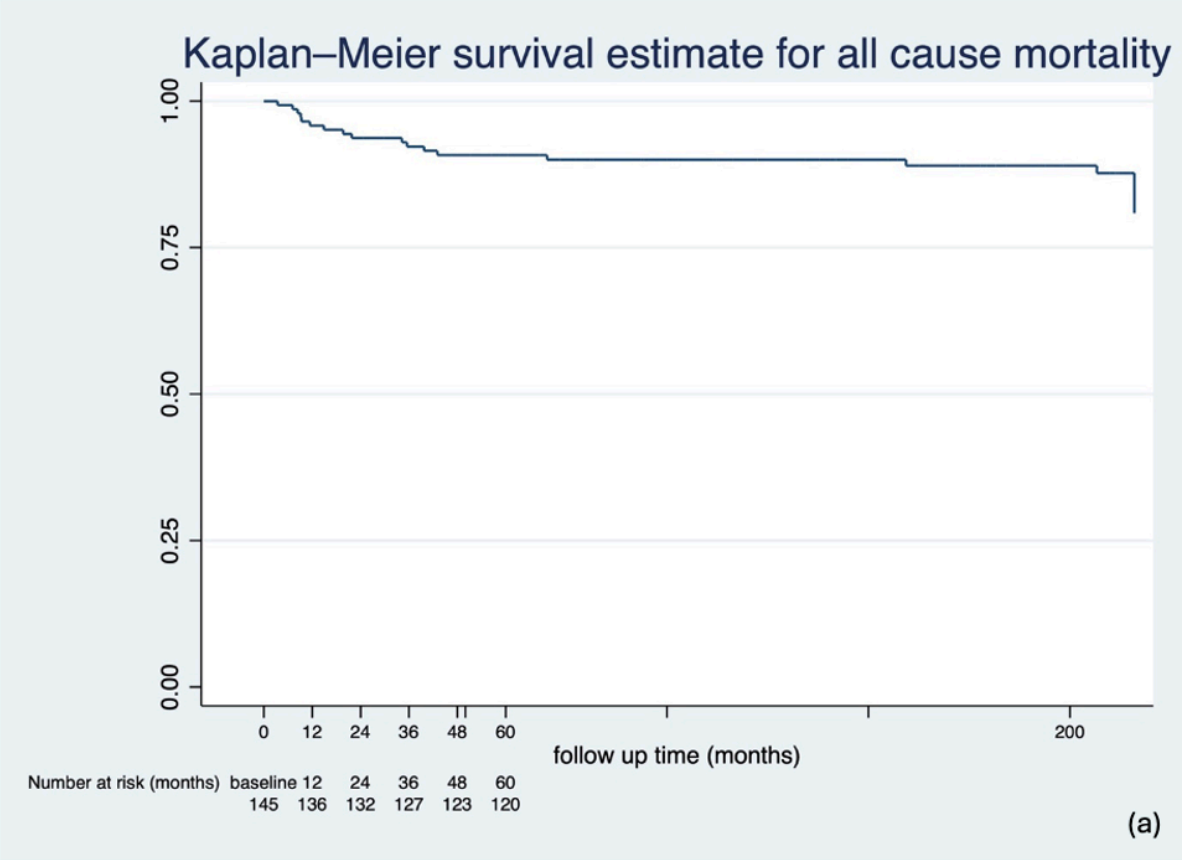
Overall survival was 96.45% (95% CI 91.69-98.51), 90.42% (95% CI 84.04-94.33) and 84.12% (95% CI 75.42-89.94) at 1, 5 and 10 years, respectively, but this varied by Rasopathy syndrome.

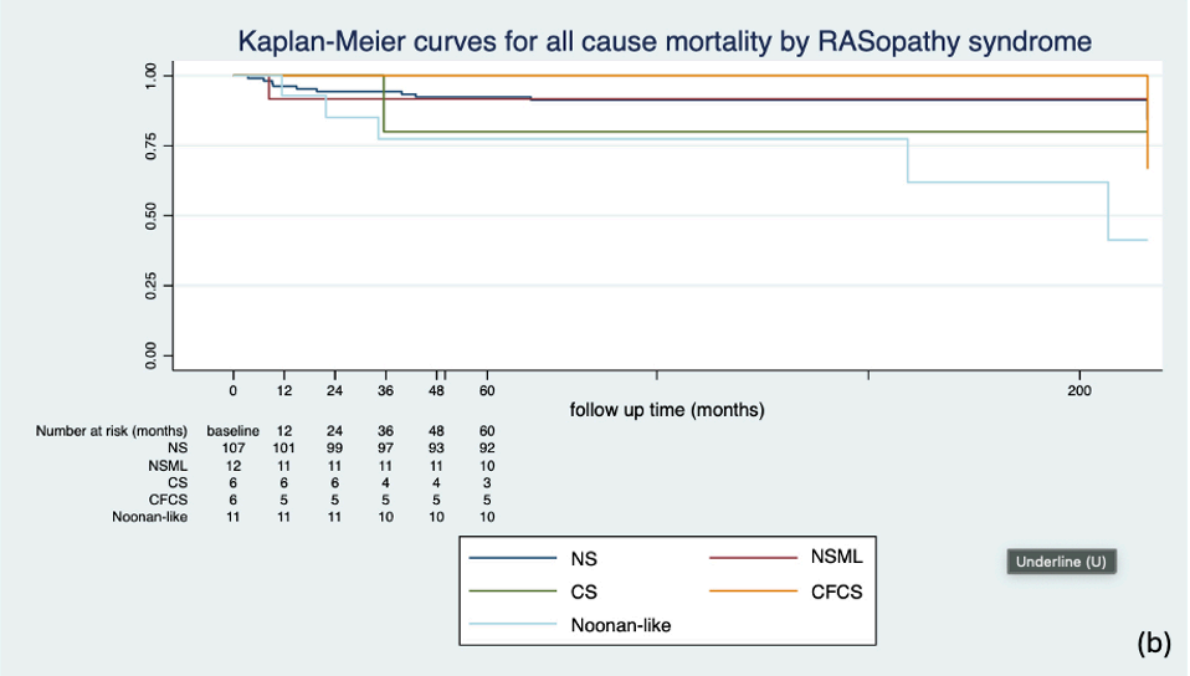
Univariate analysis identified several factors as predictors of all-cause mortality, including baseline symptoms, the presence of concomitant congenital heart disease (CHD), Rasopathy syndrome, a past medical history of congestive cardiac failure (CCF), previous CCF admissions, the presence of non-sustained ventricular tachycardia (NSVT), and moderate left ventricular wall thickness (MLVWT). These findings are summarized in Table 3-15 and Figure 3-6). Regarding SCD or equivalent event, (Figure 3.7), the presence of NSVT, past medical history of CCF, and LVOT gradient were identified as predictors on univariate analysis (Table 3-16).

Table 3-14: Survival by Rasopathy syndrome

	1 year, % (95% CI)	**5 year, % (95% CI)**	**10 year, % (95% CI)**	**15 year, % (95% CI)**
NS	94.3 (87.7 - 97.4)	91.3 (83.9 - 95.4)	91.3 (83.9 - 95.4)	91.3 (83.9 - 95.4)
NSML	91.7 (53.9 - 98.8)	91.7 (53.9 - 98.8)	91.7 (53.9 - 98.8)	91.7 (53.9 - 98.8)
CS	81.8 (23.9 - 97.2)	81.8 (23.9 - 97.2)	81.8 (23.9 - 97.2)	81.8 (23.9 - 97.2)
CFCS	100 (-)	100 (-)	50 (0.6 - 91.1)	50 (0.6 - 91.1)
Noonan-like	82.9 (47.2 - 95.5)	73.7 (32.8 - 83.3)	58.9 (32.8 - 83.3)	39.3 (7 - 72)

NS: Noonan syndrome, NSML: Noonan syndrome with multiple lentigines, CS: Costello syndrome, CFCS: cardiofaciocutaneous syndrome





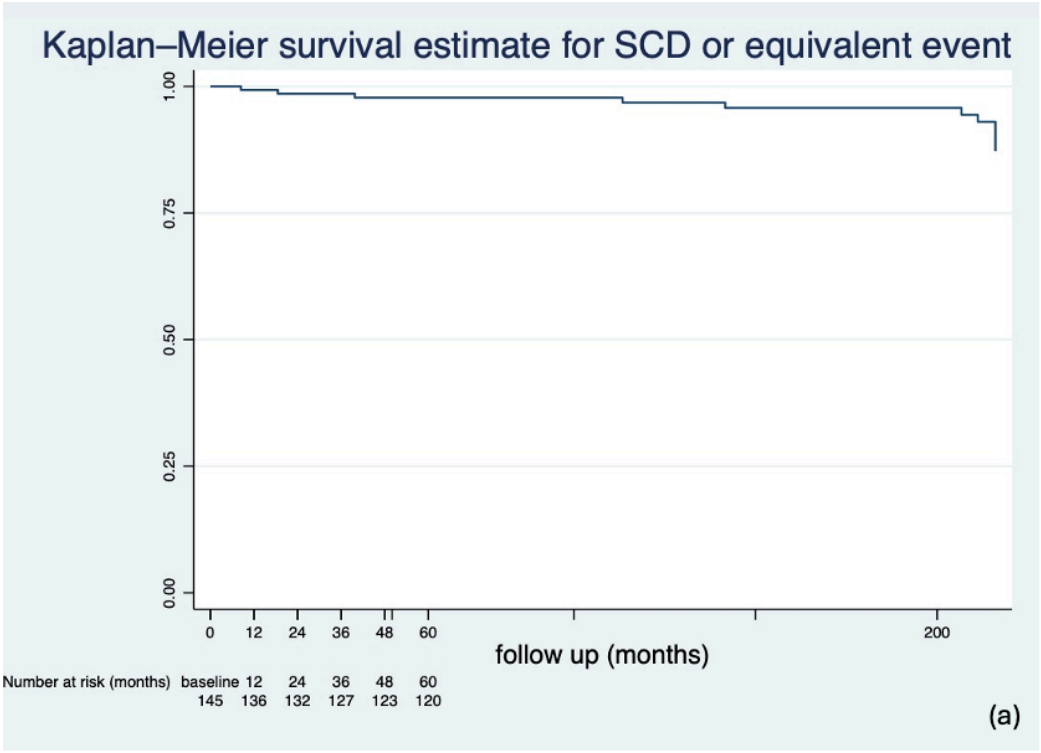
<u>Figure 3-6:</u> Kaplan-Meier curve for all-cause mortality with yearly numbers at risk for (a) whole cohort and (b) by different Rasopathy syndromes.

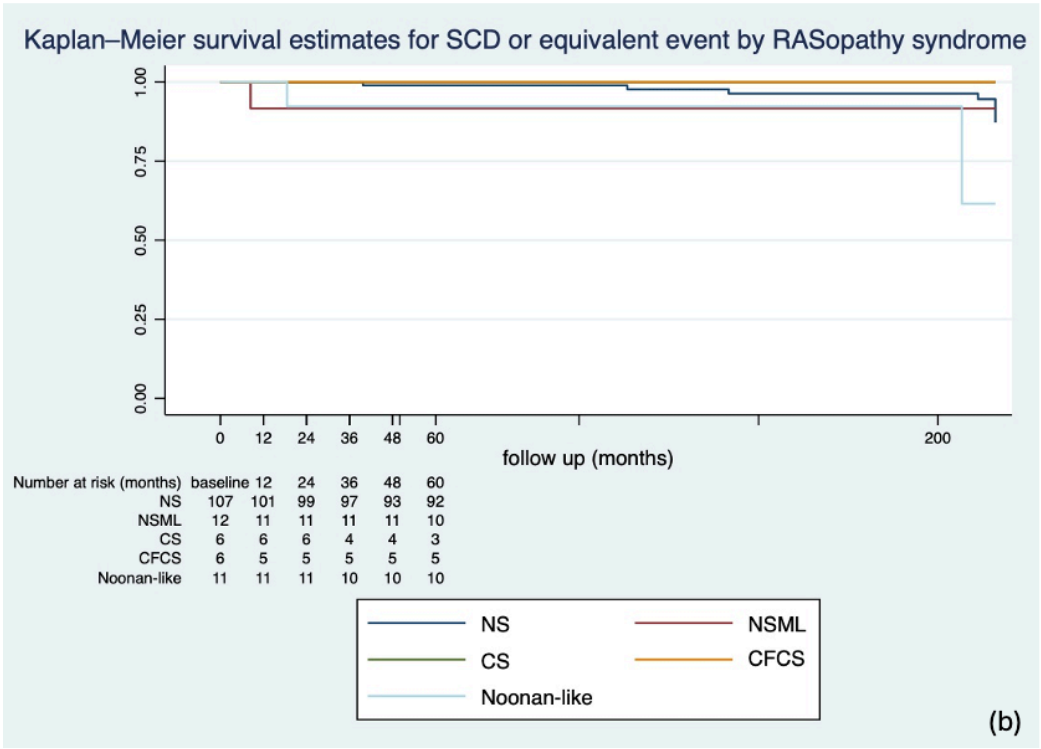
Table 3-15: Predictors of all-cause mortality

	Hazard Ratio	Std Error	95% CI	p value
Demographics and baseline clinical characteristics				
Gender	0.83	0.38	0.33-2.05	0.679
Age at diagnosis	1	0.01	0.99-1.01	0.864
Age at baseline assessment	0.99	0.01	0.98-1	0.102
PMHx CHD	2.32	1.09	0.92-5.86	**0.073**
PMHx CHF	0.45	0.21	0.18-1.14	**0.092**
PMHx arrhythmia	1.13	1.17	0.15-8.54	0.906
Symptoms	1.31	0.59	0.54-3.17	**0.017**
Medications	0.98	0.43	0.41-2.31	0.967
CHF admission	4.31	2.4	1.45-12.83	**0.009**
NSVT	5.56	4.3	1.22-25.35	**0.027**
Syndrome				**0.011**
NSML	0.68	0.71	0.09-5.22	0.714
CS	1.6	1.67	0.21-12.27	0.65
CFCS	1.46	1.52	0.019-11.16	0.715
Noonan-like	3.81	2.02	1.35-10.79	**0.012**
Gene	1.02	0.69	0.27-3.82	0.22
Echocardiographic phenotype				
LVEDD	0.956	0.36	0.89-1.03	0.225

LVEDD z score	1.02	0.04	0.95-1.1	0.533
LA diameter	0.99	0.52	0.89-1.1	0.825
LA diameter z score	1.02	0.06	0.91-1.14	0.784
MLVWT	0.85	0.07	0.73-0.99	**0.044**
MLVWT z score	0.97	0.04	0.9-1.06	0.538
LVOT gradient	0.99	0.01	0.97-1.01	0.318
RVOT gradient	0.99	0.02	0.96-1.02	0.625
Ejection fraction	1.08	0.07	0.95-1.23	0.223
Average E/E'	0.97	0.09	0.81-1.16	0.711
RVH	0.49	0.27	0.16-1.57	0.202
Mid cavity obstruction	1.56	0.87	0.52-4.68	0.428

NS: Noonan syndrome, NSML: Noonan syndrome with multiple lentigines, CS: Costello syndrome, CFCS: cardiofaciocutaneous syndrome, CHD: congenital heart defects, PMHx: past medical history, CHF: congestive heart failure, NSVT: non-sustained ventricular tachycardia, LVEDD: left ventricular end diastolic diameter, MLVWT: maximal wall thickness, LAd: left atrial diameter, LVOT: left ventricular outflow tract, SAM: systolic anterior motion of the mitral valve, RVH: right ventricular hypertrophy, RVOT: right ventricular outflow tract





<u>Figure 3-7:</u> Kaplan-Meier curve for SCD or equivalent event with yearly numbers at risk for (a) whole cohort and (b) by different Rasopathy syndromes.

Table 3-16: Predictors of SCD or equivalent event

	Hazard Ratio	Std Error	95% CI	p value
Demographics and baseline clinical characteristics				
Gender	1.47	0.89	0.45-4.84	0.522
Age at diagnosis	1	0.01	0.99-1.02	0.556
Age at baseline assessment	0.99	0.01	0.98-1.01	0.506
PMHx CHD	1.65	1.11	0.44-6.15	0.457
PMHx CHF	0.34	0.23	0.09-1.26	0.096
PMHx arrhythmia	6.42E+14	2.30E+22	-	1.000
Symptoms	1.53	0.96	0.45-5.25	0.497
Medications	0.48	0.30	0.14-1.64	0.243
CHF admission	1.75	1.83	0.22-13.68	0.596
NSVT	6.1	4.84	1.28-28.91	**0.023**
Syndrome				0.514
NSML	1.11	1.18	0.14-8.88	0.921
CS	5.08E-16	3.88E-08	-	1.000
CFCS	5.09E-16	3.45E-08	-	1.000
Noonan-like	3.07	2.45	0.64-14.6	0.159
Gene	1.24	1.04	0.24-6.41	0.82
Gene negative	1.81	1.22	0.49-6.75	0.376

Echocardiographic phenotype				
LVEDD	0.87	0.80	0.74-1.04	0.126
LVEDD z score	0.64	0.17	0.38-1.08	0.106
LA diameter	0.96	0.08	0.81-1.14	0.657
LA diameter z score	0.99	0.09	0.82-1.19	0.893
MLVWT	1.00	0.07	0.88-1.15	0.944
MLVWT z score	1.01	0.03	0.95-1.08	0.783
LVOT gradient	1.02	0.01	1-1.04	**0.031**
RVOT gradient	1.02	0.02	0.99-1.06	0.186
Ejection fraction	1.03	0.09	0.86-1.24	0.726
average E/E'	5.09E-16	3.45E-08	-	1.000
RVH	0.43	0.37	0.08-2.36	0.332
Mid cavity obstruction	0.70	0.54	0.16-3.16	0.647

NS: Noonan syndrome, NSML: Noonan syndrome with multiple lentigines, CS: Costello syndrome, CFCS: cardiofaciocutaneous syndrome, CHD: congenital heart defects, PMHx: past medical history, CCF: congestive cardiac failure, NSVT: non-sustained ventricular tachycardia, LVEDD: left ventricular end diastolic diameter, MLVWT: maximal wall thickness, LAd: left atrial diameter, LVOT: left ventricular outflow tract, SAM: systolic anterior motion of the mitral valve, RVH: right ventricular hypertrophy, RVOT: right ventricular outflow tract

3.5 Discussion

This cohort study from the UK and Ireland represents the largest analysis of the natural history of RAS-HCM. Key findings include the identification of phenotypic variations based on the specific Rasopathy syndrome, the recognition of a distinct group of patients with Noonan-like syndrome exhibiting a unique cardiac phenotype and poorer survival outcomes, and the identification of potential predictors for all-cause mortality and SCD or equivalent events.

3.5.1 Presentation and cardiac phenotype

Large registry-based studies of paediatric hypertrophic cardiomyopathy (HCM) have offered important insights into the long-term prognosis of patients with both sarcomeric and non-syndromic HCM[12,17,18,32], but the data are more limited for non-sarcomeric aetiologies. In keeping with previous reports[34,146,192], this study supports the finding that the onset of HCM in individuals with Rasopathy-related HCM typically occurs in infancy, at a significantly younger age compared to sarcomeric HCM. It also emphasizes the importance of recognizing additional cardiac "red flags" that should prompt consideration of a Rasopathy syndrome as the underlying cause of HCM in young children. These include the presence of coexisting CHD, concomitant RVH, RVOTO, and extreme QRS axis deviation, in line with previous studies[34,42,146,193,202] and as suggested by the recently published ESC guidelines for the management of cardiomyopathies[40]. Although patients with Rasopathy syndromes most commonly do not have a family history HCM[123,212], familial HCM was observed in a notable proportion of patients in our cohort, underscoring the importance of obtaining a comprehensive family history and conducting a thorough examination, even in children diagnosed with syndromic disease.

3.5.2 Correlation of clinical syndrome and genotype with cardiac phenotype

A major strength of this chapter is the high rate of genetic testing and the resulting diagnostic yield, which enabled exploration of genotype-phenotype correlations. The proportion of patients undergoing genetic testing, as well as the yield of those tests, increased significantly over time, reflecting advances in genetic knowledge and evolving clinical practices. As a result, it is possible that more nuanced genotype-phenotype associations exist than those we were able to demonstrate. Patients with variants in PTPN11

and RIT1 were diagnosed with HCM at an earlier age, which may be related to the higher prevalence of CHD in these genotypes. The suspicion of CHD likely led to earlier investigations and an earlier diagnosis of HCM via echocardiography. Although the cardiac phenotype was largely similar across the different clinical syndromes, patients with NSML exhibited the most severe LVH and the highest resting LVOT gradients. In contrast, patients with CS and CFCS had lower maximal LVWT and were less likely to have resting LVOTO. Similarly, patients with variants in PTPN11 and RAF1 had higher MLVWT, higher resting LVOT gradients, and a greater likelihood of mid-cavity obstruction, while those with HRAS variants had less LVH and a lower prevalence of resting LVOTO. This is in keeping with previous studies that have shown particularly severe cardiac phenotypes in children with NSML[224], and has implications for consideration of novel treatments such as MEK inhibitors, which have shown some promise in the treatment of severe HCM in infants with NS and NSML[205,225], as recognised by recent guidance[40,226].

A novel finding in this chapter is the identification of a distinct group of patients diagnosed with Noonan-like syndrome. Of these patients, 50% had a variant in a Rasopathy gene, which was either a variant of uncertain significance (VUS) or did not align with the clinical characteristics described in the literature. The clinical features of these patients did not fit neatly into any of the established Rasopathy syndrome categories. While their demographics and baseline clinical characteristics were similar to those of patients with other Rasopathy syndromes, they exhibited a significantly higher prevalence of extra-cardiac manifestations. The cardiac phenotype was less severe compared to other Rasopathy syndromes, with less pronounced LVH and no evidence of resting LVOTO. However, the mortality rate was high, with a 5-year survival rate of less than 60%. Although these findings should be interpreted cautiously due to the small sample size and the fact that the cause of death was unknown in 4 out of 5 patients (with the remaining death being non-cardiac), the results suggest that it is crucial to recognize this group of patients with seemingly mild HCM who nevertheless have significantly poorer outcomes compared to other Rasopathy syndromes. Given the higher prevalence of extra-cardiac manifestations in this subgroup, it is possible that non-cardiac causes of death may be more prevalent in patients with Noonan-like syndrome.

3.5.3 Survival and predictors of outcome

Survival in patients with Rasopathy-related HCM is highly dependent on age at diagnosis[12,146], a finding confirmed in this study. CCF has been reported as the most common cause of cardiac-related death in RAS-HCM[146,174,212]. This was not confirmed in our study, although it is possible that CCF-related deaths are underestimated as the cause of death was unknown in half of our cohort. In keeping with previous studies[43,146], CHD, history of CCF prior to baseline presentation and CCF requiring admission to hospital were predictors of all-cause mortality on univariate analysis in our cohort. Symptoms at baseline, NSVT and MLVWT have all been shown to be predictors of mortality in large registry studies for hypertrophic cardiomyopathy in children[17,52,227] and are now correlated with Rasopathy-associated hypertrophic cardiomyopathy specifically. Importantly, we have demonstrated for the first time that the underlying Rasopathy syndrome may be an additional potential risk factor for mortality, likely influenced by the cohort of patients with Noonan-like syndrome. These findings emphasize the importance of the underlying diagnosis in the clinical management of patients with Rasopathy. Further large international studies are needed to increase the event numbers and enable a deeper exploration of independent predictors of all-cause mortality in this population.

3.5.4 Arrhythmic events in RAS-HCM

Arrhythmic adverse events are rarely reported in patients with RAS-HCM, with reported frequencies of ventricular arrhythmias of < 2%[43,73,174,228]. The results of our study suggest that this may be a significant underestimate; nearly 5% of our cohort had a VT or VF episode, which is more in line with a recent, large (n=188), international, multicentre study[203]. These findings underscore the importance of considering the risk of ventricular arrhythmias and sudden death in individuals with Rasopathy syndromes. Currently, there are no established guidelines for assessing ventricular arrhythmia risk in patients with Rasopathy syndromes, and it remains unclear whether risk stratification algorithms used for non-syndromic HCM[14,119] are also applicable to this patient group. However, the finding in our study that potential predictors for SCD or equivalent events exist suggests that specific risk factors for ventricular arrhythmias may be present in patients with Rasopathy syndromes. Notably, one of the predictors identified in the univariate analysis, the LVOT gradient, is potentially modifiable. This finding could have implications for the treatment of

obstructive HCM in this population, even in the absence of symptoms. Future studies aimed at identifying Rasopathy-specific risk factors for ventricular arrhythmia will be crucial to address this unmet clinical need.

3.5.5 Limitations

This study is limited by the inherent challenges of retrospective research, particularly missing or incomplete data. Variations in clinical assessment and patient management were inevitable, as patients were recruited from multiple centres and across different time periods. Genetic testing was conducted at the discretion of the participating clinicians, and although a high proportion of patients with a Rasopathy syndrome had a disease-causing variant identified, it is unclear whether the genetic testing results influenced the final diagnosis or confirmed prior clinical suspicions. The exact number of patients who underwent additional genetic testing with a cardiomyopathy panel is unavailable due to the retrospective design, meaning the prevalence of a co-existing sarcomeric variant in this cohort could not be determined. Variations in echocardiographic protocols and the availability of images for retrospective assessment across centres and time periods led to missing variables. The use of a strict cut-off value of E/E' >14 to define diastolic dysfunction may have resulted in the exclusion of patients with suspected elevated filling pressures who had E/E' values between 10 and 14. Although the mortality rate is unlikely to have been significantly affected by these missing data, other phenotypic features or outcomes may have been either underestimated or overestimated. The cause of death was not documented in a substantial proportion of cases, complicating conclusions on this topic. Mortality and SCD or equivalent events were rare, so a multivariate analysis could not be performed. Data collection relied on patients being referred to collaborating paediatric cardiology centres, which may have led to the exclusion of patients with either a very mild phenotype (not requiring referral to an expert centre) or a very severe phenotype (resulting in early death in a neonatal or paediatric unit).

3.6 Conclusions

To my knowledge, this is the largest cohort of RAS-HCM encompassing various Rasopathy syndromes and genes. The findings reveal a heterogeneous clinical presentation, with different phenotypes and outcomes depending on the underlying syndrome. This was particularly evident in a distinct group of patients with Noonan-like syndrome, who

exhibited a milder HCM phenotype but had significantly worse survival. Potential predictors of all-cause mortality and SCD or equivalent events have been identified for this population, but larger studies are needed to further investigate their significance.

Chapter 4 - Resting & ambulatory electrocardiography in Rasopathy-associated hypertrophic cardiomyopathy

4.1 Introduction

The 12-lead ECG is a simple and non-invasive diagnostic tool, widely available even in low resource settings, making it an effective screening tool for a wide range of cardiac conditions. In patients with HCM[40,229], ECG abnormalities can precede the development of LVH by many years[65] and a normal ECG is usually only observed in fewer than 3% of paediatric HCM cases[66]. Although typical ECG features in RAS-HCM are recognised in clinical practice, these have not been previously systematically evaluated and their role in predicting cardiovascular outcomes in this population is unknown.

Patients with HCM are known to be more prone to arrhythmic events, both supraventricular and ventricular in origin. Supraventricular ectopy (SVE) can be commonly attributed to elevated filling pressures, leading to left atrial stretch, enlargement and fibrosis[230], but primary atrial myocardial abnormalities remain a possibility[44]. This in turn creates a predisposition to premature atrial contractions or atrial fibrillation[231]. In certain underlying aetiologies, such as due to PRKAG2 variants or LAMP2 deficiency, there are accessory pathways leading to a predisposition SVEs[68,232]. Regarding ventricular arrhythmias, which are more common than isolated SVEs in adult and paediatric patients with HCM, this can be due to myocardial fibrosis or subendocardial ischaemia[233-238]. NSVT specifically is an established risk factor for SCD in patients with non-syndromic HCM, particularly in young individuals[73,239,240]. For these reasons, regular cardiac ambulatory monitoring is performed as standard of care both in adult and paediatric patients with HCM[40,41].

There is little known about the prevalence of ectopy in patients with RAS-HCM, except for case reports[200,201] and a small sub-cohort in a larger study[43], and performing cardiac ambulatory monitoring is extrapolated from standardised practices in patients with non-syndromic HCM.

4.2 Aim

The aims of this chapter are to characterise the 12-lead resting and ambulatory ECG monitoring and to explore potential resting ECG predictors of adverse outcomes in children with RAS-HCM.

4.3 Methods

4.3.1 Population

This was a single centre (Great Ormond Street Hospital, London, United Kingdom), retrospective cohort study. Patients <18 years old with a clinical and/or genetic diagnosis of a Rasopathy syndrome and HCM were included. Exclusion criteria were the absence of a baseline ECG within a year of the first date of assessment or a poor quality ECG precluding analysis. 12-lead ECGs from a separate cohort of patients (<18 years old) with a diagnosis of HCM secondary to a pathogenic or likely pathogenic sarcomere protein gene variant (s-HCM) were used as a comparison group.

4.3.2 Resting ECG analysis and statistics

A detailed list of parameters assessed can be found in Chapter 2. Systematic ECG analysis was carried out by 2 investigators (see acknowledgements for details) using normal paediatric reference values for age[221,241,242]. After the initial ECG review, 10% of ECGs were subjected to blinded analysis by the original investigator while a separate 10% underwent blinded analysis repeated by the other investigator. To assess intra and inter observer variability in the estimation of ECG features, the differences between the measurements (mean±SD) and the Pearson correlation coefficient were calculated.

4.3.3 Ambulatory ECG analysis and statistics

Data from cardiac ambulatory monitoring were systematically collected from reports available on electronic patient records, interpreted by trained paediatric cardiac electrophysiologists. Data included length of monitoring, presence of supraventricular tachycardia (SVT), with maximal length and rate in beats and beats per minute (bpm) respectively, supraventricular ectopics (SVEs), their frequency expressed in %, episodes of VT, NSVT (3 or more consecutive ventricular beats) and ventricular ectopics (VEs) with their frequency expressed in %.

SVEs were deemed insignificant if <1%, frequent if 1-5% and significant if >5%. VEs were deemed infrequent if <30/hr and frequent if >30/hr. For analysis purposes, SVT and SVEs were grouped together as atrial arrhythmia events and VT, NSVT and VEs as ventricular arrhythmia events.

4.4 Results

4.4.1 Patient Demographics

Eighty-four patients (Figure 4-1) with RAS-HCM were included in the study and compared with 113 patients with s-HCM. The most common Rasopathy diagnosis was Noonan syndrome (NS) (N=59, 70.2%). Pathogenic/likely pathogenic variants were most commonly found in *PTPN11* (N=25, 29.8%), followed by *RAF1* (11, 13.1%) (Table 4-1). In patients with s-HCM, the two most commonly implicated genes were *MYH7* in 53 (46.9%) and *MYBPC3* in 40 (35.4%) patients (Table 4-2).

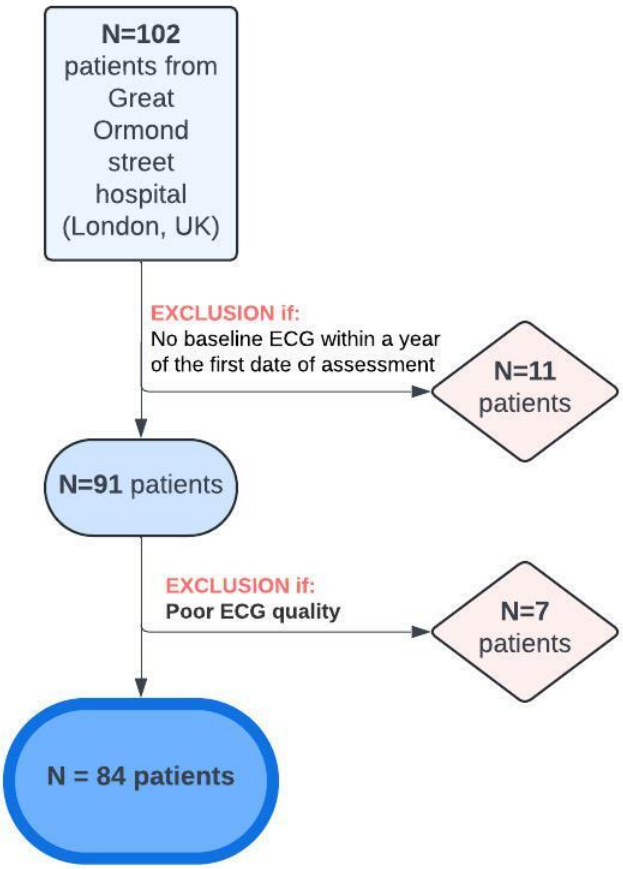


Figure 4-1: Flowchart for ECG patient cohort

Table 4-1: Distribution of Rasopathy syndrome with corresponding genotype

		NS N=59	NSML N=8	CS N=5	CFCS N=3	NLS N=9
Unknown	N=27	20	2	0	1	4
PTPN11	N=25	19	6	0	0	0
RAF1	N=11	11	0	0	0	0
RIT1	N=5	5	0	0	0	0
HRAS	N=7	0	0	5	0	2
BRAF	N=2	0	0	0	2	0
LZTR1	N=2	2	0	0	0	0
SHOC2	N=8	0	0	0	0	2
KRAS	N=3	2	0	0	0	1

NS: Noonan syndrome; NSML: Noonan syndrome with multiple lentigines; CS: Costello syndrome; CFCS: cardio-facio-cutaneous syndrome; NLS: Noonan-like syndrome

Table 4-2: Genotype of patients with non-syndromic hypertrophic cardiomyopathy

Gene	
MYBPC3	40 (35.4)
MYH7	53 (46.9)
MYL2	1 (0.9)
TNNT2	3 (2.7)
TPM1	4 (3.5)
ACTN	1 (0.9)
Troponin T	1 (0.9)
TNNI3	1 (0.9)
JPH2	2 (1.77)
MYL3	3 (2.7)

Patients with RAS-HCM had an overall younger median age at baseline assessment [1.0 years (0-3.5) vs 9.0 (3-13), p <0.001], and more commonly had concomitant cardiovascular abnormalities [N=43 (51.2%) vs N=16 (14.2%), p<0.001], of which 30 (35.7%) had pulmonary valve stenosis (PVS) as a sole defect or in combination with other defects (Table 4-3).

Table 4-3: Concomitant congenital heart defects

	RAS-HCM N=84	**nS-HCM N=113**
Valvulopathy	35 (41.7%)	5 (4.4%)
Atrial septal defect	11 (13.1%)	1 (0.9%)
Ventricular septal defect	5 (6.0%)	8 (7.1%)
Patent ductus arteriosus	6 (7.1%)	4 (3.5%)
Patent foramen ovale	6 (7.1%)	1 (0.9%)
Coarctation of aorta	2 (2.4%)	-
Hypoplastic pulmonary arteries	2 (2.4%)	-

RAS-HCM: Rasopathy associated hypertrophic cardiomyopathy; nS-HCM: non-syndromic HCM

The two groups had comparable MLVWT z-scores on echocardiogram, but patients with RAS-HCM had a larger left atrial diameter (LAd) [LAd zscore 17.4 (9.4) vs +2.8 (2.8), p <0.001] and a higher proportion of concomitant RVH [N=31 (50.0%) vs N=20 (26.9%), p<0.001]. Detailed comparison of the baseline demographics, clinical and echocardiographic characteristics of the two groups are shown in Table 4-4.

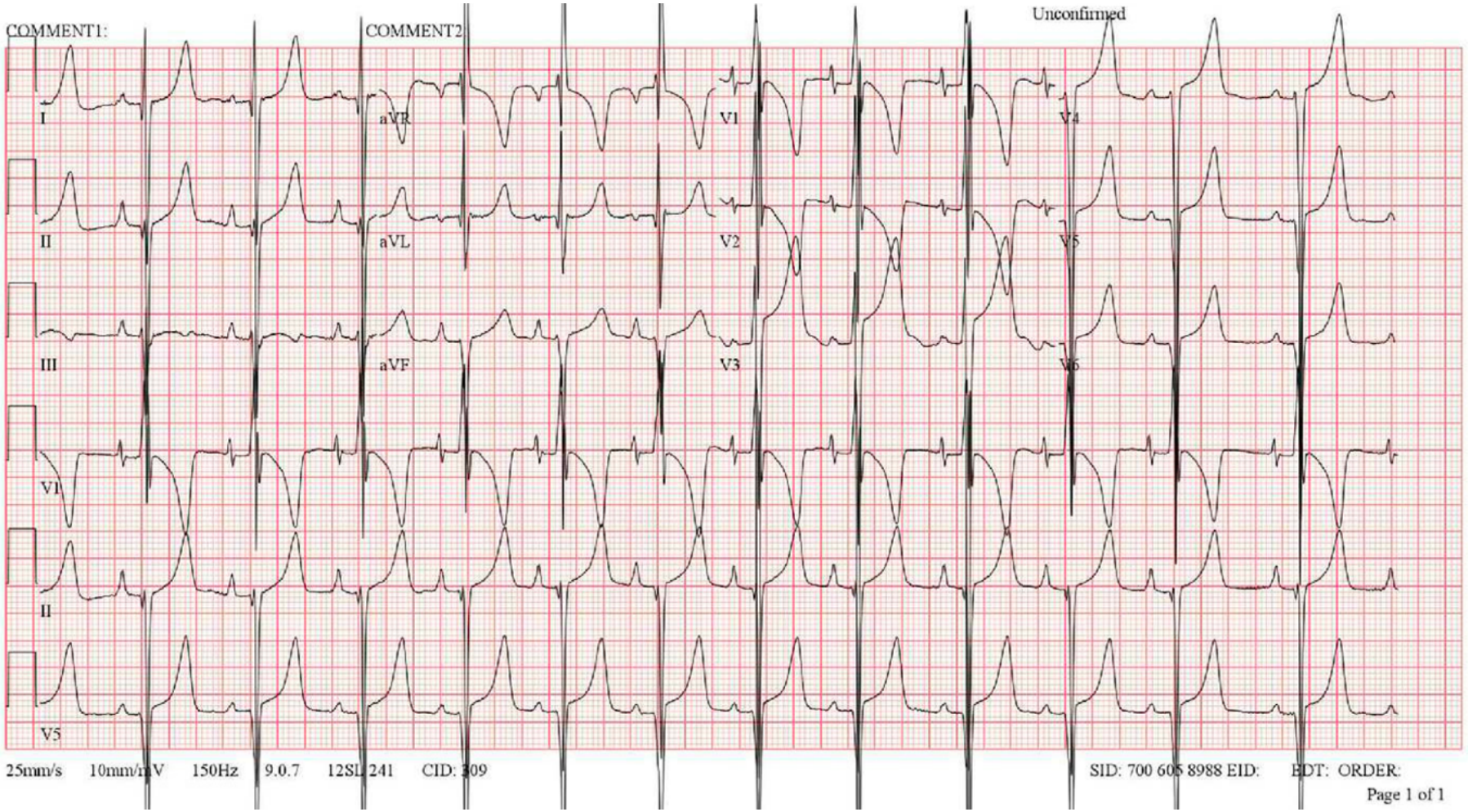
Table 4-4: Baseline demographics, clinical and echocardiographic characteristics for resting ECG cohort

	Total	s-HCM	RAS-HCM	p-value
	N=197	**N=113**	**N=84**	
Male	128 (65.0%)	73 (64.6%)	55 (65.5%)	0.90
FHx of HCM	56 (28.4%)	50 (44.2%)	6 (7.1%)	**<0.001**
Concomitant CHD	59 (29.9%)	16 (14.2%)	43 (51.2%)	**<0.001**
Age (years)	5.0 (1.0-11.0)	9.0 (3.0-13.0)	1.0 (0.0-3.5)	**<0.001**
Medication	91 (46.7%)	45 (39.8%)	46 (56.1%)	**0.025**
MLVWT (mm)	12.0 (8.0-18.0)	14.0 (9.0-20.3)	10.0 (7.0-15.0)	**<0.001**
MLVWT z score	10.7 (8.4)	10.3 (7.2)	11.6 (10.8)	0.430
LAD (mm)	25.5 (18.2-30.0)	24.2 (15.8-30.0)	27.0 (19.6-32.7)	0.360
LAD zscore	5.9 (8.1)	2.8 (3.8)	17.4 (9.4)	**<0.001**
LVOTO	59 (36.2%)	19 (17.6%)	40 (72.7%)	**<0.001**
RVH	51 (65.0%)	20 (26.9%)	31 (50.0%)	**<0.001**

s-HCM: sarcomeric hypertrophic cardiomyopathy; RAS-HCM: RAS-HCM; FHx: family history; CHD: congenital heart defects; MLVWT: maximal left ventricular wall thickness; LAD: left atrial diameter; LVOTO: left ventricular outflow tract obstruction; RVH: right ventricular hypertrophy.

4.4.2 Resting ECG Features in RAS-HCM

At baseline, the ECG of patients with RAS-HCM demonstrated a significantly higher proportion of axis deviation [N=79 (65.5%) vs N=29 (35.4%), p-value <0.001] compared to s-HCM, specifically superior axis deviation [N=25 (29.8%) vs N=3 (2.5%), p-value <0.001]. Voltage criteria for RVH were more commonly present in the ECG of patients with RAS-HCM [N=44 (52.4%) vs N=32 (28.3%), p-value <0.001], with 28 out of 30 patients (93.3%) with PVS fulfilling voltage criteria for RVH. Voltage criteria for RVH on ECG did not correlate with echocardiographic presence of RVH (p=0.596), but showed a correlation with the presence of concomitant PVS (p<0.001). Patients with s-HCM had a significantly higher prevalence of pathological Q waves [N=23 (27.4%) vs N=54 (47.8%), p-value <0.001] (Table 4-5). No significant differences in ECG features were found between underlying Rasopathy syndrome type or genotype (Table 4-6 and Table 4-7). An example ECG of a patient with RAS-HCM can be seen in Figure 4-2.



<u>Figure 4-2:</u> Example ECG of 9-year old male patient with RAS-HCM secondary to a RAF1 variant, showing superior QRS axis, evidence of right atrial enlargement, gross criteria for biventricular hypertrophy, pathological Q waves in the lateral leads ST elevation in the septal leads and giant T waves throughout.

Table 4-5: Resting ECG characteristics in RAS-HCM vs s-HCM

	Total	s-HCM	RAS-HCM	p-value
	N=197	**N=113**	**N=84**	
Abnormal axis	102 (51.8%)	73 (64.6%)	29 (34.5%)	**<0.001**
Type of axis deviation				**<0.001**
RAD	25 (12.7%)	15 (13.3%)	10 (11.9%)	
LAD	43 (21.8%)	24 (21.2%)	19 (22.6%)	
Superior Axis	28 (14.2%)	3 (2.7%)	25 (29.8%)	
Evidence of atrial enlargement				**0.006**
RAE	26 (13.2%)	7 (6.2%)	19 (22.6%)	
LAE	20 (10.2%)	13 (11.5%)	7 (8.3%)	
Bi-AE	9 (4.6%)	7 (6.2%)	2 (2.4%)	
Pathological Q waves	77 (39.1%)	54 (47.8%)	23 (27.4%)	**<0.001**
Voltage criteria LVH	108 (54.8%)	68 (60.2%)	40 (47.6%)	0.080
Voltage criteria RVH	76 (38.6%)	32 (28.3%)	44 (52.4%)	**<0.001**
Conduction delay	84 (42.6%)	46 (40.7%)	38 (45.2%)	0.52
RBBB	10 (5.1%)	8 (7.1%)	2 (2.4%)	
LBBB	8 (4.1%)	4 (3.5%)	4 (4.8%)	
ST changes >2mm	62 (31.5%)	41 (36.3%)	21 (25.0%)	0.092
TWI	85 (43.1%)	55 (48.7%)	30 (35.7%)	0.069
Giant T waves (>10 mm)	43 (21.8%)	26 (23.0%)	17 (20.2%)	0.64
Mean QTc (msec)	441.0 (32.9)	449.0 (31.3)	439.0 (35.3)	0.072
QT prolongation	29 (23.8%)	14 (23.3%)	25 (24.0%)	0.919
U waves	26 (13.2%)	12 (10.6%)	14 (16.7%)	0.21

s-HCM: sarcomeric hypertrophic cardiomyopathy; RAS-HCM: RAS-HCM; RAD: right axis deviation; LAD: left AD; RAE: right atrial enlargement; LAE: left AE; LVH: left ventricular hypertrophy; RVH; right ventricular hypertrophy; RBBB: right bundle branch block; LBBB: left BBB; TWI: T wave inversion.

Table 4-6: Resting ECG characteristics in RAS-HCM by underlying syndrome

	NS N=59	NSML N=8	CS N=5	p-value
Abnormal axis	39 (66.1%)	7 (87.5%)	2 (40.0%)	0.2114
Superior Axis	25 (42.4%)	4 (50%)	-	0.1682
Evidence of atrial enlargement	19 (32.2%)	3 (37.5%)	-	0.1018
Pathological Q waves	15 (25.4%)	2 (25.0%)	1 (20.0%)	
Voltage criteria LVH	30 (50.8%)	4 (50.0%)	1 (20.0%)	0.4252
Voltage criteria RVH	30 (50.8%)	5 (62.5%)	1 (20.0%)	0.3235
Conduction delay	25 (42.4%)	5 (62.5%)	3 (60.0%)	0.4642
RBBB	2 (3.4%)	-	-	
LBBB	4 (6.7%)	-	-	
ST changes >2mm	15 (25.4%)	-	2 (40.0%)	
TWI	21 (35.6%)	2 (25.0%)	3 (60.0%)	
Giant T waves (>10 mm)	13 (22.0%)	1 (12.5%)	-	
U waves	10 (5.9%)	1 (12.5%)	3 (60.0%)	

RAS-HCM: RAS-HCM; NS: Noonan syndrome; NSML: Noonan syndrome with multiple lentigines; CS: Costello syndrome; LVH: left ventricular hypertrophy; RVH; right ventricular hypertrophy; RBBB: right bundle branch block; LBBB: left BBB; TWI: T wave inversion.

Table 4-7: Resting ECG characteristics in RAS-HCM by underlying gene

	PTPN11 N=25	RAF1 N=11	RIT1 N=5	HRAS N=5	p-value
Abnormal axis	16 (64.0%)	8 (72.7%)	4 (80.0%)	3 (42.9%)	0.5608
Superior Axis					0.1653
Evidence of atrial enlargement	7 (28.0%)	5 (45.5%)	3 (60.0%)	1 (14.3%)	0.2263
Pathological Q waves	7 (28.0%)	4 (36.4%)	-	1 (14.3%)	0.7923
Voltage criteria LVH	12 (48.0%)	5 (45.5%)	2 (40.0%)	1 (14.3%)	0.7324
Voltage criteria RVH	12 (48.0%)	7 (63.6%)	3 (60.0%)	2 (28.6%)	0.4359
Conduction delay	15 (60.0%)	4 (36.4%)	3 (60.0%)	4 (57.1%)	0.6198
RBBB	1 (4.0%)	1 (9.1%)	-	-	0.8042
LBBB	3 (12.0%)	-	-	1 (14.3%)	
ST changes >2mm	8 (32.0%)	2 (18.2%)	-	3 (42.9%)	
TWI	9 (36.0%)	4 (36.4%)	2 (40.0%)	3 (42.9%)	0.8049
Giant T waves (>10 mm)	7 (28.0%)	2 (18.2%)	1 (20.0%)	-	
U waves	7 (28.0%)	1 (9.1%)	-	3 (42.9%)	

RAS-HCM: RAS-HCM; LVH: left ventricular hypertrophy; RVH; right ventricular hypertrophy; RBBB: right bundle branch block; LBBB: left BBB; TWI: T wave inversion.

4.4.3 Ambulatory ECG monitoring

A total of 64 cardiac ambulatory monitoring data from 42 patients with RAS-HCM was collected. Of those, 25 were repeat monitors. The median age at cardiac monitoring was 6 years (3-13). Eighteen patients had a variant in *PTPN11*, 13 in *RAF1*, 6 in *RIT1* and 5 in HRAS. Thirty-two patients (50%) in total were on b-blockers, with a higher proportion in the patients with a *RAF1* gene variant (N=11, 84.6%, p=0.041). A total of 3 patients (4.7%) had significant atrial arrhythmic events, while 17 patients (26.6%) had significant ventricular arrhythmic events. All patients that had significant ventricular arrhythmic events were on b-blockers. None of the patients in the cohort had an ICD in situ. There were no significant differences between underlying genetic variant. Results are detailed in Table 4-8.

Table 4-8: Arrhythmia in children with RAS-HCM on cardiac monitoring by underlying genetic variant

	Total (n=64)	PTPN11 (n=18)	RAF1 (n=13)	RIT1 (n=6)	HRAS (n=5)	p-value
b-blockers	32 (50)	9 (50)	11 (84.6)	4 (66.7)	1 (20)	0.041
SVT	2 (3.1)	0	1 (7.7)	0	1 (20)	0.309
NSVT	3 (4.7)	0	2 (15.4)	0	0	0.316
VT	0 (0)	0	0	0	0	
SVEs						0.879
<1%	14 (21.9)	4 (22.2)	2 (15.4)	2 (33.3)	0	
1-5%	2 (3.1)	1 (5.6)	1 (7.7)	0	0	
>5%	1 (1.6)	5 (27.8)	1 (7.7)	0	0	
VEs						0.107
<30/hr	34 (53.1)	12 (66.8)	6 (46.2)	2 (33.3)	1 (20)	
>30/hr	7 (10.9)	0	3 (23.1)	2 (33.3)	0	
Couplets	7 (10.9)	1 (5.6)	4 (30.8)	1 (16.7)	0	0.476
Atrial arrhythmia	3 (4.7)	2 (11.1)	1 (7.7)	0	0	0.959
Ventricular arrhythmia	17 (26.6)	5 (27.8)	5 (38.5)	2 (33.3)	1 (20)	0.954

4.4.4 Correlation of resting ECG with MACE in RAS-HCM

Over a median follow up period of 6.8 years (3.1-9.7), a total of 17 patients (20.2%) died of any cause in the RAS-HCM group (Table 4-9), of whom 5 (5.9%) died of cardiac causes (2 CHF-related deaths, 2 SCDs, 1 other cardiovascular-related death). There were a total of 19 (22.6%) MACE (7 CHF admissions, 5 cardiac-related deaths, 3 aborted cardiac arrests, 3 sustained VT, 1 appropriate ICD therapy).

Table 4-9: Outcomes

	Total N=197	s-HCM N=113	RAS-HCM N=84	p-value
Follow up (years)	6.4 (3.2-10.2)	6.1 (3.3-10.2)	6.8 (3.1-9.7)	0.87
LV myectomy	18 (9.1%)	9 (8.0%)	9 (10.7%)	0.51
Heart Transplant	6 (3.0%)	3 (2.7%)	3 (3.6%)	0.71
Atrial Arrhythmia	13 (6.6%)	7 (6.2%)	6 (7.1%)	0.79
Ventricular arrhythmia	36 (18.3%)	27 (23.9%)	9 (10.7%)	**0.018**
Cardiac arrest	17 (8.6%)	11 (9.7%)	6 (7.1%)	0.52
CHF Admission	10 (5.1%)	3 (2.7%)	7 (8.3%)	0.073
ICD implantation	60 (30.5%)	56 (49.6%)	4 (4.8%)	**<0.001**
Appropriate ICD Therapy	23 (11.7%)	22 (19.5%)	1 (1.2%)	
Death	20 (10.2%)	3 (2.7%)	17 (20.2%)	**<0.001**
MACE	53 (26.9%)	34 (30.1%)	19 (22.6%)	0.24

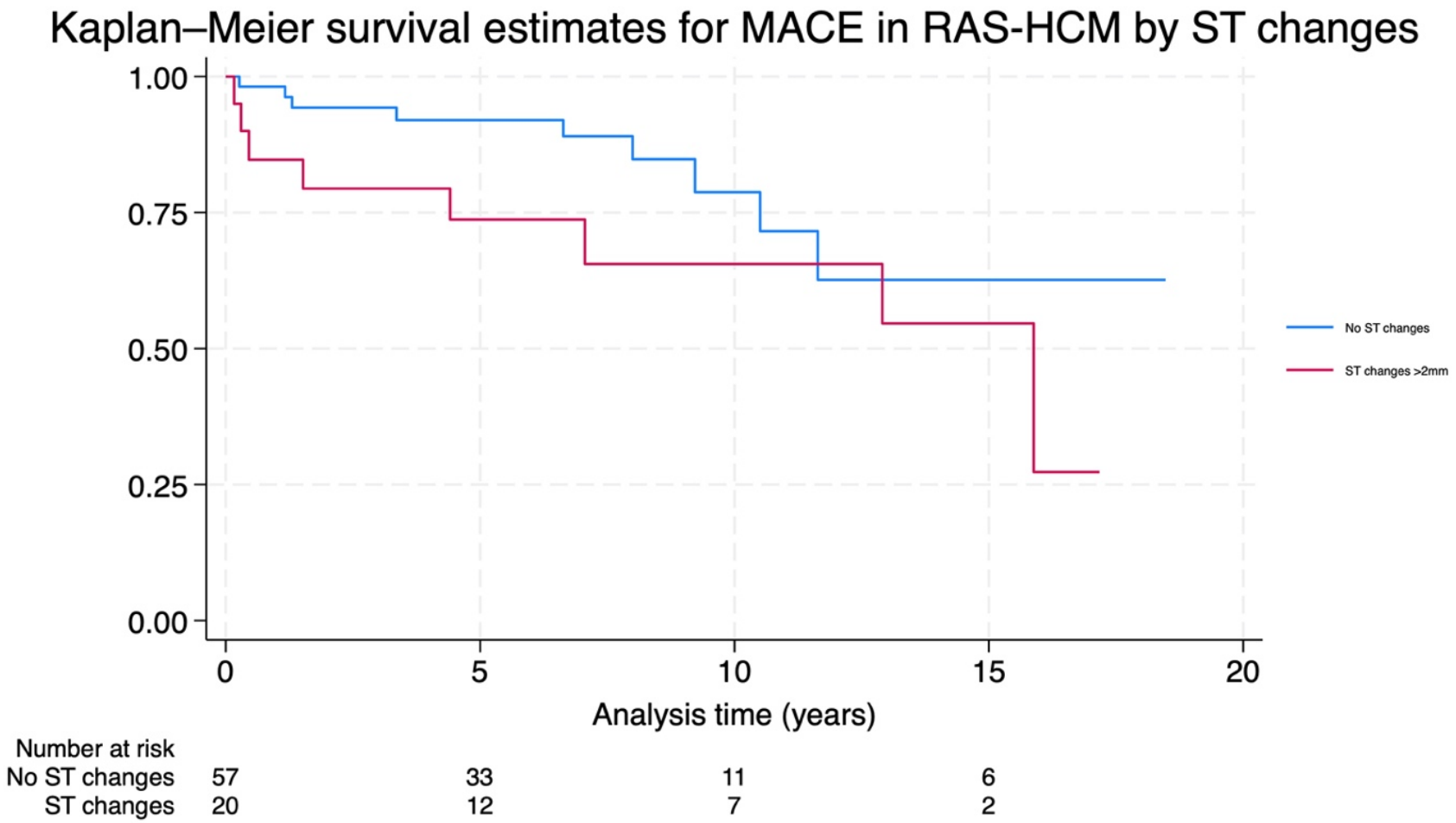
s-HCM: sarcomeric hypertrophic cardiomyopathy; RAS-HCM: RAS-HCM; LV: left ventricular; CHF: congestive heart failure; ICD: implantable cardioverter defibrillator; MACE: major adverse cardiac events.

On univariate analysis, right atrial enlargement and ST segment changes >2mm correlated significantly with MACE. After adjustment in a multivariate model, only ST segment changes >2mm remained significant (OR 3.97, 95% CI 1.33-11.92, p=0.014; adjusted OR 2.54, p-value=0.007) (Table 4-10, Figure 4-3).

Table 4-10: Logistic regression for ECG characteristics in RAS-HCM (N=84) and MACE (N=19)

					Adjusted			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**
Axis deviation	0.43	0.13	1.43	0.168				
RAD	4.00	0.82	19.42	0.086				
LAD	1.07	0.24	4.66	0.932				
Superior	0.80	0.25	2.54	0.709				
RAE	4.36	1.34	14.18	**0.014**	1.47	0.58	3.74	0.414
LAE	4.50	0.84	23.99	0.078				
Bi-AE	1.00	-	-	-				
Q waves present	2.53	0.30	21.59	0.397				
Voltage criteria LVH	1.71	0.61	4.80	0.311				
Voltage criteria RVH	1.77	0.62	5.06	0.288				
Conduction delay	1.47	0.53	4.09	0.463				
RBBB	3.56	0.21	59.69	0.378				
LBBB	1.15	0.11	11.72	0.907				
ST changes >2mm	3.97	1.33	11.91	**0.014**	2.54	1.29	5.02	**0.007**
TWI	0.57	0.18	1.78	0.335				
Giant T waves (>10mm)	2.27	0.71	7.26	0.169				
QTc (msec)	0.99	0.98	1.02	0.716				
QTc prolongation	6.29	0.75	52.68	0.090				
U waves	0.92	0.23	3.71	0.907				

RAS-HCM: Rasopathy-associated hypertrophic cardiomyopathy; MACE: major adverse cardiac events; OR: odds ratio; CI: confidence intervals; RAD: right axis deviation; LAD: left AD; RAE: right atrial enlargement; LAE: left AE; LVH: left ventricular hypertrophy; RVH; right ventricular hypertrophy; RBBB: right bundle branch block; LBBB: left BBB; TWI: T wave inversion.



<u>Figure 4-3:</u> Kaplan-Meier survival curve for major arrhythmic cardiac events (MACE) in Rasopathy-associated hypertrophic cardiomyopathy (RAS-HCM), grouped by the presence of ST segment changes >2mm on ECG. Follow up time in years.

4.5 Discussion

This study shows that children with RAS-HCM can have distinct ECG features, including superior axis deviation, evidence of atrial enlargement and voltage criteria for RVH. While there were no significant associations with underlying syndrome and genotype, ST segment changes on baseline ECG emerged as an independent predictor for MACE.

Superior axis deviation has long been considered to be a feature specific to RAS-HCM and is included as a 'red flag' raising suspicion of Rasopathies as the underlying aetiology for paediatric HCM[40,243]; however, this is, to our knowledge, the first study that documents this in comparison to children with s-HCM. Another notable aspect is the high prevalence of RVH voltage criteria, which is supported by data from a recent study[244]. RAS-HCM is known to have higher rates of biventricular involvement[43,193], which could be explained by the higher prevalence of concomitant congenital heart defects in RAS-HCM, specifically right heart lesions such as pulmonary valve stenosis.

No significant association was found between ECG phenotype and underlying Rasopathy syndrome or genotype, but there were several ECG features emerging as more common in certain syndromes or genotypes, such as T-wave abnormalities and U-wave more often found in Costello syndrome; interventricular conduction and repolarization abnormalities more often observed in HRAS variant. These results were not statistically significant but represent a trend. Taking into account the smaller representation of other clinical syndromes apart from Noonan syndrome, these should be repeated in a larger cohort to explore their significance.

There was a significant association between the presence of ST segment changes >2mm with MACE in RAS-HCM. Microvascular ischaemia has been associated with MACE in adults with HCM[245,246], but data in paediatric HCM populations, and in particular RAS-HCM, are lacking. The mechanisms of coronary ischaemia thought to play a part in s-HCM are also present in RAS-HCM, including microstructural abnormalities such as impaired coronary blood flow due to small vessel disease[247] and microvascular dysfunction[245], haemodynamic alterations related to LVOTO [82,179,243,248], impaired diastolic function[243,249], myocardial hypercontractility[96], and increased oxygen demand creating an energy mismatch[250,251]. In addition, patients with Rasopathy syndromes commonly have concomitant congenital heart defects[174,179,243], which may place an additional ischaemic burden on the myocardium, and additional contributing mechanisms such as coronary artery ectasia[252,253] may also play a role.

The assessment of microvascular disease in childhood HCM is challenging, due to the patchy nature of the disease and technical difficulties related to heart rate, but the finding that ST changes on the 12-lead ECG are associated with adverse outcomes suggests that further efforts to evaluate this are warranted.

In this chapter is described, for the first time systematically, the arrhythmic burden, as evidenced by ambulatory cardiac monitoring, in patients with RAS-HCM. They appear to have frequent ventricular ectopy, mostly in the form of frequent isolated ventricular ectopic beats, despite medical therapy with b-blockers.

While the presence of NSVT is an established risk factor for SCD in paediatric and adult patients with non-syndromic HCM[73,239,240], this has only been identified, as detailed in chapters 3 and 4, as a potential risk factor of SCD in children with RAS-HCM. Thus, the presence of VT or NSVT on ambulatory monitoring can lead to interventions like ICD placement[40,41,233], making it an important clinical tool in this population.

Frequent isolated monomorphic ventricular ectopy is known to be more prevalent in HCM[233-238] but the correlation with adverse cardiac outcomes has not been systematically evaluated.

However, ectopic beats, whether supraventricular or ventricular in origin, can cause patient symptoms such as palpitations, dizziness, or syncope, which can significantly impact the quality of a patient's life[254]. As such, cardiac monitoring in patients with RAS-HCM remains a useful tool.

4.5.1 Limitations

This study is limited by the relatively small sample size which means that it may not be powered to detect potentially important differences in the ECG, specifically in exploring genotype-phenotype correlations, where we have observed trends towards certain associations. Recruitment was from a tertiary paediatric cardiology unit, and so the patients may represent the more severe end of the HCM spectrum, contributing to selection bias. Given the retrospective study design, some clinical data may be incomplete, particularly in relation to genetic testing, which varies according to era. MACE is a composite outcome encompassing cardiac mortality, heart failure and SCD equivalent events. Our study, due to the rare nature of the condition and population, did not have enough individual events to study these outcomes in isolation. Reversible causes of arrhythmias such as electrolyte

imbalances were not assessed in this study. In light of our findings highlighting the significance of ventricular arrhythmias in this population, this study should be repeated in a larger cohort, to facilitate comparisons and investigate the potential contribution to adverse cardiac outcomes.

4.6 Conclusions

This study demonstrates distinctive ECG features in children with RAS-HCM, including superior axis deviation and voltage criteria for RVH, which could help distinguish RAS-HCM in clinical practice. An important proportion of children with RAS-HCM have ventricular ectopy, most commonly in the form of frequent isolated ventricular ectopics, which may have an impact on symptoms and quality of life. ST segment changes are an independent predictor of MACE in this population, which could have potential implications for the prediction of adverse outcomes, but larger studies are needed to investigate this further.

Chapter 5 - Sudden cardiac death risk assessment in Rasopathy-associated hypertrophic cardiomyopathy

5.1 Introduction

SCD is the most common cause of death in childhood HCM)after the first year of life[12,17,32], with higher annual rates compared to adults with HCM[20,227]. The identification of children at high risk of SCD, who would benefit most from the implantation of a primary prevention ICD, is a cornerstone of HCM management in childhood. A recently published validated model, HCM Risk-Kids, provides an estimated 5-year risk for SCD in children with HCM based on clinical parameters: MLVWT, LAd, LVOT gradient, unexplained syncope, and NSVT[14,116]. 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[12,17,255]. Although traditionally the risk of SCD in children with RAS-HCM has been considered to be low, recent data suggest a prevalence of SCD of up to 4%[17,255], with a recent, large, international, multicentre study showing comparable rates to children with non-syndromic HCM[203] . Despite this, there are no published risk factors for SCD in this patient cohort.

5.2 Aim

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 RAS-HCM, with a secondary aim to investigate predictors of SCD in this population.

5.3 Methods

Patient selection methods have been previously described. They were consecutively evaluated between January 1, 1985, and December 31, 2020, in 13 paediatric cardiology centres in the United Kingdom (see Table 5-1), Our Lady's Children's Hospital in Dublin, Ireland, and the German Heart Centre in Munich.

Table 5-1: List of collaborating centres with corresponding number of patients contributed

Centre	Number of patients*
Great Ormond Street Hospital, London	98
German Heart Centre, Munich	29
Bristol Royal Hospital for Children	15 (8 & 7)
Birmingham Children's Hospital	10 (7 & 3)
University Hospital of Wales, Cardiff	12 (8 & 4)
Royal Brompton Hospital, London	11 (6 & 5)
Glenfield Hospital, Leicester	8 (3 & 5)
Royal Hospital for Children, Glasgow	8 (2 & 6)
Evelina Children's Hospital, London	6 (2 & 4)
Southampton General Hospital	4 (1 & 3)
Alder Hey, Liverpool	2 (1 & 1)
Freeman's Hospital, Newcastle	2 (2 & 0)
Leeds General Infirmary	2 (2 & 0)
Our Lady's Children's Hospital, Dublin	2 (0 & 2)
John Radcliffe Hospital, Oxford	1 (0 & 1)

**The numbers add up to more than the total number of patients in this study – this is because some patients were seen in the local paediatric cardiology centre as well as Great Ormond Street Hospital as a national reference centre and were not included twice in the study numbers. In the parenthesis there is the breakdown of numbers, first number is patients only seen at the local centre, second number is patients seen in both the local and reference centre*

HCM Risk-Kids[14] 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 ≥ 3 consecutive ventricular beats at a rate of ≥ 120 beats per minute lasting ≤ 30 s on ambulatory ECG monitoring), MLVWT *Z* score[219], LAd *Z* score[220], 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). LVOTO was defined as a peak instantaneous gradient ≥ 30 mmHg[5].

5.3.1 Study endpoints

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[14]. SCD was defined as a witnessed sudden death with or without documented cardiac failure, death within 1 hour of new symptoms, or a nocturnal death with no history of worsening symptoms[5]. Outcomes were determined by the treating cardiologist at each centre without knowledge of the HCM Risk-Kids estimates.

5.3.2 Missing data

Patients with more than three missing values in the predictor variables used in the HCM Risk-Kids model were excluded from the validation cohort. Logistic regression was employed to identify predictors of missingness, and the data were found to be missing at random. To handle the missing data, we used multiple imputation by chained equations, performing 100 imputations for the missing values of baseline variables and clinical parameters. The imputation model included all predictors of missingness, the outcome, all prespecified predictors from the HCM Risk-Kids model, and the estimate of the cumulative hazard function. Each imputation iteration was set to 500. The imputation model incorporated potential predictors of missingness, the outcome, and SCD risk predictor variables. A total of 100 imputed datasets were generated, and estimates from these datasets were combined using Rubin's rule. Trace plots and Kernel density plots for the observed and imputed data are provided in Figure 5-1 and Figure 5-2, respectively.

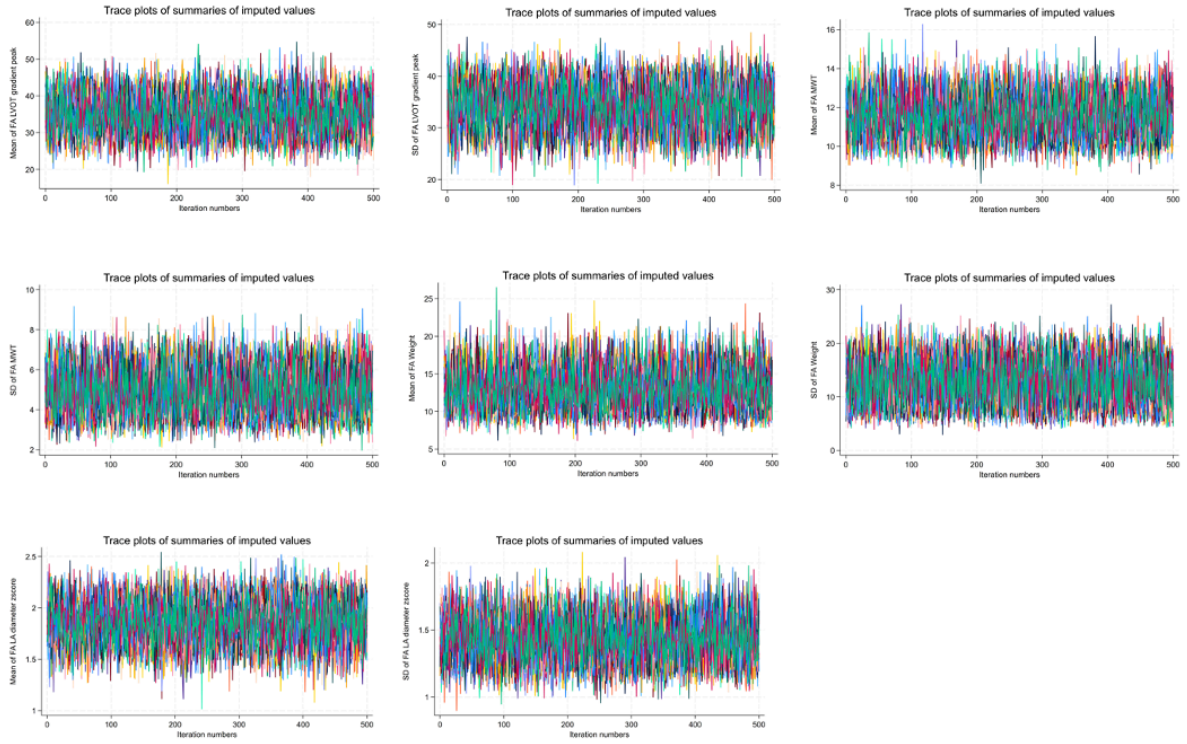


Figure 5-1: Trace plot summaries of imputed values

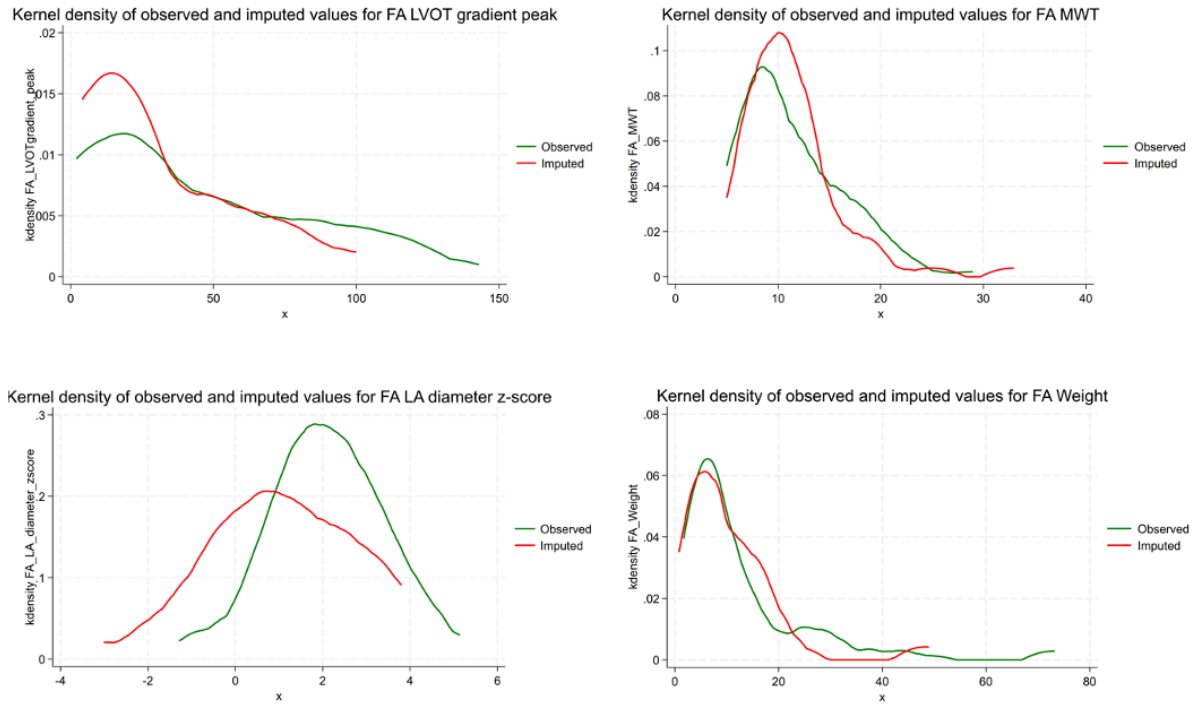


Figure 5-2: Kernel density for observed and imputed values

5.3.3 Validation of HCM Risk-Kids

The estimated 5-year risk of SCD was calculated for each individual patient using the HCM Risk-Kids model[14]:

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

where prognostic index =

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

To evaluate the predictive performance of the SCD risk score, both discrimination and calibration measures were employed. Discrimination, which refers to the model's ability to distinguish between high-risk and low-risk patients, was assessed using Harrell's overall concordance C-statistic[256], which ranges from 0.5 (indicating no predictive discrimination) to 1.0 (indicating perfect discrimination). 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 predicted 5-year probability of SCD and observed outcomes, a calibration plot was used. This plot compares the predicted probabilities (from the HCM Risk-Kids score) with the observed risk of SCD. For evaluating calibration accuracy, two optimal cutoff values (0.04 and 0.06) were used to categorize patients into low-risk, medium-risk, and high-risk groups[116].

5.4 Results

5.4.1 Baseline data and demographics

The study cohort included 169 patients, of whom 8 (4.7%) were first assessed between 1981-1990, 24 (14.2%) between 1991-2000, 60 (35.5%) between 2001-2010, and 77 (45.6%) between 2011-2020. Sixteen patients (13.7%) out of the 117 for whom this information was available were diagnosed antenatally. For 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 was 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 symptoms of congestive heart failure

(CHF), and 10 (6.7%) due to a murmur detected on auscultation. Eighteen patients (10.7%) had a family history of HCM, 2 (1.2%) had a family history of sudden cardiac death (SCD), and 8 patients (8% of 100 patients for whom this information was available) had a family history of a Rasopathy syndrome, of whom 3 also had a family history of HCM. Table 5-2 provides a detailed summary of baseline demographic, clinical, and echocardiographic characteristics for the entire cohort, as well as separately for patients with and without an SCD-equivalent event.

Table 5-2: Demographic and clinical characteristics of patients based on sudden cardiac death endpoints

	Whole cohort	Patients With SCD-equivalent	Patients without SCD-equivalent	
Variable	**N = 169**	***n = 11***	***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 (IQR)	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)	
MLVWT (mm), median (IQR)	10.5 (8.0-13.5)	7.0 (6.0-8.0)	11.0 (8.0-14.0)	0.012[2]
MLVWT z-score, median (IQR)	9.2 (5.6-15.8)	6.6 (3.9-8.1)	9.2 (5.7-15.8)	0.245[2]
LAd (mm), median (IQR)	25.0 (20.0-29.0)	22.0 (18.0-28.0)	26.0 (20.0-30.0)	0.260[2]
LAd z-score, median (IQR)	2.0 (1.0-2.9)	1.4 (0.5-2.2)	2.0 (1.0-3.0)	0.239[2]
LVOT peak gradient, median (IQR)	28.5 (10.0-61.5)	9.0 (4.0-100.0)	30.0 (10.0-60.0)	0.360[2]
LVOTO, n (%)	63 (37.5)	6 (54.5)	57 (36.3)	0.334[1]

[1] Fisher's exact test, [2] Mann–Whitney U-test

Abbreviations: IQR, interquartile range; NSVT, non-sustained ventricular tachycardia; NYHA, New York Heart Association; MLVWT, maximal left ventricular wall thickness; LAd, left atrial diameter; LVOT, left ventricular outflow tract; LVOTO, LVOT obstruction

5.4.2 Genetics

One hundred and three patients (60.9%) had a gene variant identified in the RAS-MAPK pathway, of which 61 (59.2%) were classified as pathogenic, 5 (4.9%) as likely pathogenic, and 5 (4.9%) 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%) had a variant in *RIT1*. A detailed table of the genetic variants, including nucleotide and protein changes identified for each syndrome, is provided in Table 5-3. Additionally, five patients had an additional variant in a cardiomyopathy-related gene identified: one with a likely pathogenic (LP) variant in *MAP2K2* and a pathogenic (P) variant in *MYH7* (familial), one with an LP variant in *RAF1* and a VUS in *MYH7*, one with a pathogenic variant in *PTPN11* and a VUS in *MYH7*, one with an unknown RAS-MAPK variant and a VUS in *FLH1*, and one with a pathogenic variant in KRAS and an additional VUS in *MEK1*.

Table 5-3: Clinical syndrome by gene affected, nucleotide and protein change

Clinical syndrome	Affected Gene	N (%)	Nucleotide	Protein	N (%)	Significance
NS	PTPN11	27 (21.1)	c.923A>G	p.Asn308Ser	4 (14.8)	P
			c.922A>G	p.Asn308Asp	3 (11.1)	P
			c.836A>G	p.Tyr279Cys	3 (11.1)	P
			c.1528C>G	p.Gln510Glu	2 (7.4)	P
			c.124A>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.188A>G	p.Tyr63Cys	1 (3.7)	P
			c.218C>T	p.Thr73Ile	1 (3.7)	P
			c.236A>G	p.Glu79Arg	1 (3.7)	P
			c.317A>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.854T>C	p.Phe285Ser	1 (3.7)	P
			c.923A>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.775T>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.766A>G	p.Arg256Gly	1 (3.9)	LP
	RIT1	11 (8.6)	c.170C>G	p.Ala57Gly	2 (18.2)	P
			c.244T>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.244T>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.346A>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 unidentified	24 (18.8)				
NSML	PTPN11	12 (63.2)	c.836A>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 unidentified	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.466T>C	p.Phe156Leu	1 (11.1)	P
			c.64C>A	p.Gln22Lys	1 (11.1)	LP
	Variant unidentified	1 (10.0)				
CFCS	BRAF	3 (50.0)	c.1782T>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 unidentified	1 (16.7)				
NS_LAH	SHOC2	3 (50.0)	c.4A>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.

5.4.3 Outcomes

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 (IQR) 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 (25^{th}-75^{th} 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 5-4).

Table 5-4: 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**	**1,277.1**	**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				
NS	128 (75.7)	9	1021.2	0.88 (0.46-1.69)
NSML	19 (11.2)	1	137.6	0.73 (0.10-5.16)
CS	10 (5.9)	1	48.7	2.05 (0.29-14.58)
CFCS	6 (3.6)	0	52.3	
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)

NYHA: New York Heart Association; NS: Noonan syndrome; NSML: Noonan syndrome with multiple lentigines; CS: Costello syndrome; CFCS: cardio-facio-cutaneous syndrome; NS_LAH: Noonan syndrome with loose anagen hair; NSVT: non-sustained ventricular tachycardia; LV: left ventricular

5.4.4 Missing data

Eighty-four patients (49.7%) had one or more missing data points for the predictor variables in the HCM Risk-Kids model: 30 patients (17.8%) had one missing variable, 29 patients (17.2%) had two missing variables, and 25 patients (14.8%) had three missing variables. Table 5-5 provides further details on the missing data for each variable.

Table 5-5: Distribution of missing values

Variable	No. Missing values n, (%)	No. Non-missing values, n (%)
Gender	0 (0.00)	169 (100.00)
Family history	0 (0.00)	169 (100.00)
NYHA/Ross classification	5 (2.96)	164 (97.04)
Clinical syndrome	0 (0.00)	169 (100.00)
Affected Gene	0 (0.00)	169 (100.00)
Unexplained syncope	0 (0.00)	169 (100.00)
NSVT	0 (0.00)	169 (100.00)
LV outflow tract obstruction	1 (0.59)	168 (99.41)
Age at diagnosis	61 (36.09)	108 (63.91)
Age at first assessment	2 (1.18)	167 (98.82)
Maximal left ventricular wall thickness (MLVWT)	25 (14.79)	144 (85.21)
MLVWT z score	49 (28.99)	120 (71.01)
LA diameter	56 (33.14)	113 (66.86)
LA z score	65 (38.46)	104 (61.54)
LV outflow tract peak gradient	49 (28.99)	120 (71.01)

5.4.5 Validation of HCM Risk-Kids

The performance of the HCM Risk-Kids model in predicting the 5-year risk in this cohort was evaluated. Harrell's C index was 0.60 (95% CI 0.5-1), indicating moderate discriminatory

ability. When assessing the ability of the risk score to differentiate between high and low risk using a 6% cutoff, the sensitivity was 9.4%, specificity was 63.9%, positive predictive value (PPV) was 1.7%, and negative predictive value (NPV) was 91%. Figure 5- illustrates the survival outcomes for the entire cohort and by risk category (low, medium, high) as defined by the HCM Risk-Kids score, showing considerable overlap between the different risk categories. The clinical syndrome, genetic information, and HCM Risk-Kids score parameters for individuals with an SCD equivalent event are summarized in Table 5-6. Notably, 6 out of 11 (54.5%) patients who experienced an event were classified in the low-risk category.

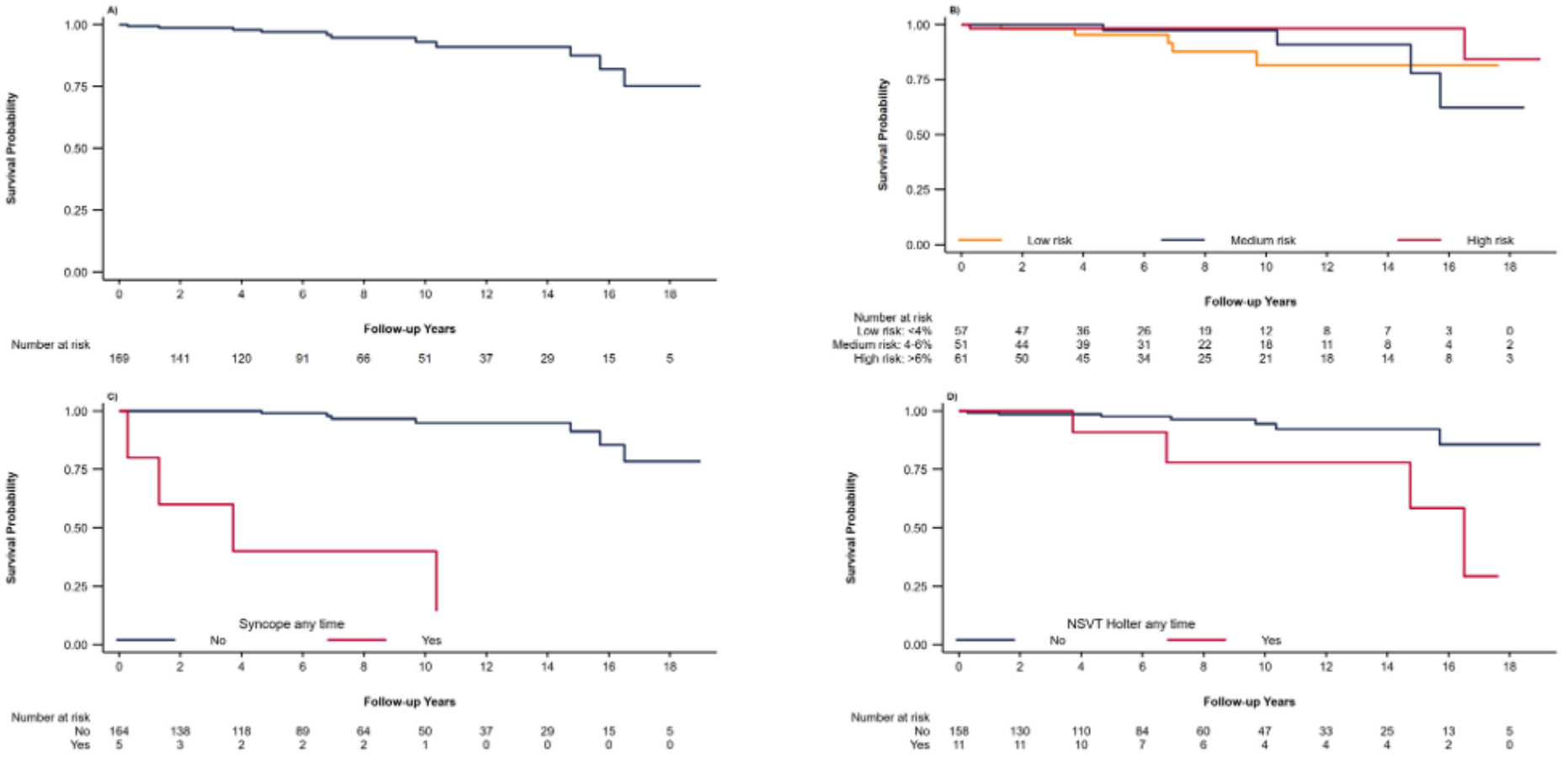


Figure 5-3: Kaplan-Meier survival curves for sudden cardiac death equivalent for whole cohort (A), by risk category (B), in patients with and without a history of syncope (C) and in patients with and without evidence of NSVT on holter monitoring (D) in follow up time (years)

Table 5-6: 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)
NS	Unknown	Low risk	2.31	+3.4	+1.4	No	No	4
NS	Unknown	Low risk	2.74	+2.4	+1.4	Yes	No	14
NS	Unknown	Low risk	3.42	+8.2	-0.5	No	No	16
NS	RAF1	Low risk	3.56	+6.2	+1	No	No	10
NS	RIT1	Low risk	3.60	+6.5	+1.8	Yes	Yes	110
CS	HRAS	Low risk	1.56	+1.4	+0.5	No	Yes	8
NS	PTPN11	Medium risk	4.21	+6.7	+2.2	No	No	4
NS	LZTR1	Medium risk	4.37	+3.9	+2.6	Yes	No	2
NS	Unknown	High risk	6.12	+8.1	+2	No	Yes	10
NS	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

5.4.6 Predictors of SCD in RAS-HCM

Unexplained syncope (HR 42.17, 95% CI 10.49-169.56, $p < 0.001$) and the presence of non-sustained ventricular tachycardia (NSVT) on Holter monitoring (HR 5.48, 95% CI 1.58-19.03, $p < 0.007$) were identified as significant predictors of sudden cardiac death (SCD) or an equivalent event in univariate analysis (see Table 5-7). Figure 5-3(C, D) illustrates the event-free survival for patients with and without unexplained syncope, and with and without NSVT, highlighting the increased risk associated with these predictors in this cohort.

Table 5-7: 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
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

5.5 Discussion

To my knowledge, this is the first validation of 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 RAS-HCM.

5.5.1 Prevalence of SCD

The reported prevalence of SCD in children with RAS-HCM has been estimated at 4%[17,255]. 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[12,17,32]. 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 represent a more severe phenotype. This could also explain the findings of a recent study suggesting a similar cumulative incidence of SCD in children with RAS-HCM and those with non-syndromic disease[203]. Nevertheless, the findings highlight the fact that SCD can occur in patients with RAS-HCM, emphasizing the importance of SCD risk prediction in this group of patients.

5.5.2 Validation of HCM-Risk Kids

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 in children with RAS-HCM, and it has very low specificity and positive predictive value. This is further supported by the fact that the majority of patients who experienced a SCD equivalent event were classified as low risk based on the 5-year estimated SCD risk. Additionally, individuals with RAS-HCM exhibit a distinct phenotype compared to patients with sarcomeric gene variants[12,17,32]. Despite a comparable prevalence of SCD equivalent events compared to the original HCM Risk-Kids cohort[14], our group was more symptomatic at baseline evaluation, had unexplained syncope less frequently, and were more likely to have LVOTO. The poor performance of the HCM Risk-Kids model in children with RAS-HCM may be related to the relatively small sample size in this study, as supported by the finding that 2 of the variables included in the model (NSVT and syncope) appear to be predictors of SCD in this population

as well. Nonetheless, the findings suggest that the use of the HCM Risk-Kids model for 5-year SCD prediction may not be appropriate in this population based on current evidence, and larger multicentre studies are needed to further investigate this.

5.5.3 Predictors of SCD in RAS-HCM

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 non-syndromic HCM[14,77,119]. Syncope in patients with HCM may be related to arrhythmic causes, haemodynamic abnormalities such as LVOTO, or abnormal vascular responses[257]; our findings suggest that these mechanisms may also be important in patients with RAS-HCM. Similarly, NSVT is an established risk factor for SCD in patients with non-syndromic HCM, particularly in young individuals[73,239,240] and the findings in the present study suggest that this may also be the case in children with RAS-HCM. In contrast, MLVWT and LAd did not emerge as predictors of SCD in this cohort [258]. These findings highlight the need to identify specific risk factors in RAS-HCM and explore independent predictors in this population.

5.5.4 Limitations

This study is limited by its retrospective design, which inherently involves missing or incomplete data. To ensure robustness in the imputation of missing data, we incorporated all relevant predictors into the imputation model that we considered important for explaining missingness. The proportion of missing data was similar to that observed in the HCM Risk-Kids cohort, and imputation diagnostics, including comparisons of means and distributions of predictors before and after imputation, confirmed that the data had not been distorted.

Additionally, we are investigating a rare condition with a low number of events, which is lower than the paediatric sarcomeric cohort for which the HCM Risk-Kids model was originally developed. Variations in clinical assessment and patient management were inevitable, as patients were recruited from multiple centres and over different time periods. Genetic testing was performed at the discretion of participating clinicians, and while a high proportion of patients with a Rasopathy syndrome had a disease-causing variant identified, it is unclear whether the genetic test results influenced the final diagnosis or merely confirmed previous clinical suspicions.

Variations in echocardiographic protocols and the availability of images for retrospective assessment across different centres and time periods also led to missing variables. Data collection relied on patients being referred to collaborating paediatric cardiology centres, meaning that those with mild phenotypes or severe, early mortality may not have been included. As a result, the true incidence of SCD events in RAS-HCM is unknown. While this study provides an event rate, it may not accurately represent the broader population prevalence.

The small sample size and low event rate in our cohort resulted in wide confidence intervals for the C-index values, reflecting uncertainty in the estimates. This limitation also precluded a multivariate analysis to investigate independent predictors of SCD. Additionally, this study focuses on a paediatric cohort, and the findings may not necessarily apply to older adolescents or young adults with RAS-HCM.

The limitations of the study design could be addressed through future prospective, large multicentre studies aimed at identifying predictors of SCD in patients with Rasopathy syndromes and HCM. Further investigations into the role of additional imaging modalities (such as echocardiography and cardiac MRI), electrocardiographic findings, and circulating biomarkers in SCD risk prediction could provide valuable insights into improving risk assessment in this population.

5.6 Conclusions

This study demonstrates that sudden cardiac death (SCD) and malignant ventricular arrhythmias can occur in children with RAS-HCM. The HCM Risk-Kids SCD risk prediction model, however, does not appear to have good discriminatory ability or calibration for this population, suggesting that it may not be suitable for predicting SCD risk in children with RAS-HCM. Unexplained syncope and the presence of non-sustained ventricular tachycardia (NSVT) seem to be potential predictors of SCD in these patients. However, larger multicenter studies are needed to further explore and validate these findings.

Chapter 6 - Disease progression in Rasopathy-associated hypertrophic cardiomyopathy

6.1 Introduction

Despite histological similarities to sarcomeric HCM[259], RAS-HCM has distinct pathophysiological mechanisms, primarily involving cell-cycle dysregulation[189,260]. The clinical phenotype and natural history of RAS-HCM differ substantially from sarcomeric HCM[43,202], characterized by earlier onset, frequent biventricular involvement, and common association with CHD. Patients with RAS-HCM typically present with smaller LV chambers and higher rates of LVOTO[43,202,243]. Notably, mortality in the first year of life approaches 60% and is predominantly due to CHF[179,243]. Longitudinal data examining the evolution over time of the RAS-HCM phenotype are limited, but small reports have suggested spontaneous regression of LVH in up to 17% of cases and progression in approximately 34%[43,174,194]. The mechanisms underlying these divergent trajectories—whether representing true myocardial remodelling or relative changes during somatic growth—remain poorly understood.

6.2 Aim

The aim of this chapter is to describe the long-term changes in cardiac phenotype in a large, multicentre cohort of childhood-onset RAS-HCM.

6.3 Methods

6.3.1 Study population

This was a retrospective multicentre study of childhood-onset RAS-HCM. Data were collected on patients presenting to a collaborating paediatric cardiology centre (Table 6-1) under the age of 18 years with a diagnosis of HCM and a clinical and/or genetic diagnosis of a Rasopathy syndrome were collected. Patients were excluded if they lacked data at baseline assessment or if they did not have more than 1 follow up timepoint.
The collaborators from each participating centre guaranteed the integrity of data from their institution. Eligible patients were identified by the principal investigator at each

collaborating site. Data were collected independently at each participating centre and each local investigator provided data on all consecutive patients with RAS-HCM from their centre.

Table 6-1: Collaborating Centres

Center	Number of patients (%)
Great Ormond Street Hospital, London, UK	100 (49.8)
Naples, Italy	37 (18.4)
German Heart Center, Munich, Germany	34 (16.9)
Alder Hey, Liverpool, UK	14 (7.0)
Murcia, Spain	6 (3.0)
Glasgow Children's Hospital, Glasgow, UK	5 (2.5)
Birmingham Children's Hospital, Birmingham, UK	2 (1.0)
Southampton General Hospital, Southampton, UK	2 (1.0)
Leeds General Infirmary, Leeds, UK	1 (0.5)

6.3.2 Patient assessment and data collection

Data were collected at predefined intervals: baseline and 1, 2, 5, 10, and 20 years of follow-up. Data included demographics, underlying syndrome, genotype, heart failure symptoms (NYHA[214]/Ross functional classification[215], cardiac medication, and 2D transthoracic echocardiogram findings. Assessment was made according to methods described in chapter 2.

6.3.3 Outcomes

The primary outcome was a composite of MACE: SCD or equivalent event, hospitalization due to CHF symptoms, or cardiac transplantation. SCD equivalent event was defined as appropriate ICD therapy, aborted cardiac arrest, or sustained VT with haemodynamic compromise. Outcomes were determined by the treating cardiologist at each site.

6.3.4 Statistical Analysis

NYHA/Ross functional class was analysed as class I versus II-IV and I-II versus III-IV. Changes in MLVWT z-scores were categorized for analysis purposes as decreased (<-2), stable (-2 to 2), or increased (>2) based on average rate of change per year. End of follow-up was defined

as last clinical follow up. Follow-up periods were predetermined and categorized into clinically relevant intervals: baseline, 0-1.5, 1.5-2.5, 2.5-7.5, 7.5-15, and 15-35 years. Continuous variables are presented as median (interquartile range) or mean ± standard deviation based on distribution, and categorical variables as frequencies (percentages). Between-group comparisons utilized Mann-Whitney U test or Student's t-test for continuous variables and chi-square or Fisher's exact test for categorical variables. Disease progression was assessed using mixed-effects models with random intercepts and slopes, accounting for within-subject correlation and between-centre variability. Time-to-event analyses employed Kaplan-Meier methods and Cox proportional hazards models. Variables for multivariable models were selected based on clinical relevance and univariate $p < 0.10$. The final model includes all significant variables at $p<0.10$. Statistical analyses were performed using Stata version 18.0 (StataCorp). Two-sided $p < 0.05$ was considered significant, without adjustment for multiple comparisons in secondary analyses.

6.3.5 Missing data

Missing data patterns were systematically evaluated across all follow-up time points. The average follow-up included 3.9 visits per patient (range: 1-7 visits). Analysis of missing data mechanisms revealed no significant differences in baseline characteristics between patients with complete and incomplete data (maximal wall thickness Z-score: p=0.54; NYHA classification: p=0.099). Dropout analysis showed no significant association between missingness and clinical variables (p=0.36), suggesting a missing at random mechanism.

6.4 Results

6.4.1 Population

Two-hundred-and-seventeen (217) patients were identified, of whom 3 were excluded due to lack of baseline assessment data, and a further 13 were excluded due to <2 follow up timepoints. The final study cohort consisted of 201 patients, of whom 155 (77.1%) had a diagnosis of NS, 25 (12.4%) NSML, 12 (6.0%) CS, 4 (3.0%) CFCS and 4 (3.0%) NS-LAH. A breakdown of Rasopathy syndrome by gene identified can be found in Table 6-2.

Table 6-2: Rasopathy syndrome by gene identified

Syndrome, total N (%)	Gene	N (%)
Noonan syndrome, 155 (77.1)	PTPN11	46 (29.5)
	RAF1	36 (23.1)
	RIT1	15 (9.6)
	LZTR1	5 (3.2)
	KRAS	3 (1.9)
	Unidentified	7 (4.5)
	Untested	37 (23.7)
Noonan syndrome with multiple lentigines, 25 (12.4)	PTPN11	22 (88.0)
	Untested	3 (12.0)
Costello syndrome, 12 (6.0)	HRAS	10 (83.3)
	KRAS	1 (8.3)
	BRAF	1 (8.3)
Cardio-facio-cutaneous syndrome, 4 (3.0)	MAP2K2	1 (33.3)
	MEK1	1 (33.3)
	KRAS	1 (33.3)
	Unidentified	1 (25.0)
Noonan syndrome with loose anagen hair, 4 (3.0)	SHOC2	4 (100)

The median age at diagnosis of HCM was 0.4 years (0.03-2.73) and median age at baseline assessment was 1.01 years (0.35-4.62). Forty-nine patients (24.6%) presented with heart failure symptoms (NYHA/Ross functional class > I) and 99 patients (51.3%) were taking one or more cardiac medications. Eighty-four patients (48.6%) had concomitant right ventricular hypertrophy and 39 (28.1%) had evidence significant LVOTO. Sixty-seven patients (33.3%) had concomitant CHD. Further information on the baseline characteristics of the whole cohort can be found in Table 6-3.

Table 6-3: Baseline characteristics of whole cohort (N=201)

Male	117 (58.2)
Female	84 (41.8)
BSA	0.54 (0.37)
Age at diagnosis of HCM (years)	0.40 (0.03-2.73)
Age at baseline assessment (years)	1.01 (0.35-4.62)
NYHA/Ross > I	49 (24.6)
Medication	99 (51.3)
LVEDD (mm)	23.0 (18.1-31.1)
LVEDD z score	-1.89 (1.89)
IVST (mm)	10.0 (7.0-12.7)
IVST z score	+9.6 (7.0)
LVPWT (mm)	6.8 (5.0-10.0)
LVPWT z score	+5.1 (5.7)
LAd (mm)	24.5 (20.0-30.0)
LAd z score	+9.8 (7.4)
MLVWT (mm)	11.0 (8.0-13.0)
MLVWT z score	+10.5 (7.1)
LVOT gradient (mmHg)	27.0 (7.0-60.0)
LVOTO > 30mmHg	64 (46.4)
LVOTO > 50mmHg	39 (28.1)
Mid-cavity obstruction	45 (54.2)
RVH	84 (48.6)
RVOT gradient (mmHg)	19.5 (6.5-50.0)
RVOTO	42 (51.9)
Average E/E'	11.4 (8.2-16.0)
Systolic dysfunction	1 (1.8)
Hyperdynamic systolic function	49 (87.5)
Diastolic impairment	30 (33.0)

BSA: body surface area; NYHA: New York Heart Association; LVEDD: left ventricular end diastolic diameter; IVST: intraventricular septal thickness; LVPWT: LV posterior wall thickness; LAd: left atrial diameter; MLVWT: maximal LV wall thickness; LVOT: left ventricular outflow tract; LVOTO: LVOT obstruction; RVH: right ventricular hypertrophy; RVOT: right VOT; RVOTO: RVOT obstruction;

6.4.2 Survivors vs non-survivors

Clinical and echocardiographic parameters of surviving patients were compared to those of non-surviving patients at baseline assessment (N=173 vs N=18) and at one year of follow up (N=117 vs N=17). Non-survivors were younger [0.3 (0.3-1.0) years vs 1.2 (0.4-5.4) years, p=0.019] and smaller at baseline assessment [BSA 0.3 (0.3-0.4) vs 0.4 (0.3-0.7), p=0.020]. At one year of follow up, a higher proportion of non-survivors was symptomatic [NYHA/Ross > I N=6 (40.0%) vs N=15 (13.3%), p=0.009] and on cardiac medication [N=13 (86.7%) vs N=60 (53.6%), p=0.015], and had a higher left ventricular posterior wall thickness (LVPWT) [7.5mm (6.0-10.2) vs 6.1mm (4.9-9.0), p=0.004] (Table *6-4*).

Table 6-4: *Clinical and Echocardiographic Characteristics of Rasopathy-HCM Patients: Survivors Versus Non-Survivors at baseline and 1 year of age*

	Baseline				**1 year**			
	Total	**Survivors**	**Non-survivors**	**p-value**	**Total**	**Survivors**	**Non-survivors**	**p-value**
	N=191	**N=173**	**N=18**		**N=134**	**N=117**	**N=17**	
Sex				0.86				0.65
Male	113 (59.2%)	102 (59.0%)	11 (61.1%)		80 (59.7%)	69 (59.0%)	11 (64.7%)	
Female	78 (40.8%)	71 (41.0%)	7 (38.9%)		54 (40.3%)	48 (41.0%)	6 (35.3%)	0.34
Age at diagnosis (months)	6.4 (1.0-37.7)	6.8 (1.0-45.9)	3.5 (0.1-8.8)	0.21				
Age (years)	1.1 (0.4-4.8)	1.2 (0.4-5.4)	0.5 (0.3-1.0)	**0.019**	2.1 (1.3-6.1)	2.2 (1.4-6.4)	1.5 (0.9-2.5)	**0.049**
BSA	0.4 (0.3-0.7)	0.4 (0.3-0.7)	0.3 (0.3-0.4)	**0.020**	0.5 (0.4-0.8)	0.5 (0.4-1.0)	0.5 (0.4-0.6)	0.16
NYHA/Ross > I	43 (22.8%)	36 (21.1%)	7 (38.9%)	0.086	21 (16.4%)	15 (13.3%)	6 (40.0%)	**0.009**
Medication	90 (49.2%)	80 (47.9%)	10 (62.5%)	0.26	73 (57.5%)	60 (53.6%)	13 (86.7%)	**0.015**
LVEDD (mm)	23.4 (18.5-31.3)	23.4 (18.5-31.8)	22.9 (18.1-25.2)	0.44	26.0 (21.2-32.9)	26.5 (21.3-34.0)	24.0 (18.9-27.6)	0.46
LVIDD z score	-1.8 (1.8)	-1.9 (1.8)	-1.0 (1.8)	0.13	-2.0 (1.9)	-1.9 (1.9)	-2.3 (1.8)	0.52
IVST (mm)	10.0 (7.0-12.5)	10.0 (7.0-12.8)	9.0 (6.5-11.6)	0.48	9.2 (7.0-13.5)	9.6 (7.0-14.0)	8.8 (7.5-11.2)	0.96
IVST z score	9.4 (7.0)	9.3 (6.9)	10.1 (8.5)	0.71	7.6 (6.2)	7.6 (6.3)	7.5 (5.0)	0.13
LVPWT (mm)	6.8 (4.9-9.8)	7.0 (5.0-10.0)	5.5 (4.1-5.8)	0.370	6.2 (4.9-9.0)	6.1 (4.9-9.0)	7.5 (6.0-10.2)	**0.004**
LVPWT zscore	4.8 (5.3)	5.1 (5.3)	1.9 (4.1)	0.056	3.5 (4.8)	3.0 (3.8)	7.4 (8.4)	0.27
LAd (mm)	25 (20-30)	25 (21-31)	23 (15-25)	0.19	25 (21-30)	25 (21-31)	24 (20-29)	0.19
LAd z score	10 (7)	10 (7)	5 (6)	0.16	11 (8)	12 (8)	8 (5)	0.82
MLVWT (mm)	10 (7-13)	11 (8-13)	10 (7-12)	0.64	10 (8-14)	10 (8-15)	10 (8-14)	0.31
MLVWT z score	10 (7)	10 (7)	12 (7)	0.47	9 (6)	9 (6)	11 (7)	0.27

	Baseline				1 year			
	Total N=191	Survivors N=173	Non-survivors N=18	p-value	Total N=134	Survivors N=117	Non-survivors N=17	p-value
MLVWT z score difference					0 (5)	0 (4)	-4 (7)	0.120
MLVWT z score category								0.16
Stable					57 (50.9%)	53 (53.0%)	4 (33.3%)	
Improving					29 (25.9%)	23 (23.0%)	6 (50.0%)	
Worsening					26 (23.2%)	24 (24.0%)	2 (16.7%)	
RVH	77 (46.4%)	69 (44.8%)	8 (66.7%)	0.14	60 (50%)	51 (49.9%)	9 (64.3%)	0.074
LVOT gradient (mmHg)	27 (7-60)	23 (7-60)	35 (11-45)	0.64	20 (6-55)	15 (6-45)	54 (10-95)	0.057
LVOTO >30mmHg	61 (45.9%)	52 (43.3%)	9 (69.2%)	0.075	40 (40.4%)	33 (38.4%)	7 (53.8%)	0.29
LVOTO >50mmHg	37 (27.6%)	34 (28.1%)	3 (23.1%)	0.70	25 (25.2%)	20 (23.3%)	5 (28.5%)	0.24
LVOTO category								
Stable					119 (88.8%)	106 (89.9%)	14 (82.4%)	
Improved					10 (7.5%)	10 (8.5%)	0 (0%)	
Worsened					5 (3.7%)	2 (1.7%)	3 (17.6%)	
Mid cavity obstruction	43 (53.8%)	38 (55.1%)	5 (45.5%)	0.55	38 (52.8%)	32 (53.3%)	6 (50.0%)	0.83
RVOT gradient (mmHg)	18 (6-48)	17 (6-50)	21 (16-40)	0.77	21 (4-52)	18 (4-50)	41 (20-62)	0.16
RVOTO	39 (50.6%)	35 (50.0%)	4 (57.1%)	0.72	32 (55.2%)	26 (52.0%)	6 (75.0%)	0.22
Diastolic impairment	28 (31.8%)	26 (32.1%)	2 (28.6%)	0.85	26 (32.5%)	22 (30.6%)	4 (50.0%)	0.27
Systolic dysfunction	1 (1.9%)	1 (2.0%)	0 (0.0%)	0.78	3 (5.9%)	3 (6.2%)	0 (0.0%)	
Hyperdynamic systolic function	47 (87.0%)	43 (86.0%)	4 (100.0%)	0.42	36 (70.6%)	33 (68.8%)	3 (100.0%)	0.25

	Baseline				1 year			
	Total	**Survivors**	**Non-survivors**	**p-value**	**Total**	**Survivors**	**Non-survivors**	**p-value**
	N=191	**N=173**	**N=18**		**N=134**	**N=117**	**N=17**	

BSA: body surface area; NYHA: New York Heart Association; LVEDD: left ventricular end diastolic diameter; IVST: intraventricular septal thickness; LVPWT: LV posterior wall thickness; LAd: left atrial diameter; MLVWT: maximal LV wall thickness; LVOT: left ventricular outflow tract; LVOTO: LVOT obstruction; RVH: right ventricular hypertrophy; RVOT: right VOT; RVOTO: RVOT obstruction;

6.4.3 Outcomes and predictors

Patients were followed up over a median of 7.3 years (3.1-12.6). During follow up, 18 patients (8.9%) died at a median age of 2.2 years (0.6-10.0) and 4 patients (2.7%) received a heart transplant at a median age of 1.9 years (0.6-4.1). Twenty patients (8.7%) underwent a septal myectomy at a median age of 3.3 years (1.1-13.2), while 15 patients (6.5%) underwent surgery for CHD repair and a further 7 (3.1%) had a PV repair. Forty-two patients (18.3%) had a MACE (incidence 1.401/100 patient years) and 16 (7.0%) had a SCD or equivalent event (incidence 0.577/100 patient years) (Table 6-5).

Univariate analysis identified NYHA/Ross functional class > I, LVEDD z score, LVPWT z score, MLVWT z score, LVOT gradient and RVH as potential predictors of MACE; on backwards elimination multivariable analysis, NYHA/Ross functional class > I remained an independent predictor of MACE [HR 7.08 (1.1-43.9) p = 0.035] (Table Table 6-6, Figure 6-1).

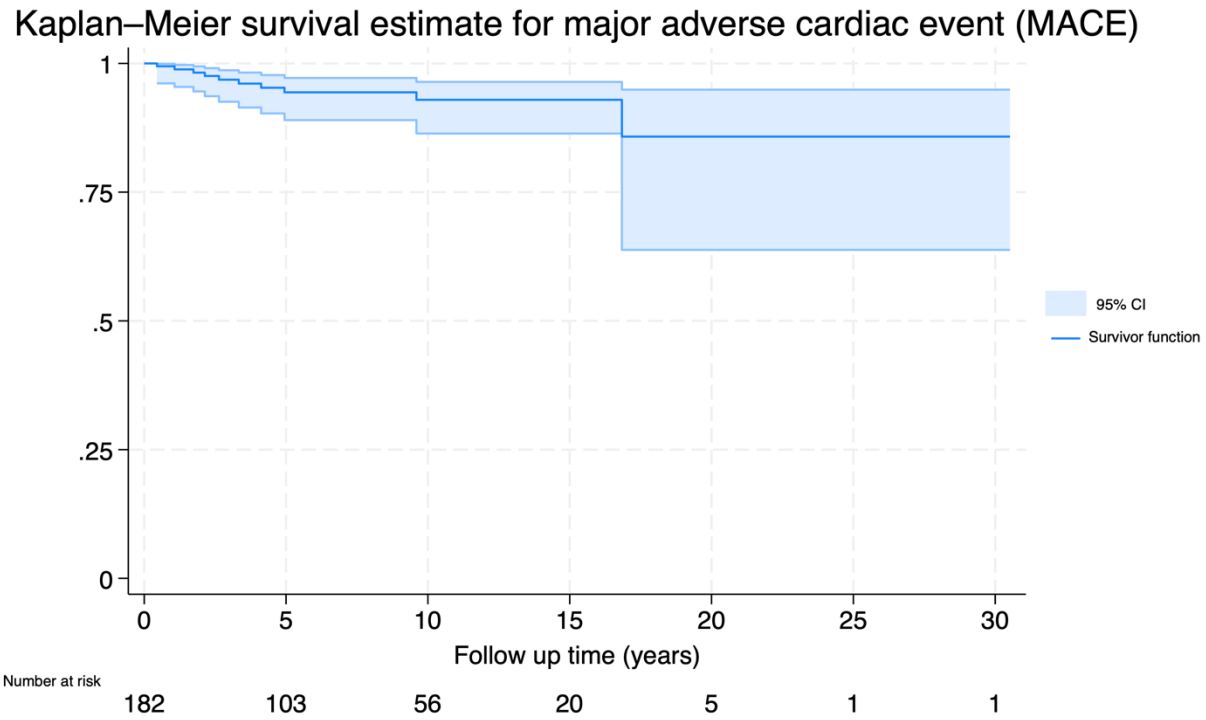


Figure 6-1: Long-term freedom from major adverse cardiac events in paediatric Rasopathy-associated hypertrophic cardiomyopathy with follow up time (years)

Table 6-5: Outcomes

VT/VF	13 (7.0%)
Cardiac arrest	5 (4.5%)
CVS death	24 (10.4%)
SCD	2 (8.3%)
CHF	7 (29.2%)
Other CVS	2 (8.3%)
CHF requiring hospitalisation	27 (18.2%)
Heart transplant	4 (2.7%)
SCD equivalent event	16 (7.0%)
MACE	42 (18.3%)
Atrial arrhythmia	11 (4.8%)
NSVT	12 (5.2%)
Cardiac device insertion	22 (9.6%)
ICD	14 (6.1%)
ILR	6 (2.6%)
PPM	1 (0.4%)
ILR and ICD	2 (0.9%)
LV myectomy	20 (8.7%)
MV repair	2 (0.9%)
RV myectomy	5 (2.2%)
PV surgery	7 (3.1%)
Other CHD surgery	15 (6.5%)

VT: ventricular tachycardia; VF: ventricular fibrillation; CVS: cardiovascular; SCD: sudden cardiac death; CHF: congestive heart failure; MACE: major adverse cardiac event; NSVT: non-sustained VT; ICD: implantable cardioverter-defibrillator; ILR: implantable loop recorder; PPM: permanent pacemaker; LV: left ventricle; MV: mitral valve; RV: right ventricle; PV: pulmonary valve; CHD: congenital heart defect

Table 6-6: Univariate Cox regression for MACE

	HR	95% CI	p-value
BSA	0.55	0.0-15.4	0.305
Sex	1.85	0.2-20.1	0.613
NYHA/Ross >I	14.07	1.7-114.5	**0.013**
Medication	5.45	0.3-112.1	0.272
Age at diagnosis	0.93	0.5-1.6	0.804
LVEDD z score	0.67	0.4-1.13	**0.132**
IVST z score	1.03	0.9-1.1	0.542
LVPWT z score	1.17	1.0-1.4	**0.049**
LAd z score	0.84	0.6-1.3	0.408
MLVWT z score	1.09	1.0-1.2	**0.097**
LVOT gradient (mmHg)	1.05	1-1.1	**0.039**
LVOTO >50mmHg	3.89	0.2-76.9	0.373
RVH	11.58	0.7-184.6	**0.083**
RVOT gradient (mmHg)	1.02	1.0-1.1	0.391
RVOTO	7.66	0.12-487.0	0.337
Average E/E’	1.15	0.9-1.5	0.263
Diastolic dysfunction	7.34	0.2-263.6	0.275
Previous CHD surgery	4.37	0.3-59.7	0.269

BSA: body surface area; NYHA: New York Heart Association; LVEDD: left ventricular end diastolic diameter; IVST: intraventricular septal thickness; LVPWT: LV posterior wall thickness; LAd: left atrial diameter; MLVWT: maximal LV wall thickness; LVOT: left ventricular outflow tract; LVOTO: LVOT obstruction; RVH: right ventricular hypertrophy; RVOT: right VOT; RVOTO: RVOT obstruction; CHD: congenital heart defect

6.4.3.1 Complex atrial arrythmias

Among the twenty patients (9.9%) who were followed up beyond the age of 18 years into adulthood, four patients (20%) had an episode of complex atrial arrhythmia. All events [paroxysmal atrial fibrillation (N=2), flutter (N=1) or prolonged atrial tachycardia (N=1)] were recorded on a cardiac monitor at a median age of 22.6 years (22.2-24.5) after a median follow-up time of 3.4 years (1.9-6.4). The patients' clinical and echocardiographic characteristics at the time of the event are recorded in Table 3-7. Of note, all four patients had dilated atria, 3 out of four had moderate mitral regurgitation and elevated average E/E' at the time of the event

6.4.4 Phenotypic progression in survivors

Overall, symptomatic status improved [NYHA > I at baseline N=39 (20.9%) vs N=16 (15.4%) at 10 years of follow up, p=0.009] while a higher proportion were on cardiac medication [N=89 (49.4%) at baseline vs N=26 (56.5%) at follow up, p=0.015]. MLVWT z score [+10.3 (7.3) at baseline vs +8.9 (8.6) at 20 years of follow up, p=0.039], median LVOT gradient [23 (7-60)mmHg vs 7 (5-25)mmHg at 20 years of follow up, p=0.019] and median RVOT gradient [17 (6-50)mmHg vs 5 (2-7)mmHg at 20 years of follow up, p=0.001] all improved during follow up. LAd z score progressively worsened [+10.6 (7.5) at baseline vs +25.7 (10.2) at 20 years follow up, p<0.001]. (Table 6-8, Figure 6-2).

When applying a mixed effects model to estimate change per year of follow up in echocardiographic measurements, LAd z score was predicted to increase by +1.17 (95% CI 0.93-1.31, p<0.001) and average E/E' to increase by +0.39 (95% CI 0.01-0.77, p=0.047), while RVOT gradient was predicted to decrease by -1.25mmHg (95% CI -1.95 – 0.55, p<0.001). (Table 6-9).

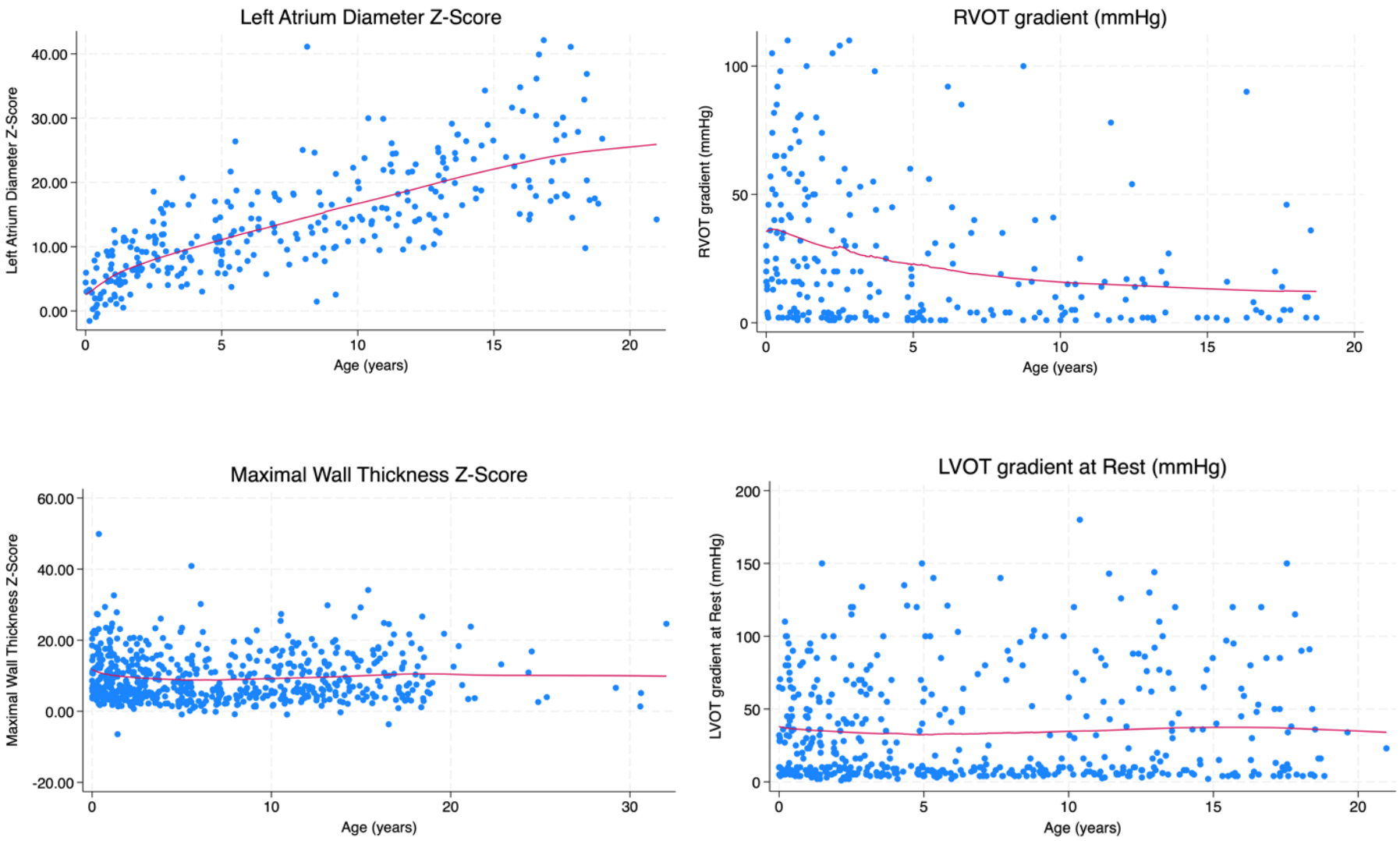
6.4.5 Symptomatic neonates

A separate analysis was conducted for patients who presented at baseline assessment with significant symptoms of CHF (NYHA/Ross functional class III-IV). Of a total of 15 (7.5%) such patients with a median age at baseline of 0.4 years (0.0-1.0), 5 (33.3%) died. Non-survivors had a significantly smaller LVEDD z score [-4.2 (0.1) vs -0.9 (1.0), p=0.023] compared to surviving patients (Table 6-10).

Table 6-7: Clinical and echocardiographic characteristics of patients with complex atrial arrhythmias

	Patient 1	Patient 2	Patient 3	Patient 4
Gender	Male	Female	Female	Male
Event	AT	AFL	PAF	PAF
Age at event (y)	29.71	20.17	22.52	20.65
Palpitations	No	Yes	Yes	Yes
NYHA	I	I	II	I
Meds	No	b-blockers, amiodarone	b-blockers	b-blockers
LVEDD (mm)	47	23	39	50
MLVWT (mm)	26	18	10	12
LAd (mm)	52	42	45	44
LVOT (mmHg)	127	51	5	12
MR grade	Severe	Mild	Moderate	Moderate
EF (%)	73	75	85	53
E/A	1.34	1.21	1.02	1.18
Average E/E'	24	25	24	6.6
RVH	No	Yes	No	No
Estimated RVSP (mmHg)	32		27	18
Device	ICD	ICD	ICD	No
LV myectomy	No	No	No	Yes

AT: atrial tachycardia; AFL: atrial flutter; PAF: paroxysmal atrial fibrillation; NYHA: New York Heart Association; LVEDD: left ventricular end diastolic diameter; MLVWT: maximal LV wall thickness; LAd: left atrial diameter; LVOT: left ventricular outflow tract; MR: mitral valve regurgitation; RVH: right ventricular hypertrophy; RVSP: right ventricular systolic pressure (+ right atrial pressure)



<u>Figure 6-2:</u> Progressive Changes in left atrial diameter (z-score), maximal left ventricular wall thickness (z-score), and left and right outflow tract gradients in childhood Rasopathy-associated hypertrophic cardiomyopathy with increasing age (years)

Table 6-8: Progression through follow up for survivors

	Baseline N=189	1 year N=117	2 years N=91	5 years N=140	10 years N=90	20 years N=23	p value
BSA	0.43 (0.31-0.77)	0.52 (0.42-0.98)	0.63 (0.54-1.04)	0.77 (0.66-1.00)	1.06 (0.94-1.39)	1.55 (1.42-1.82)	**0.000**
NYHA/Ross > I	39 (20.9)	15 (13.3)	12 (13.9)	21 (15.4)	16 (18.6)	3 (13.0)	**0.009**
Medication	89 (49.4)	60 (53.6)	46 (54.8)	68 (50.0)	26 (56.5)	12 (54.6)	**0.015**
LVEDD (mm)	23.6 (19.0-32.0)	26.5 (21.3-34.0)	29.0 (25.5-37.5)	30.9 (27.8-34.0)	35.5 (32.0-39.9)	41.4. (34.7-44.8)	**0.000**
LVIDD z score	-1.9 (1.9)	-1.9 (1.9)	-1.5 (1.6)	-1.7 (1.9)	-1.9 (2.9)	-1.7 (1.5)	0.798
IVST (mm)	10.0 (7.0-14.0)	9.6 (7.0-14.0)	9.5 (7.8-13.1)	10.4 (8.0-15.4)	14.2 (10.0-20.2)	10.9 (9.2-15.7)	**0.000**
IVST z score	+9.9 (7.4)	+7.6 (6.3)	+7.1 (5.4)	+8.5 (7.2)	+10.5 (7.8)	+7.1 (8.3)	0.180
LVPWT (mm)	7.0 (5.0-10.6)	6.1 (4.9-9.0)	6.2 (5.5-9.0)	7.3 (6.0-10.3)	9.0 (7.0-12.1)	10.0 (8.0-12.0)	**0.000**
LVPWT zscore	+5.3 (5.3)	+2.9 (3.8)	+2.8 (3.5)	+3.9 (4.4)	+4.2 (3.8)	+4.3 (4.8)	0.105
LAd (mm)	25.6 (21.0-31.1)	25.2 (20.1-31.0)	28.4 (24.4-34.5)	29.2 (25.4-35.2)	34.1 (29.2-41.1)	36.8 (31.1-48.8)	**0.000**
LAd z score	+10.6 (7.5)	+11.5 (7.8)	+13.9 (7.7)	+14.9 (6.9)	+20.7 (7.4)	+25.7 (10.2)	**0.000**
MLVWT (mm)	11 (8-14)	10 (8-15)	11 (8-14)	11 (5-15)	14 (11-20)	15 (11-19)	**0.000**
MLVWT z score	+10.3 (7.3)	+8.8 (6.4)	+8.1 (5.6)	+8.3 (6.5)	+10.5 (7.1)	+8.9 (8.6)	**0.039**
MLVWT z score difference		-0.5 (-1.8-+1.9)	-0.5 (-1.3-+0.7)	-0.14 (-0.7-+0.6)	+0.0 (-0.5-+0.8)	-0.1 (-0.2-+0.1)	0.693
MLVWT z score category							0.160
Stable		53 (53.0)	59 (72.8)	95 (84.8)	63 (87.5)	16 (88.9)	

Improving		23 (23.0)	14 (17.3)	7 (6.3)	4 (5.6)	2 (11.1)	
Worsening		24 (24.0)	8 (9.9)	10 (8.9)	5 (6.9)	0 (0.0)	
LVOT gradient							
(mmHg)	23 (7-60)	15 (6-45)	10 (6-43)	9 (6-50)	36 (8-88)	7 (5-25)	**0.019**
LVOTO >30mmHg	56 (42.4)	33 (38.4)	27 (34.6)	30 (31.3)	26 (59.1)	3 (27.3)	0.290
LVOTO >50mmHg	38 (28.8)	20 (23.3)	17 (21.8)	22 (22.9)	18 (42.9)	2 (18.2)	0.240
LVOTO category							**0.022**
Stable		105 (89.7)	83 (91.2)	129 (92.1)	85 (94.4)	20 (87.0)	
Improved		10 (8.5)	7 (7.7)	8 (5.7)	3 (3.3)	2 (8.7)	
Worsened		2 (1.7)	1 (1.1)	3 (2.1)	2 (2.2)	1 (4.3)	
RVH	76 (46.1)	51 (49.0)	35 (42.7)	51 (40.2)	26 (39.4)	6 (31.6)	0.280
RVOT gradient							
(mmHg)	17 (6-50)	19 (4-50)	15 (2-36)	4 (1-21)	6 (2-17)	5 (2-7)	**0.001**
RVOTO	37 (50.0)	26 (52.0)	16 (32.0)	11 (27.5)	6 (25.0)	0 (0.0)	0.220
Average E/E’	12.2 (8.2-15.3)	12.2 (8.6-16.1)	12.0 (8.7-18.8)	10.7 (8.2-17.9)	15.3 (10.4-25.1)	10.9 (8.1-13.7)	0.082
Diastolic							
impairment	30 (34.1)	22 (30.6)	24 (35.8)	33 (39.8)	25 (62.5)	4 (36.4)	0.270
Systolic dysfunction	2 (3.7)	3 (6.3)	0 (0.0)	0 (0.0)	2 (4.1)	0 (0.0)	0.660
Hyperdynamic							
systolic function	46 (83.6)	33 (68.8)	23 (67.7)	46 (65.7)	32 (65.3)	9 (60.0)	0.250

BSA: body surface area; NYHA: New York Heart Association; LVEDD: left ventricular end diastolic diameter; IVST: intraventricular septal thickness; LVPWT: LV posterior wall thickness; LAd: left atrial diameter; MLVWT: maximal LV wall thickness; LVOT: left ventricular outflow tract; LVOTO: LVOT obstruction; RVH: right ventricular hypertrophy; RVOT: right VOT; RVOTO: RVOT obstruction;

Table 6-9: Temporal Evolution of Cardiac Structure and Function in Paediatric RAS-HCM

	Coefficient	95% Confidence interval		p-value
LVEDD (mm)	1.052472	0.8768147	1.2281292	**0.000**
LVEDD z score	-0.0116443	-0.0683194	0.0450307	0.687
IVST (mm)	0.3643576	0.2351896	0.4935257	**0.000**
IVST z score	-0.0526351	-0.1942272	0.0889569	0.466
LVPWT (mm)	0.1965385	0.1161815	0.2768956	**0.000**
LVPWT z score	-0.0740062	-0.1524461	0.0044338	0.064
LAd (mm)	1.0814559	0.8056242	1.3572875	**0.000**
LAd z score	1.1735362	0.9330838	1.4139885	**0.000**
MLVWT (mm)	0.3598424	0.2584094	0.4612753	**0.000**
MLVWT z score	-0.0555402	-0.1635077	0.0524272	0.313
LVOT gradient (mmHg)	0.04818	-0.7632386	0.8595986	0.907
RVOT gradient (mmHg)	-1.2494336	-1.9514087	-0.5474585	**0.000**
Average E/E’	0.3891165	.0045577	.7736754	**0.047**

LVEDD: left ventricular end diastolic diameter; IVST: intraventricular septal thickness; LVPWT: LV posterior wall thickness; LAd: left atrial diameter; MLVWT: maximal LV wall thickness; LVOT: left ventricular outflow tract; RVOT: right VOT

Table 6-10: Comparison of Symptomatic Neonates With Rasopathy-HCM: Outcomes Based on Clinical and Echocardiographic Parameters

	Total N=15	**Survivors N=10**	**Non-survivors N=5**	**p-value**
Sex				0.26
Male	9 (60.0%)	7 (70.0%)	2 (40.0%)	
Female	6 (40.0%)	3 (30.0%)	3 (60.0%)	
Age at HCM diagnosis (months)	2.8 (0.3-10.6)	2.1 (0.0-3.9)	3.5 (0.6-17.4)	0.55
Age at baseline (years)	0.4 (0.0-1.0)	0.5 (0.3-1.0)	0.4 (0.0-0.4)	0.066
BSA	0.3 (0.2-0.3)	0.3 (0.3-0.4)	0.2 (0.2-0.3)	0.11
NYHA/Ross				0.52
III	13 (86.7%)	8 (80.0%)	5 (100.0%)	
IV	2 (13.3%)	2 (20.0%)	0 (0.0%)	
Medication	12 (80.0%)	7 (70.0%)	5 (100.0%)	0.17
LVEDD (mm)	19.2 (15.4-21.4)	21.4 (19.2-38.0)	14.7 (14.0-15.4)	0.083
LVEDD z score	-2.2 (1.9)	-0.9 (1.0)	-4.2 (0.1)	**0.023**
IVST (mm)	11.5 (9.9-16.3)	12.5 (8.4-19.0)	11.2 (10.2-14.3)	1
IVST z score	16.1 (7.6)	15.7 (8.8)	16.9 (5.7)	0.82
LVPWT (mm)	9.5 (5.3-11.0)	11.0 (6.7-11.1)	5.8 (5.2-6.5)	0.19
LVPWT z score	8.1 (4.3)	9.1 (4.1)	4.2 (2.1)	0.15
LAd (mm)	24 (15-42)	33 (24-42)	15 (15-15)	0.22
LAd z score	10 (13)	15 (13)	1 (.)	0.53
MLVWT (mm)	12 (10-16)	12 (10-19)	11 (10-14)	0.67
MLVWT z score	16 (7)	16 (8)	16 (6)	0.97
RVH	10 (71.4%)	6 (60.0%)	4 (100.0%)	0.13
LVOT gradient (mmHg)	58 (42-75)	58 (10-143)	55 (44-70)	1
LVOTO > 30mmHg	6 (85.7%)	2 (66.7%)	4 (100.0%)	0.21
LVOTO >50mmHg	4 (57.1%)	2 (66.7%)	2 (50.0%)	0.66
RVOT (mmHg)	24 (16-58)	14 (14-14)	30 (19-85)	0.18
RVOTO	3 (75.0%)	0 (0.0%)	3 (100.0%)	

BSA: body surface area; NYHA: New York Heart Association; LVEDD: left ventricular end diastolic diameter; IVST: intraventricular septal thickness; LVPWT: LV posterior wall thickness; LAd: left atrial diameter; MLVWT: maximal LV wall thickness; LVOT: left ventricular outflow tract; LVOTO: LVOT obstruction; RVH: right ventricular hypertrophy; RVOT: right VOT; RVOTO: RVOT obstruction;

6.5 Discussion

This is a large, multicentre study using serial data to evaluate disease progression in paediatric RAS-HCM. The major finding is the demonstration of progressive LA dilatation and diastolic impairment associated with complex atrial arrythmias in early adulthood. Symptomatic status and a smaller LV cavity are predictors of MACE and non-surviving symptomatic patients presenting in infancy, respectively.

6.5.1 Long-term cardiac phenotype evolution

A major novel finding in the present study was the progressive development of LA dilatation and diastolic impairment in patients with RAS-HCM, despite no increase in LVH or LVOTO, and the high prevalence of complex atrial arrhythmias in early adult life [261,262], albeit that the numbers are small. Recent data from the European Cardiomyopathy and Myocarditis Registry have highlighted inadequate utilisation of anticoagulation in adult patients with HCM, despite a high prevalence of AF and stroke[263]; the findings in the present study suggest that similar vigilance and early consideration of anticoagulation may also be necessary in young adults with RAS-HCM.

In contrast to sarcomeric HCM, where MLVWT increases during adolescence and early adulthood[45,48,264 44], the degree of LVH and LVOT gradients remain stable over time in childhood-onset RAS-HCM. As MLVWT contributes to risk prediction for SCD in non-syndromic HCM[14,265,266], it is possible that the lack of progression of LVH may partly explain the lower reported SCD rates in RAS-HCM. In keeping with previous reports of improving pulmonary valve stenosis in children with Rasopathies[181,182], the RVOT gradient was found to improve with time in our cohort.

6.5.2 Functional status as a predictor of outcome

Another novel finding in this study is the identification of CHF symptoms as a time-independent predictor of MACE in RAS-HCM. While NYHA functional class > I at baseline assessment has been shown to be a predictor of adverse cardiac outcomes in adults with HCM[267], this has not previously been serially assessed in children with RAS-HCM. As NYHA/Ross functional class assessment is a reproducible clinical tool, a change in functional status should prompt closer surveillance and management.

6.5.3 Risk factors for early mortality

Patients with RAS-HCM are known to have a higher mortality rate during the early disease course, especially during the first 6 months of life, attributable to CHF[43,179]. In keeping with previous studies[179], younger age at presentation and concomitant CHD requiring surgery were risk factors for early mortality. In addition, in the present chapter, symptomatic neonatal patients who did not survive had significantly smaller LV cavities. This may contribute to reduced LV stroke volume[268] leading to a smaller functional reserve in those symptomatic neonates. This finding, if confirmed in larger studies, may allow better selection of patients who may benefit from early consideration of treatment, including with novel therapies such as mTOR and MEKi[269].

6.5.4 Limitations

This chapter is limited by inherent problems of retrospective studies, in particular, missing or incomplete data. The nature of a rare condition such as RAS-HCM resulted in a relatively small population sample with low event rates for independent outcomes, although this is the largest clinical cohort of RAS-HCM reported to date. This prevented investigation of independent predictors of cardiac mortality or SCD using a multivariate analysis. A small proportion of patients were followed up into adulthood, so the finding of complex atrial arrhythmias would need to be corroborated in a larger scale study in the adult population. Symptomatic neonates included in this chapter were small in number and thus the comparative findings should be interpreted with caution and re-investigated in a larger scale study aimed at this population. Data collection for this cohort relied on patients being referred to collaborating paediatric cardiology centres. Therefore, it is possible that patients who either had a very mild phenotype, not warranting referral to an expert centre, or, conversely, had a very severe phenotype resulting in early death in a neonatal or paediatric unit, may not have been included in this chapter.

6.6 Conclusions

Patients presenting with RAS-HCM in childhood develop progressive diastolic dysfunction and LA dilatation, resulting in complex atrial arrhythmias in early adulthood. NYHA/Ross functional class >I is an independent predictor of MACE.

Chapter 7 – Conclusions, overall limitations and future work

7.1 Summary of findings

This thesis provides a comprehensive, chapter-wise evaluation of the natural history, phenotypic expression, and risk profile of paediatric Rasopathy-associated hypertrophic cardiomyopathy (RAS-HCM), using a robust, multicentre international cohort.

Chapter 3 presents the first systematic characterisation of the natural history of RAS-HCM in children. The chapter demonstrates that, while overall survival has improved in recent decades, morbidity remains substantial. Key features of this cohort include early age at diagnosis, predominantly infancy, frequent biventricular hypertrophy, and a high burden of congenital heart defects. Disease severity varied significantly by genotype and syndrome subtype, with RAF1 and RIT1 mutations conferring a more severe cardiac phenotype. Concomitant congenital heart disease, infantile presentation, and impaired functional class emerged as predictors of worse outcomes.

Chapter 4 evaluates resting and ambulatory ECG features. The study identifies distinct electrocardiographic features in RAS-HCM, namely left axis deviation, repolarization abnormalities, and increased arrhythmia burden. NSVT was observed in a significant subset of patients and was associated with MACE. Moreover, specific ECG patterns, such as pathological T-wave inversion and ST depression, correlated with adverse outcomes.

Chapter 5 focuses on sudden cardiac death (SCD) risk stratification, providing the first external validation of the HCM Risk-Kids model in a syndromic HCM population. The findings revealed a modest predictive performance, highlighting that the model, which was developed for non-syndromic HCM, should not be used to predict risk in RAS-HCM. Furthermore, NSVT and unexplained syncope were significantly associated with SCD-equivalent events in RAS-HCM, while the presence of pathogenic variants did not confer added predictive value.

Chapter 6 investigates longitudinal disease progression, showing that structural and functional cardiac parameters often evolve over time, with left atrial enlargement and

worsening diastolic dysfunction being the most consistent markers of deterioration, while LVH remains overall stable. In a smaller subset followed up into adulthood, a high prevalence of complex atrial arrythmias was noted, highlighting the need for further research into this finding.

7.2 Overall limitations

The principal limitations of this study are related to its retrospective, multicentre design. Variability in imaging protocols, data completeness, and genetic testing strategies across institutions and over time introduced potential biases. The inability to perform multivariate analysis for rare outcomes such as SCD limits the robustness of risk prediction modelling. Moreover, some sub-analyses were underpowered due to small sample sizes, particularly for specific genotypes and long-term follow-up beyond early adulthood. The selection of patients from tertiary paediatric cardiology centres may skew the cohort toward more severe phenotypes. However, this is the largest and most complete cohort to date of paediatric patients with RAS-HCM leading to ability to perform investigative and predictive analyses that have not been previously published.

7.3 Future work

The results of this thesis highlight a number of important areas for future research in RAS-HCM. Building on the limitations of retrospective data and the novel risk factors identified here, several directions are both feasible and necessary.

A key priority is the establishment of prospective multicentre cohort studies to validate the risk factors identified in this work and to allow for the development of robust multivariable models of SCD prediction. A prospective design would reduce the biases inherent to retrospective analyses, enable uniform outcome adjudication, and allow for systematic collection of multimodal data. Such studies are critical to provide the time-to-event information needed to refine risk stratification in this patient group.

Equally important is the development of syndrome-specific risk stratification tools. This thesis has shown that existing non-syndromic models underperform in RAS-HCM, emphasising the need for tailored approaches. Future models should incorporate genetic determinants, detailed imaging features, functional measures such as cardiopulmonary

exercise testing, and electrocardiographic parameters. Integrating these domains into a single framework would improve clinical decision-making and align with precision cardiology strategies.

Further work is required to understand the long-term natural history of RAS-HCM. The current findings suggest that atrial arrhythmias, diastolic dysfunction, and left atrial dilatation become more relevant with advancing age, yet long-term follow-up into adulthood remains limited. Extended longitudinal studies would clarify the arrhythmic burden, thromboembolic risk, and progression to heart failure, and would guide surveillance and preventative management in older survivors.

The role of novel biomarkers also warrants investigation. Imaging techniques such as myocardial strain analysis, T1 mapping, and extracellular volume quantification may reveal early myocardial changes not captured by standard measures. Pilot data are already available from a multicentre cohort of 47 children with RAS-HCM who underwent CMR. Compared with s-HCM, patients demonstrated a higher indexed LV mass but a lower prevalence of LV LGE, supporting distinct pathophysiological mechanisms. Notably, RAF1 variants were associated with more severe hypertrophy, higher LV mass index, and hyperdynamic function, underscoring genotype-specific phenotypic differences. Over seven years of follow-up, potential CMR predictors of MACE included reduced LV end-diastolic volume, low LV cardiac output, and the presence of RV hypertrophy. These findings highlight the utility of advanced imaging biomarkers for refining risk stratification and identifying high-risk phenotypes in RAS-HCM, but further in-depth analysis of prospective raw CMR data is needed.

The analysis of CPET data would also be of interest – this has not been previously described in RAS-HCM and its use in predicting outcomes has not been explored. Pilot data is available from 55 children with RAS-HCM undergoing CPET and compared to s-HCM demonstrating that children with RAS-HCM have reduced exercise tolerance relative to healthy peers, with lower prevalence of exercise-induced arrhythmias and ischaemia compared to s-HCM. CPET is feasible and informative in symptomatic patients, supporting its use in clinical assessment and providing pilot data for future studies evaluating prognostic value and exercise guidance in paediatric RAS-HCM.

Similarly, circulating markers of fibrosis, myocardial injury, and pathway dysregulation could complement imaging to provide dynamic risk assessment. Incorporating such biomarkers

into longitudinal studies may improve both phenotypic characterisation and monitoring of disease progression. Pilot data for circulating biomarkers of 36 patients with RAS-HCM, 36 patients with s-HCM, 13 with a Rasopathy syndrome but no HCM and 26 gene negative controls suggest that there are differences between those groups, but further samples and analysis are needed to delineate if a biomarker panel would be viable.

Finally, translational efforts should focus on targeted therapies. Early clinical experience with MEK inhibitors in high-risk genotypes such as RAF1 and RIT1 has shown encouraging results, including regression of hypertrophy and improved haemodynamics. However, these observations remain preliminary. Carefully designed, genotype-informed clinical trials are needed to evaluate efficacy, safety, and timing of intervention, ideally within international collaborative frameworks to overcome the challenges of small patient numbers. Other pathway modulators, such as mTOR inhibitors, may also warrant exploration in selected patient groups.

In summary, future research should combine prospective clinical studies, biomarker development, and therapeutic innovation. Together, these efforts have the potential to move RAS-HCM management beyond extrapolation from non-syndromic cohorts and towards tailored, evidence-based strategies for risk prediction and treatment.

7.4 Conclusions

This thesis establishes RAS-HCM as a genetically and clinically heterogeneous disease with significant implications for prognosis and management. Compared to sarcomeric HCM, RAS-HCM is characterized by earlier onset, frequent biventricular involvement and progressive atrial dilation. The identification of modifiable and time-independent predictors of adverse events provides a framework for clinical risk stratification and intervention. The lack of applicability of standard SCD prediction models further underscores the necessity of a dedicated risk assessment paradigm for this population. Overall, this work enhances our understanding of RAS-HCM and proposes practical, clinically relevant strategies for its long-term management.

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Appendix

Academic output during PhD

Peer-reviewed publications:

Boleti O, Roussos S, Monda E, Norrish G, Field E, Cervi E, et al. Childhood-onset RASopathy-associated hypertrophic cardiomyopathy is associated with progressive left atrial dilatation, diastolic impairment and complex atrial arrhythmias. (manuscript accepted for publication for publication, European Heart Journal November 2025, DOI: 10.1093/eurheartj/ehaf1012).

Boleti O, Sunjaya A, Field E, Norrish G, Tollit J, Cervi E, Kaski JP. Characterisation and prognostic implications of the 12-lead electrocardiogram in children with RASopathy-associated hypertrophic cardiomyopathy. Heart. 2025 Oct 7:heartjnl-2025-326268. doi: 10.1136/heartjnl-2025-326268. Epub ahead of print. PMID: 41057251.

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