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THE HEART IN RASOPATHIES

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THE HEART IN RASOPATHIES

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ABSTRACT

The cardiovascular phenotype associated with RASopathies has expanded far beyond the original descriptions of pulmonary valve stenosis by Dr. Jaqueline Noonan in 1968 and hypertrophic cardiomyopathy by Hirsch *et al* in 1975. Because of the common underlying RAS/MAPK pathway dysregulation, RASopathy syndromes usually present with a typical spectrum of overlapping cardiovascular anomalies, although less common cardiac defects can occur. The identification of the causative genetic variants has enabled the recognition of specific correlations between genotype and cardiac phenotype. Characterization and understanding of genotype–phenotype associations is not only important for counselling a family of an infant with a new diagnosis of a RASopathy condition but is also critical for their clinical prognosis with respect to cardiac disease, neurodevelopment and other organ system involvement over the lifetime of the patient.

This review will focus on the cardiac manifestations of the most common RASopathy syndromes, the relationship between cardiac defects and causal genetic variation, the contribution of cardiovascular abnormalities to morbidity and mortality and the most relevant follow-up issues for patients affected by RAS/MAPK pathway diseases, with respect to cardiac clinical outcomes and management, in children and in the adult population.

KEYWORDS

RASopathy, Congenital heart disease, Hypertrophic cardiomyopathy, Noonan syndrome, Cardio-facio-cutaneous syndrome, Costello syndrome, Noonan syndrome with multiple lentigines.

INTRODUCTION

Congenital heart defects (CHDs) and hypertrophic cardiomyopathy (HCM) are common features in RASopathies, with a prevalence from 60 to 90% in affected patients, as previously reported by several studies (Calcagni et al., 2020; Jhang et al., 2016; Lin et al., 2011; Linglart & Gelb, 2020; Prendiville et al., 2014).

As for other genetic syndromes, the presence of a cardiac disease can be the clinical finding that leads to the diagnosis, and RASopathies should always be considered in the differential diagnosis of children with HCM, in particular when other systemic or cardiac features of these traits are present (*e.g.*, short stature, hypertelorism, cryptorchidism, pulmonary valve stenosis) (Limongelli et al., 2020).

The prenatal recognition of some cardiac defects (*e.g.*, pulmonary valve stenosis and/or HCM), especially when associated with certain specific ultrasound findings, such as increased nuchal translucency or nuchal fold, polyhydramnios, cystic hygroma, hydrops fetalis, ascites/thoracic effusion or lymphatic dysplasia, can help to guide the differential diagnosis of RASopathies and define the indication for molecular genetic testing (Digilio et al., 2011; Myers et al., 2014; Scott et al., 2021).

In general, all patients should undergo a thorough cardiac assessment after the diagnosis, including ECG and two-dimensional color Doppler echocardiography, followed by regular cardiac surveillance based on the cardiac phenotype and on the specific genetic cause (Linglart & Gelb, 2020).

This review will focus on: (a) the cardiac manifestations of the most common RASopathy syndromes, (b) the relationship between cardiac defects and causal genetic variation, (c) the contribution of cardiovascular abnormalities to morbidity and mortality and (d) the most relevant follow-up issues for patients affected by RAS/MAPK pathway diseases, with respect to cardiac clinical outcomes and management, in children and in the adult population.

1. CONGENITAL HEART DEFECTS IN RASOPATHIES

1.1 Cardiovascular anomalies and genotype-phenotype correlation

RASopathy syndromes are a heterogeneous group of genetic multisystemic disorders caused by germline mutations in genes that encode proteins of the RAS/MAPK signal transduction pathway. Because of the common underlying RAS/MAPK pathway dysregulation, these syndromes have overlapping cardiac features and usually present with a typical spectrum of CHDs (Aoki et al., 2016; Rauen, 2013; Tartaglia & Gelb, 2010).

Among RASopathies, the most common syndromes are Noonan syndrome (OMIM PS163950), cardio-facio-cutaneous syndrome (CFCS, OMIM #115150), Costello syndrome (OMIM #218040), and Noonan syndrome with multiple lentigines (NSML, OMIM #151100).

The most common CHDs shown to be associated with these RASopathies include pulmonary valve stenosis (PVS), atrioventricular septal defect (AVSD) and atrial septal defect (ASD) (Calcagni et al., 2017; Digilio et al., 2013; Linglart & Gelb, 2020).

PVS represents the most recurrent CHD, reported in about 50% of individuals affected by Noonan syndrome (Bell et al., 2021; Roberts et al., 2013). The stenotic PV often has typical anatomic features, showing a dysplastic phenotype with myxomatous thickening and poorly mobile leaflets, resulting in severe right ventricular outflow tract obstruction. In some cases, PVS is supra-annular, with fusion of valvular cusps with the wall of the pulmonary artery (Digilio et al., 2009). In this regard, a thorough echocardiographic assessment of the site of obstruction and valvular morphology is fundamental to choose the optimal type of repair between balloon valvuloplasty and surgical treatment (Lin et al., 2008; McCrindle, 1994; Prendiville et al., 2014).

The reported prevalence of PVS in CFCS is ranging from 31% to 44% based on different cohorts (Allanson et al., 2011; Lin et al., 2011; Pierpont et al., 2014; Rodriguez-Viciana et al., 2006). In Costello syndrome, PVS is present in 15-20% of cases, associated with sub-valvular and supravalvular pulmonary stenosis. PVS may frequently be the result of sub-pulmonary muscular obstruction related to HCM. Rarely, severe forms of sub-valvular pulmonary stenosis have been described as “double-chambered right ventricle” (Gripp et al., 2019; Lin et al., 2011). Compared to other RASopathies, patients with Costello syndrome are less likely to have a severe form of PVS (Lin et al., 2011). Similarly, PVS in NSML is rare, mostly associated with HCM (Sarkozy et al., 2008).

AVSD represents a relatively common feature in Noonan syndrome with prevalence of about 15% (Linglart & Gelb, 2020; Marino et al., 1999), most frequently reported as partial AVSD

and more rarely as complete AVSD (Digilio et al., 2013; Pradhan et al., 2013). AVSD in Noonan syndrome may be associated with other cardiac defects including subaortic stenosis, structural mitral valve (MV) anomalies, PVS and HCM (Digilio et al., 2013; Marino et al., 1995, 1999; Pradhan et al., 2013).

In patients with Noonan syndrome, left-sided obstructive cardiac lesions have also been reported in the absence of HCM spectrum. In particular, anatomic obstructions have been described at valvular or sub-valvular level (Burch et al., 1993), in subaortic location, as a result of left ventricular valve anomalies (Marino et al., 1995) or as coarctation of the aorta (CoA) (Digilio et al., 1997, 1998). Data on the prevalence of left-sided obstructions in Noonan syndrome vary widely in the different reported cohorts, ranging from 2% to 12.5% for CoA and 2% to 17% for left-sided valve abnormalities (Colquitt & Noonan, 2014; Digilio et al., 1998; Digilio & Marino, 2001; Prendiville et al., 2014).

In addition, atypical CHD have been described, also as isolated cardiovascular lesions (Calcagni et al., 2020; Leoni et al., 2022; Linglart & Gelb, 2020), including mitral and aortic valve dysfunction, abnormalities of ascending and descending aorta, coronary artery dilation, enlargement of the left atrial appendage and isolated pulmonary branches diseases. MV abnormalities most frequently occur as a minor valvular dysfunction without clinical relevance, due to redundant MV leaflets and/or elongated chords (Leoni et al., 2022). However, moderate-to-severe regurgitation can also occur, in case of dysplastic leaflets and/or significant MV prolapse (Calcagni et al., 2020; Linglart & Gelb, 2020). Since MV abnormalities might present as isolated valve disorder, specifically without concomitant HCM, this raises the concern that RAS/MAPK pathway dysregulation may independently affect the morphogenesis of the MV apparatus.

Since 2001 when *PTPN11* gene missense mutations were found to be causative of Noonan syndrome (Tartaglia et al., 2001), several studies have described the association between mutations in genes encoding components of the RAS/MAPK signalling pathway and RASopathies (Aoki et al., 2016). Congenital heart anomalies occur with different frequency among RASopathy syndromes as a result of mutations in different genes, making it possible to delineate specific correlations between genotype and cardiac phenotype.

PVS is the most frequent cardiovascular defect in patients with Noonan syndrome due to variants of *PTPN11,* with an approximate prevalence of 70% (Calcagni et al., 2017; Digilio et al., 2010; Prendiville et al., 2014); specifically, an association between PVS and mutation on codon 308 of the gene has been recognized (Sarkozy et al., 2003; Tartaglia et al., 2002). In these patients, a high prevalence of a severe form of pulmonary stenosis, both at valvular and supravalvular levels and in association with dysplastic PV, has been described (Leoni et al., 2022). Atrial septal defect

(ASD), isolated or in association with PVS, is commonly detected in individuals with Noonan syndrome-*PTPN11* abnormalities, with a specific correlation of mutations on exon 3 of the gene (Sarkozy et al., 2003). Hemodynamically significant MV anomalies and AVSD can also be observed in patients with *PTPN11* variants, whereas tetralogy of Fallot, ventricular septal defect, patent ductus arteriosus and left-sided obstructions are less frequently reported (Digilio et al., 2013; Leoni et al., 2022; Marino et al., 1995; Prendiville et al., 2014). Conversely, *PTPN11* mutations on exon 7, 12 and 13 are associated with a small subset of CHDs in patients with NSML (Kauffman et al., 2021; Sarkozy et al., 2003). *PTPN11* is the most commonly mutated gene in patients with RASopathies and atypical CHD, such as aortic insufficiency, coronary artery dilation (particularly in patients with NSML), left atrial appendage dilatation and isolated pulmonary arteries anomalies (Calcagni et al., 2020).

Among patients with *SOS1* variants, PVS of mild degree, often associated with ASD, is the most commonly described cardiac abnormality, followed by different types of valve diseases (Leoni et al., 2022; Roberts et al., 2007; Tartaglia et al., 2007). The cardiac phenotype associated with *SOS2* pathogenic variants is similar to the one described in association with *SOS1*, with pulmonary stenosis and septal defects being the most recurrent diseases (Cordeddu et al., 2015; Yamamoto et al., 2015). In individuals harboring pathogenic variants in *KRAS*, the heart is involved in the majority of cases without correlation with a specific cardiac phenotype, even though PVS seems to have a slightly greater prevalence over the other cardiac defects (Leoni et al., 2022; Pierpont & Digilio, 2018). In the subgroup of subjects with causal variation in *RAF1*, CHDs are poorly represented, with PVS and ASD being the most common defects (Kobayashi et al., 2010; Pandit et al., 2007; Razzaque et al., 2007). The prevalence of cardiovascular involvement in individuals harboring *RIT1* alleles ranges between 90 and 100%, with a strong correlation with PVS (Aoki et al., 2013; Yaoita et al., 2016). *LZTR1* cardiac phenotype includes different types of CHDs, most often ASD and PVS (Chinton et al., 2020; Umeki et al., 2019; Yamamoto et al., 2015).

Noonan syndrome with loose anagen hair (NSLAH) due to *SHOC2* gene variants seems to have correlation with PVS, MV dysplasia and septal defects (Cordeddu et al., 2009; Komatsuzaki et al., 2010). Less than half of patients with *HRAS* pathogenic variants, which underlie Costello syndrome, shows CHDs, particularly PVS and MV anomalies, mostly in association with HCM (Lin et al., 2011). Finally, the most frequent CHD in CFCS caused by *BRAF, MAP2K1* and *MAP2K2* variants is PVS, followed by ASD (Allanson et al., 2011; Armour & Allanson, 2007; Yaoita et al., 2016).

Although many correlations between genetic variants and CHDs have been established, others may not have emerged due to the small patient numbers, indicating that further research is needed.

1.2 Management options and outcome

The management of CHDs in RASopathies depends on the nature of the specific heart defect (**Figure 1**). However, when considering cardiac outcomes and necessities during the follow-up period, cardiac defects can vary in terms of spectrum and severity, and consequently, their clinical involvement is quite heterogeneous.

In Noonan syndrome, PVS shows differing degrees of severity: mild in ~60% of patients, moderate in ~10% and severe in ~30% (Colquitt & Noonan, 2014; Shaw et al., 2007). Usually, the mild form of PVS is nonprogressive and is unlikely to require intervention (Colquitt & Noonan, 2014). Conversely, moderate-to-severe stenosis carries a higher rate of intervention, as a consequence of a higher degree of dysplasia of the valve leaflets. Patients with severe PVS very often undergo therapeutic procedure, often within two years of the diagnosis. Due to the distinct anatomic features of the pulmonary valve, the standard approach using percutaneous balloon valvuloplasty has been showed to be rarely successful in these patients (Linglart & Gelb, 2020), who need to undergo percutaneous re-intervention or surgical treatment (either valvotomy or valve leaflet excisions) (Hemmati et al., 2019; Lin et al., 2008; McCrindle, 1994; Prendiville et al., 2014). In 2018, Holzmann and colleagues reported their results concerning the immediate response to primary balloon pulmonary valvuloplasty. These results appeared sub-optimal in terms of reduction of right ventricle-pulmonary artery gradient with a higher reintervention rate when compared to non-syndromic patients (Holzmann et al., 2018). Therefore, with regard to risk of re-operation, a second procedure is frequently required, mostly due to the reoccurrence of PVS (Burch et al., 1993; Calcagni et al., 2017). Except for severe forms of PVS or PVS associated with other CHDs, the limited data available in literature on cardiac surgical prognosis in Noonan syndrome report that the early postoperative outcomes for these patients with PVS are comparable to those of non-syndromic patients (Hemmati et al., 2019).

Patients with AVSD frequently require an earlier intervention compared to individuals with Noonan syndrome affected by other cardiac anomalies (Calcagni et al., 2017). The concurrence of MV and/or aortic valve abnormalities in patients with AVSD results in a more complex and severe cardiac phenotype, deserving a careful evaluation for a more appropriate surgical approach (Calcagni et al., 2017).

Left-sided obstructive cardiac lesions usually require surgical treatment. Indications for intervention and surgical results vary widely and depend on the severity of the stenosis, the presence of multilevel left heart obstruction, other associated cardiac lesions or other non-cardiac risk factors. There is evidence that structural abnormalities of the MV may not only contribute to the development of a subaortic gradient in patients with obstructive HCM and mild septal hypertrophy but might also affect the surgical outcome in patients with CHDs (Calcagni et al., 2017). Another risk factor for morbidity and mortality is the occurrence of subaortic stenosis, probably due to the presence of accessory fibrous connective tissue and/or anomalous MV insertion or abnormality of the left ventricular papillary muscles (Digilio et al., 1998; Marino et al., 1995).

Among atypical CHD, increased awareness of the significance of MV anomalies has ensured that MV morphology and function are carefully investigated for their possible clinical relevance, allowing an early detection of valvular dysfunction. Interestingly, recent studies highlighted the concomitance of congenital dysplasia of two or more cardiac valves, described as "congenital polyvalvular disease", suggesting a new distinct cardiovascular phenotype of the RASopathies, with implications for diagnosis and management (Leoni et al., 2022; Matalon et al., 2021). All these data raise the concern that also atypical CHD need to be carefully investigated and continuously monitored for their possible impact on the clinical outcome (Calcagni et al., 2020; Romano et al., 2010; Wolf et al., 2022). Most frequently, cardiac surgery is not required, as minor CHDs have often a favorable outcome (Calcagni et al., 2020). However, when a minor lesion is associated to major cardiac defects, the latter will direct the need for intervention and the short-term and long-term outcomes.

Overall mortality in patients with RASopathies is low, being less than 2.5% in the overall population and less than 3% in the subgroup with cardiac disease, with flat survival curves (Calcagni et al., 2017). Linglart and Gelb found a similar length of hospital stay comparing patients with and without an associated syndrome (Linglart & Gelb, 2020). With respect to mortality, the adverse event generally occurs in the first two years of life, or during the adulthood. Overall mortality in the atypical CHD subgroup is reduced when compared to typical cardiac diseases.

For adults with RASopathies, clear evidence is still lacking in the current literature. Nonetheless, a previous study by Pierpont and Digilio highly recommended close follow-up for such patients (Pierpont & Digilio, 2018). In their adult cohort, almost one-half needed cardiac surgery and almost 3.5% experienced an arrhythmic event. In patients with PVS, long term sequelae of chronic pulmonary regurgitation might be expected after surgical or catheter intervention. Even in the absence of specific data in literature, the management of these patients should be similar to non-syndromic ones, needing pulmonary revalvulation later in life, when patients become

symptomatic or when progressive RV dilatation or dysfunction occurs (Baumgartner et al., 2021; Stout et al., 2019). Although cardiac complications are common findings in the adult population, these heart diseases are usually stable and non-progressive after the surgical procedure (Smpokou et al., 2012).

Some adults with RASopathies were rarely found to have cardiac abnormalities other than structural CHDs, such as aortic root aneurysm, dilation of the ascending aorta, aortic dissection and giant aneurysms of the sinuses of Valsalva, in particular in Noonan syndrome patients with *PTPN11* mutations (Morgan et al., 1989; Power et al., 2006; Purnell et al., 2005; Shachter et al., 1984). In a retrospective study, Cornwall et al reported that aortic root aneurysms (defined as z-score ≥2) were prevalent in Noonan syndrome patients (~20%), often presenting during childhood, detected by routine screening and progressing over time (Cornwall et al., 2014). These findings imply that some individuals with Noonan syndrome may have connective tissue disorder-like vascular changes in adulthood, suggesting that all adults with Noonan syndrome should have lifelong cardiac follow-up.

Coronary artery dilation, either isolated or with HCM, has also been reported in patients with RASopathies (Calcagni et al., 2016, 2020; Pacileo et al., 2006). In the setting of HCM, coronary artery ectasia likely reflects the consequences of increased myocardial mass, left ventricle outflow tract obstruction and diastolic dysfunction (Limongelli et al., 2007). Conversely, in patients without HCM or any other coexistent cardiovascular defects, coronary artery ectasia could be related to the RAS-MAPK system dysregulation itself (Calcagni et al., 2020). Although the clinical significance and long-term outcome of this finding remain to be clarified, clinicians should be aware of the increased cardiovascular risk in these patients, and careful coronary multimodality imaging, including coronary CT angiography or MRI angiography, is mandatory to monitor whether this anomaly may progress. Especially in adulthood, it is essential to prevent risk factors for myocardial infarction, such as systemic hypertension and hypercholesterolemia, which could accelerate atherosclerotic coronary artery disease. In such cases, use of antiplatelet or anticoagulant to prevent coronary artery thrombosis might be considered.

Of note, non-cardiac comorbidities may influence the cardiac surgery outcome, such as lymphatic abnormalities resulting in chylothorax in up to 10% (Hemmati et al., 2019) and bleeding diathesis, widely ranging in prevalence from 50% to 89% when considering either a history of bleeding and/or abnormal hemostatic lab results (Briggs & Dickerman, 2012; Artoni et al., 2014). Indeed, a wide spectrum of bleeding abnormalities including coagulation factor deficiency and platelet dysfunction has been described in patients with RASopathies, leading to possible bleeding complications during and after surgical procedures (Di Candia et al., 2021; Ruiz-Llobet et al., 2020). Thus, it is essential to investigate the coagulation system in these patients.

2. HYPERTROPHIC CARDIOMYOPATHY IN RASOPATHIES

2.1 Cardiovascular anomalies and genotype-phenotype correlation

In recent longitudinal cohorts of pediatric patients with HCM, RASopathies represent a common underlying etiology (approx. 20% of cases), with the highest prevalence of HCM in infancy (up to 42% of cases) (Alexander et al., 2018; Norrish et al., 2019) and a significant morbidity and mortality among affected individuals (Lioncino et al., 2022).

The occurrence of HCM is heterogeneous among the different RASopathies. The prevalence is highest in NSML, where HCM is diagnosed in up to 80% of patients, generally occurring during infancy (Limongelli et al., 2007). On the other hand, it occurs less frequently in the other RASopathies: 65% in Costello syndrome, 40% in CFCS, 20-25% in Noonan syndrome (Monda, Rubino, et al., 2021).

It has been suggested that the pathophysiology of HCM is related to a hyperactivation of the RAS-MAPK cascade, responsible for cardiomyocyte hypertrophy and myocardial disarray. However, this pathophysiological mechanism cannot be generalized to all RASopathies. For example, variants in *PTPN11* associated with Noonan syndrome are different from those related to NSML (Gelb & Tartaglia, 2011). While Noonan syndrome-related variants behave as a gain-of-function alleles with increased basal phosphatase activity (Keilhack et al., 2005), NSML-related variants are responsible for catalytic impairment (Lauriol & Kontaridis, 2011). Thus, in *PTPN11* Noonan syndrome-related variants the mechanism of HCM development is the upregulation of MAPK signaling, while *PTPN11* hypomorphic mutants associated with NSML cause enhanced signal flow through the PI3K-AKT-mTOR pathway. The elucidation of the pathophysiology of RASopathy-related HCM has significant clinical relevance for the possible development of targeted therapies.

2.2 Clinical features and diagnosis

HCM in RASopathies has higher risk of death and transplantation when compared to non-syndromic forms. When presenting below the 6 months of age with symptoms of heart failure, there is a higher risk of mortality, reaching early 22% at 1 year (6-fold higher than non-syndromic

forms). While in surviving subjects without symptoms of heart failure, sudden cardiac death (SCD) is more frequent among adolescents and young adults (Alexander et al., 2018).

HCM in RASopathies is characterized by a more severe left ventricular hypertrophy (LVH) and a higher prevalence and severity of left ventricular outflow tract obstruction (LVOTO) compared with non-syndromic forms (Cerrato et al., 2008). Several factors contribute to generating LVOTO, including systolic anterior motion (SAM) of the MV, the displacement of papillary muscles, the anomalous insertion of mitral chordae, and an accessory fibrous connective tissue that can cause subaortic stenosis. These complex mechanisms for LVOTO result in a high risk for reintervention and death (Calcagni et al., 2017). Biventricular hypertrophy, due to the coexistence of HCM and PVS, is relatively common and may represent a specific red flag for RASopathies (Limongelli et al., 2020). Coronary artery abnormalities are commonly identified (up to 30%) and contribute to myocardial ischemia, worsening the imbalance between myocardial oxygen supply and demand (Calcagni et al., 2020). In less than 6% of cases, MV abnormalities cause severe mitral regurgitation, making more prone to symptoms for heart failure (Marino et al., 1995). Decreased height-for-age and lower left ventricular fractional shortening z-score are independent predictors of mortality in patients with Noonan syndrome with HCM.

Several ECG abnormalities have been reported, with signs of LVH and diffuse repolarization abnormalities representing the most common findings. In addition, extreme right axis deviation (a "superior" QRS axis) represents a specific disease marker, commonly identified in patients with Noonan syndrome with biventricular hypertrophy (Limongelli et al., 2020; Rapezzi et al., 2013). Other ECG abnormalities that could be encountered are pseudo-infarction q waves and prolonged QT interval (Limongelli et al., 2008). Atrial tachyarrhythmias are commonly experienced by patients with Costello syndrome (in more than 50%), but the natural history is usually benign, with a high rate of responsiveness to medical therapy and spontaneous regression within the first year of life (Levin et al., 2018). However, atrial tachycardia is not an exclusive feature of the Costello Syndrome (Lin et al., 2011). Non-reentrant atrial tachycardias (such as multifocal atrial tachycardia and ectopic atrial tachycardia) have also been reported in patients with Noonan syndrome (with *RAF1*, *SOS1* and *PTPN11* mutated genes). Furthermore, patients with mutation of *PTPN11* gene in the spectrum of NSML may present with atrial disorders (Levin et al., 2018). Even rare, these atrial arrhythmias may appear in early infancy or in the first 1-2 months of life. These forms present with a high ventricular rate and are often a challenge to be controlled by the medical treatment.

Atrial tachycardia in RASopathy patients may occur in the presence or absence of HCM. In addition, these atrial arrhythmias could cause tachycardia-induced cardiomyopathy with a reduced

cardiac function or they may a consequence of cardiomyopathy itself. Patients without HCM frequently experience a hyperdynamic left ventricle which probably may be related to the increased intracellular calcium. Disorders of intracellular calcium homeostasis have also been reported in RASopathies and may influence the management of antiarrhythmic therapy (Wehrens et al., 2004).

A recent study investigated the morphology of the ventricular septum determined by echocardiography, comparing patients with NSML and Noonan syndrome patients. In this study, a sigmoid septum and a ventricular septal bulge were observed predominantly in NSML patients, whereas biconvex septa were more common in Noonan syndrome patients. Furthermore, each cardiac phenotype showed association with specific genotypes and the clearest genotype-cardiac phenotype association occurred in patients carrying variants affecting specific exons of *PTPN11* (Kauffman et al., 2021). A more recent study confirmed the sigmoid-shaped ventricular septum morphology in a small subset of patients of its cohort of 116 cases, occurring in different RASopathies and associated with pathogenic variants involving multiple genes (Delogu et al., 2022). Whether ventricular septum morphology represents a distinct cardiac phenotype in RASopathies with correlations between echocardiographic features and the involved gene/variant remains to be addressed with further research.

The diagnostic algorithm for the diagnosis of HCM-related RASopathy is described in **Figure 2**. In summary, in patients fulfilling the diagnostic criteria for HCM (maximal left ventricular wall thickness ≥15 mm or ≥13 mm, without or with family history for HCM in adults, respectively, or ≥2 z-score in children), attention should be paid to identifying diagnostic clues suggestive for RASopathies (Authors/Task Force members et al., 2014; Limongelli et al., 2022; Ommen et al., 2020). In these patients, genetic testing for the identification of the disease-causing mutation is required for the diagnosis.

2.3 Management options and outcomes

The diagnosis of HCM represents a major prognostic determinant in patients with RASopathies since the severity of the cardiac phenotype is associated with a low survival rate and high risk of death (Calcagni et al., 2017).

The risk for SCD appears to be significantly lower compared with patients with sarcomeric variants, but risk stratification for SCD in patients with RASopathies is challenging (Monda, Lioncino, Rubino, et al., 2022). In non-syndromic HCM, a previous history of sudden cardiac arrest, sustained or non-sustained ventricular tachycardia, unexplained syncope, and massive LVH have been suggested as risk factors for SCD, and in their presence, implantable cardioverter

defibrillation (ICD) implantation may be considered (Monda, Lioncino, Rubino, et al., 2022; Ommen et al., 2020). The relevance of these clinical features in RASopathy patients need to be confirmed.

Medical therapy in patients with RASopathy-related HCM is mainly focused on managing symptoms associated with LVOTO (Limongelli et al., 2022) (**Figure 3**). Non-vasodilating beta-blockers represent the first line and should be titrated to the maximum tolerated dose to obtain a LVOT gradient target <50 mmHg (i.e., the threshold for invasive strategy) (Authors/Task Force members et al., 2014; Monda, Lioncino, Palmiero, et al., 2022; Ommen et al., 2020). Non-vasodilating calcium antagonists should be considered when beta-blockers are contraindicated or not tolerated. However, their use should be carefully monitored since a rare association with severe bradycardia or heart failure worsening in infants treated with verapamil has been reported (Moran & Colan, 1998). Disopyramide may be considered in addition to beta-blockers to reduce the degree of obstruction and improve symptoms. This drug has proved to be effective also in Noonan syndrome, but the magnitude of reduction should be tempered because the effect is temporary (O'Connor et al., 2018). Surgical myectomy is the treatment of choice for patients with LVOTO who remain symptomatic despite optimal medical therapy. Patients with biventricular obstruction with severe PVS usually manifest severe heart failure and symptoms refractory to medical therapy. Pulmonary valvuloplasty is often ineffective in patients with RASopathies, and surgical repair is generally required.

Orthotopic heart transplantation is rarely required in patients with RASopathies. It should be considered in patients with severe heart failure and refractoriness to medical therapy, intractable ventricular arrhythmias, cardiogenic shock requiring inotropes, severe diastolic dysfunction or in patients with severe LVOTO when surgical myectomy is not effective or feasible (Limongelli et al., 2022; Monda, Lioncino, et al., 2021). The evaluation for indication to transplant should assess the cardiac and non-cardiac risk (Gajarski et al., 2009). Knowledge of specific mutation should be of particular value in risk assessment: *PTPN11 p.Gln510Glu* mutation should be considered for an earlier evaluation for transplant. Also, *PTPN11*- and *RIT1*-associated Noonan syndrome patients have a known coagulopathy risk. Other mutations carry a higher risk for malignancies. This information should be taken into account when assessing the individual risk prior to transplant listing. Growth issues and gastrostomy feeding are also commonly encountered in post-transplant management (McCallen et al., 2019).

Treatment of RASopathies with therapies targeting the RAS/MAPK cascade (in Noonan syndrome) or the PI3K/AKT/mTor pathway (in NSML) are limited to case reports suggesting a beneficial effect of these therapeutic approaches in improving clinical status and resulting in LVH

regression (Andelfinger et al., 2019; Marin et al., 2011; Nakano et al., 2022; Mussa et al., 2021). MEK inhibition, specifically, has also been reported as a treatment for arrythmia and for lymphatic dysplasia, each of which can be isolated or comorbid conditions in children with RASopathies and cardiomyopathy, further supporting the efficacy of targeted therapy in RASopathy-associated conditions (Meisner et al., 2021; Dori et al., 2020; Nakano et al., 2022). However, the absence of clinical trials or large studies evaluating the risk and benefits of these drugs limits their use in clinical practice.

CONCLUSIONS AND PERSPECTIVES

As this review demonstrates, the cardiovascular phenotype associated with RASopathies has expanded far beyond the original descriptions of pulmonary valve stenosis by Dr. Jaqueline Noonan in 1968 and hypertrophic cardiomyopathy by Hirsch *et al* in 1975 (Noonan, 1968; Hirsch et al., 1975). Yet, we still can appreciate the importance of these two cardiac findings with respect to disease burden and morbidity among individuals with RASopathy disorders. Our understanding of the phenotypes associated with RAS pathway gene variants has continued to expand at a rapid pace with a great deal of interest in the associated cardiovascular phenotypes based on the specific gene (Pierpont & Digilio, 2018). The common and overlapping cardiovascular phenotypes among all of the RASopathies underscores the recognized common pathophysiology of this group of conditions which generally speaking results in activating RAS/MAPK signal transduction. Still, there are clearly systemic—morphologic and other organ system—differences that are clear when one compares genotype groups. For example, patients with *PTPN11*-associated Noonan syndrome are distinguishable from patients with *RAF1*-associated Noonan syndrome, and their risk for cardiovascular disease also diverge slightly, with *PTPN11* conferring higher risk for pulmonary valve stenosis and less risk for hypertrophic cardiomyopathy, the converse being true for *RAF1*. Determination and understanding of genotype is not only important for counselling a family of an infant with a new diagnosis of a RASopathy condition but is also critical for their clinical prognosis with respect to cardiac disease, neurodevelopment and other organ system involvement over the lifetime of the patient.

Equally important is our better understanding of the prevalence of RASopathy disorders in patients with these common cardiac phenotypes, individually and in various combinations: pulmonary valve stenosis, infantile hypertrophic cardiomyopathy, polyvalvular dysplasia, and incidentally detected coronary artery ectasia. While pediatric cardiologists have, as a specialty, become quite knowledgeable about common syndromic forms of congenital heart disease and the relevance of genetic diagnosis in patients with certain types of congenital heart defects and

cardiomyopathy, much more is still to be learned about how to use genetic diagnosis to improve clinical outcomes. While barriers still exist to collecting genetic information from medical records datasets, future research will depend on the ability to determine hospital and surgical outcomes based on genetic etiology of diseases such as RASopathies. This data collection and analysis is necessary for understanding outcomes for individuals with RASopathies and providing evidence-based precision care. Better understanding of new cardiovascular phenotypes is another area that warrants further investigation. While treatments of pulmonary valve stenosis or hypertrophic cardiomyopathy are well studied, and clinical guidelines established, mildly dysplastic heart valves and coronary ectasia/aneurysm attributable to RAS pathway variants are two examples of cardiovascular disease for which there are no standards of care for monitoring or treatment. The prevalence and associated morbidity of these findings is entirely unknown.

Efforts to improve our understanding of genotype-cardiac phenotype correlations in RASopathies will be critical for optimal medical and surgical management. Genotype can for example to some degree predict risk for associated bleeding disorders, lymphatic dysplasia, malignancy and other comorbidities that can have significant impact on outcome of a cardiac procedure, and on quality of life for the individual. While the collective literature on RASopathies and the associated cardiovascular features is expansive, large systematic population-based and long-term outcomes research are lacking, and especially needed to truly understand how genotype can best inform clinical care in patients with RASopathy-associated cardiovascular disease. Of great interest is the application of FDA-approved and investigational RAS/MAPK pathway inhibitors, such as trametinib and sirolimus, in the treatment of hypertrophic cardiomyopathy and other morbid complications of RASopathies, such as lymphatic disease and malignancy. Understanding of the influence of various gain-of function variants in the RAS/MAPK pathway will be critical to understand the utility and efficacy of these treatments in children with Noonan syndrome and related RASopathies. While only a handful of publications exist that describe isolated experiences with these pharmacologic agents, they are being used widely throughout the Unites States, Canada and Europe under investigational/compassionate use or off-label. Real-world collection of this collective experience is likely to shape the next decade of clinical research in RASopathy conditions and will be a paradigm of personalized medicine for monogenic disease in the modern era.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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FIGURE LEGENDS

Figure 1. Diagnosis–treatment flow-chart for congenital heart defects associated with RASopathies.

Abbreviations: CHDs, congenital heart defects; PVS, pulmonary valve stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; MV, mitral valve; ECG, electrocardiogram.

Figure 2. Diagnostic flow-chart for hypertrophic cardiomyopathy associated with RASopathies.

Abbreviations: ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHDs, congenital heart defects; CS, Costello syndrome; MV, mitral valve; NGS, next-generation sequencing; NSML, Noonan syndrome with multiple lentigines; PVS, pulmonary valve stenosis; VSD, ventricular septal defect.

Figure 3. Determinants and management of left ventricular outflow tract obstruction in hypertrophic cardiomyopathy associated with RASopathies.

THE HEART IN RASOPATHIES

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ABSTRACT

The cardiovascular phenotype associated with RASopathies has expanded far beyond the original descriptions of pulmonary valve stenosis by Dr. Jaqueline Noonan in 1968 and hypertrophic cardiomyopathy by Hirsch *et al* in 1975. Because of the common underlying RAS/MAPK pathway dysregulation, RASopathy syndromes usually present with a typical spectrum of overlapping cardiovascular anomalies, although less common cardiac defects can occur. ~~Because of the common underlying RAS/MAPK pathway dysregulation, RASopathy syndromes have overlapping cardiac features and usually present with a typical spectrum of cardiovascular anomalies, although less common cardiac defects can occur.~~ The identification of the causative genetic variants has enabled the recognition of specific correlations between genotype and cardiac phenotype. Characterization~~Determination~~ and understanding of genotype–phenotype associations is not only important for counselling a family of an infant with a new diagnosis of a RASopathy condition but is also critical for their clinical prognosis with respect to cardiac disease, neurodevelopment and other organ system involvement over the lifetime of the patient.

This review will focus on the cardiac manifestations of the most common RASopathy syndromes, the relationship between cardiac defects and causal genetic variation, the contribution of cardiovascular abnormalities to morbidity and mortality and the most relevant follow-up issues for patients affected by RAS/MAPK pathway diseases, with respect to cardiac clinical outcomes and management, in children and in the adult population.

KEYWORDS

RASopathy, Congenital heart disease, Hypertrophic cardiomyopathy, Noonan syndrome, Cardio-facio-cutaneous syndrome, Costello syndrome, Noonan syndrome with multiple lentigines.

INTRODUCTION

Congenital heart defects (CHDs) and hypertrophic cardiomyopathy (HCM) are common features in RASopathies, with a prevalence from 60 to 90% in affected patients, as previously reported by several studies (Calcagni et al., 2020; Jhang et al., 2016; Lin et al., 2011; Linglart & Gelb, 2020; Prendiville et al., 2014).

As for other genetic syndromes, the presence of a cardiac disease can be the clinical finding that leads to the diagnosis, and RASopathies should always be considered in the differential diagnosis of children with HCM, in particular when other systemic or cardiac features of these traits are present (*e.g.*, short stature, hypertelorism, cryptorchidism, pulmonary valve stenosis) (Limongelli et al., 2020).

The prenatal recognition of some cardiac defects (*e.g.*, pulmonary valve stenosis and/or HCM), especially when associated with certain specific ultrasound findings, such as increased nuchal translucency or nuchal fold, polyhydramnios, cystic hygroma, hydrops fetalis, ascites/thoracic effusion or lymphatic dysplasia, can help to guide the differential diagnosis of RASopathies and define the indication for molecular genetic testing (Digilio et al., 2011; Myers et al., 2014; Scott et al., 2021).

~~As a rule of thumb~~In general, all patients should undergo a thorough cardiac assessment after the diagnosis, including ECG and two-dimensional color Doppler echocardiography, followed by regular cardiac surveillance based on the cardiac phenotype and on the specific genetic cause (Linglart & Gelb, 2020).

This review will focus on: (a) the cardiac manifestations of the most common RASopathy syndromes, (b) the relationship between cardiac defects and causal genetic variation, (c) the contribution of cardiovascular abnormalities to morbidity and mortality and (d) the most relevant follow-up issues for patients affected by RAS/MAPK pathway diseases, with respect to cardiac clinical outcomes and management, in children and in the adult population.

1. CONGENITAL HEART DEFECTS IN RASOPATHIES

1.1 Cardiovascular anomalies and genotype-phenotype correlation

RASopathy syndromes are a heterogeneous group of genetic multisystemic disorders caused by germline mutations in genes that encode proteins of the RAS/MAPK signal transduction pathway. Because of the common underlying RAS/MAPK pathway dysregulation, these syndromes have overlapping cardiac features and usually present with a typical spectrum of CHDs (Aoki et al., 2016; Rauen, 2013; Tartaglia & Gelb, 2010).

Among RASopathies, the most common syndromes are Noonan syndrome (~~NS,~~ OMIM PS163950), cardio-facio-cutaneous syndrome (CFCS, OMIM #115150), Costello syndrome (~~CS,~~ OMIM #218040), and Noonan syndrome with multiple lentigines (NSML, OMIM #151100).

The most common CHDs shown to be associated with these RASopathies include pulmonary valve stenosis (PVS), atrioventricular septal defect (AVSD) and atrial septal defect (ASD) (Calcagni et al., 2017; Digilio et al., 2013; Linglart & Gelb, 2020).

PVS represents the most recurrent CHD, reported in about 50% of individuals affected by Noonan syndrome~~S~~ (Bell et al., 2021; Roberts et al., 2013). The stenotic PV often has typical anatomic features, showing a dysplastic phenotype with myxomatous thickening and poorly mobile leaflets, resulting in severe right ventricular outflow tract obstruction. In some cases, PVS is supra-annular, with fusion of valvular cusps with the wall of the pulmonary artery (Digilio et al., 2009). In this regard, a thorough ~~–~~echocardiographic assessment of the site of obstruction and valvular morphology is fundamental to choose the optimal type of repair between balloon valvuloplasty and surgical treatment (Lin et al., 2008; McCrindle, 1994; Prendiville et al., 2014).

The reported prevalence of PVS in CFCS is ranging from 31% to 44% based on different cohorts (Allanson et al., 2011; Lin et al., 2011; Pierpont et al., 2014; Rodriguez-Viciana et al., 2006). In Costello syndrome~~CS~~, PVS is present in 15-20% of cases, associated with sub-valvular and supravalvular pulmonary stenosis. PVS may frequently be the result of sub-pulmonary muscular obstruction related to HCM. Rarely, severe forms of sub-valvular pulmonary stenosis have been described as "double-chambered right ventricle" (Gripp et al., 2019; Lin et al., 2011). Compared to other RASopathies, patients with Costello syndrome~~CS~~ are less likely to have a severe form of PVS (Lin et al., 2011). Similarly, PVS in NSML is rare, mostly associated with HCM (Sarkozy et al., 2008).

AVSD represents a relatively common feature in Noonan syndrome ~~NS~~ with prevalence of about 15% (Linglart & Gelb, 2020; Marino et al., 1999), most frequently reported as partial AVSD

and more rarely as complete AVSD (Digilio et al., 2013; Pradhan et al., 2013). AVSD in Noonan syndrome~~NS~~ may be associated with other cardiac defects including subaortic stenosis, structural mitral valve (MV) anomalies, PVS and HCM (Digilio et al., 2013; Marino et al., 1995, 1999; Pradhan et al., 2013).

In patients with Noonan syndrome~~NS~~, left-sided obstructive cardiac lesions have also been reported in the absence of HCM spectrum. In particular, anatomic obstructions have been described at valvular or sub-valvular level (Burch et al., 1993), in subaortic location, as a result of left ventricular valve anomalies (Marino et al., 1995) or as coarctation of the aorta (CoA) (Digilio et al., 1997, 1998). Data on the prevalence of left-sided obstructions in Noonan syndrome~~NS~~ vary widely in the different reported cohorts, ranging from 2% to 12.5% for CoA and 2% to 17% for left-sided valve abnormalities (Colquitt & Noonan, 2014; Digilio et al., 1998; Digilio & Marino, 2001; Prendiville et al., 2014).

In addition, atypical ~~cardiac defects~~CHD ~~(ACDs)~~ have been described, also as isolated cardiovascular lesions (Calcagni et al., 2020; Leoni et al., 2022; Linglart & Gelb, 2020), including mitral and aortic valve dysfunction, abnormalities of ascending and descending aorta, coronary artery ~~(CA)~~ dilation, enlargement of the left atrial appendage and isolated pulmonary branches diseases. MV abnormalities most frequently occur as a minor valvular dysfunction without clinical relevance, due to redundant MV leaflets and/or elongated chords (Leoni et al., 2022). However, moderate-to-severe regurgitation can also occur, in case of dysplastic leaflets and/or significant MV prolapse (Calcagni et al., 2020; Linglart & Gelb, 2020). Since MV abnormalities might present as isolated valve disorder, specifically without concomitant HCM, this raises the concern that RAS/MAPK pathway dysregulation may independently affect the morphogenesis of the MV apparatus.

Since 2001 when *PTPN11* gene missense mutations were found to be causative of Noonan syndrome~~NS~~ (Tartaglia et al., 2001), several studies have described the association between mutations in genes encoding components of the RAS/MAPK signalling pathway and RASopathies (Aoki et al., 2016). Congenital heart anomalies occur with different frequency among RASopathy syndromes as a result of mutations in different genes, making it possible to delineate specific correlations between genotype and cardiac phenotype.

PVS is the most frequent cardiovascular~~ac~~ defect~~isease~~ in patients with Noonan syndrome~~NS~~ due to variants of *PTPN11,* with an approximate prevalence of 70% (Calcagni et al., 2017; Digilio et al., 2010; Prendiville et al., 2014); specifically, an association between PVS and mutation on codon 308 of the gene has been recognized (Sarkozy et al., 2003; Tartaglia et al., 2002). In these patients, a high prevalence of a severe form of pulmonary stenosis, both at valvular

and supravalvular levels and in association with dysplastic PV, has been described (Leoni et al., 2022). Atrial septal defect (ASD), isolated or in association with PVS, is commonly detected in individuals with Noonan syndrome~~NS~~-*PTPN11* abnormalities, with a specific correlation of mutations on exon 3 of the gene (Sarkozy et al., 2003). Hemodynamically significant MV anomalies and AVSD can also be observed in patients with *PTPN11* variants, whereas tetralogy of Fallot, ventricular septal defect, patent ductus arteriosus and left-sided obstructions are less frequently reported (Digilio et al., 2013; Leoni et al., 2022; Marino et al., 1995; Prendiville et al., 2014). Conversely, *PTPN11* mutations on exon 7, 12 and 13 are associated with a small subset of CHDs in patients with NSML (Kauffman et al., 2021; Sarkozy et al., 2003). *PTPN11* is the most commonly mutated gene in patients with RASopathies and atypical CHD ~~ACDs~~, such as aortic insufficiency, coronary artery ~~CA~~ dilation (particularly in patients with NSML), left atrial appendage dilatation and isolated pulmonary arteries anomalies (Calcagni et al., 2020).

Among patients with *SOS1* variants, PVS of mild degree, often associated with ASD, is the most commonly described cardiac abnormality, followed by different types of valve diseases (Leoni et al., 2022; Roberts et al., 2007; Tartaglia et al., 2007). The cardiac phenotype associated with *SOS2* pathogenic variants is similar to the one described in association with *SOS1*, with pulmonary stenosis and septal defects being the most recurrent diseases (Cordeddu et al., 2015; Yamamoto et al., 2015). In individuals harboring pathogenic variants in *KRAS*, the heart is involved in the majority of cases without correlation with a specific cardiac phenotype, even though PVS seems to have a slightly greater prevalence over the other cardiac defects (Leoni et al., 2022; Pierpont & Digilio, 2018). In the subgroup of subjects with causal variation in *RAF1*, CHDs are poorly represented, with PVS and ASD being the most common defects (Kobayashi et al., 2010; Pandit et al., 2007; Razzaque et al., 2007). The prevalence of cardiovascular involvement in individuals harboring *RIT1* alleles ranges between 90 and 100%, with a strong correlation with PVS (Aoki et al., 2013; Yaoita et al., 2016). *LZTR1* cardiac phenotype includes different types of CHDs, most often ASD and PVS (Chinton et al., 2020; Umeki et al., 2019; Yamamoto et al., 2015).

Noonan syndrome with loose anagen hair (NSLAH) due to *SHOC2* gene variants seems to have correlation with PVS, MV dysplasia and septal defects (Cordeddu et al., 2009; Komatsuzaki et al., 2010). Less than half of patients with *HRAS* pathogenic variants, which underlie Costello syndrome~~CS~~, shows CHDs, particularly PVS and MV anomalies, mostly in association with HCM (Lin et al., 2011). Finally, the most frequent CHD in CFCS caused by *BRAF, MAP2K1* and *MAP2K2* variants is PVS, followed by ASD (Allanson et al., 2011; Armour & Allanson, 2007; Yaoita et al., 2016).

Although many correlations between genetic variants and CHDs have been established, others may not have emerged due to the small patient numbers, indicating that further research is needed.

1.2 Management options and outcome

The management of CHDs in RASopathies depends on the nature of the specific heart defect (**Figure 1**). However, when considering cardiac outcomes and necessities during the follow-up period, cardiac defects can vary in terms of spectrum and severity, and consequently, their clinical involvement is quite heterogeneous.

In Noonan syndrome~~NS~~, PVS shows differing degrees of severity: mild in ~60% of patients, moderate in ~10% and severe in ~30% (Colquitt & Noonan, 2014; Shaw et al., 2007). Usually, the mild form of PVS is nonprogressive and is unlikely to require intervention (Colquitt & Noonan, 2014). Conversely, moderate-to-severe stenosis carries a higher rate of intervention, as a consequence of a higher degree of dysplasia of the valve leaflets. Patients with severe PVS very often undergo therapeutic procedure, often within two years of the diagnosis. Due to the distinct anatomic features of the pulmonary valve, the standard approach using percutaneous balloon valvuloplasty has been showed to be rarely successful in these patients (Linglart & Gelb, 2020), who need to undergo percutaneous re-intervention or surgical treatment (either valvotomy or valve leaflet excisions) (Hemmati et al., 2019; Lin et al., 2008; McCrindle, 1994; Prendiville et al., 2014). In 2018, Holzmann and colleagues reported their results concerning the immediate response to primary balloon pulmonary valvuloplasty. These results appeared sub-optimal in terms of reduction of right ventricle-pulmonary artery gradient with a higher reintervention rate when compared to non-syndromic patients (Holzmann et al., 2018). Therefore, with regard to risk of re-operation, a second procedure is frequently required, mostly due to the reoccurrence of PVS (Burch et al., 1993; Calcagni et al., 2017). Except for severe forms of PVS or PVS associated with other CHDs, the limited data available in literature on cardiac surgical prognosis in Noonan syndrome~~NS~~ report that the early postoperative outcomes for these patients with PVS are comparable to those of non-syndromic patients (Hemmati et al., 2019).

Patients with AVSD frequently require an earlier intervention compared to individuals with Noonan syndrome~~NS~~ affected by other cardiac anomalies (Calcagni et al., 2017). The concurrence of MV and/or aortic valve abnormalities in patients with AVSD results in a more complex and severe cardiac phenotype, deserving a careful evaluation for a more appropriate surgical approach (Calcagni et al., 2017).

Left-sided obstructive cardiac lesions usually require surgical treatment. Indications for intervention and surgical results vary widely and depend on the severity of the stenosis, the presence of multilevel left heart obstruction, other associated cardiac lesions or other non-cardiac risk factors. There is evidence that structural abnormalities of the MV may not only contribute to the development of a subaortic gradient in patients with obstructive HCM and mild septal hypertrophy but might also affect the surgical outcome in patients with CHDs (Calcagni et al., 2017). Another risk factor for morbidity and mortality is the occurrence of subaortic stenosis, probably due to the presence of accessory fibrous connective tissue and/or anomalous MV insertion or abnormality of the left ventricular papillary muscles (Digilio et al., 1998; Marino et al., 1995).

Among atypical CHD~~ACDs~~, increased awareness of the significance of MV anomalies has ensured that MV morphology and function are carefully investigated for their possible clinical relevance, allowing an early detection of valvular dysfunction. Interestingly, recent studies highlighted the concomitance of congenital dysplasia of two or more cardiac valves, described as "congenital polyvalvular disease", suggesting a new distinct cardiovascular phenotype of the RASopathies, with implications for diagnosis and management (Leoni et al., 2022; Matalon et al., 2021). All these data raise the concern that also atypical CHD~~ACDs~~ need to be carefully investigated and continuously monitored for their possible impact on the clinical outcome (Calcagni et al., 2020; Romano et al., 2010; Wolf et al., 2022). Most frequently, cardiac surgery is not required, as minor CHDs have often a favorable outcome (Calcagni et al., 2020). However, when a minor lesion is associated to major cardiac defects, the latter will direct the need for intervention and the short-term and long-term outcomes.

Overall mortality in patients with RASopathies is low, being less than 2.5% in the overall population and less than 3% in the subgroup with cardiac disease, with flat survival curves (Calcagni et al., 2017). Linglart and Gelb found a similar length of hospital stay comparing patients with and without an associated syndrome (Linglart & Gelb, 2020). With respect to mortality, the adverse event generally occurs in the first two years of life, or during the adulthood. Overall mortality in the atypical CHD~~ACDs~~ subgroup is reduced when compared to typical cardiac diseases.

For adults with RASopathies, clear evidence is still lacking in the current literature. Nonetheless, a previous study by Pierpont and Digilio highly recommended close follow-up for such patients (Pierpont & Digilio, 2018). In their adult cohort, almost one-half needed cardiac surgery and almost 3.5% experienced an arrhythmic event. In patients with PVS, long term sequelae of chronic pulmonary regurgitation might be expected after surgical or catheter intervention. Even in the absence of specific data in literature, the management of these patients should be similar to

non-syndromic ones, needing pulmonary revalvulation later in life, when patients become symptomatic or when progressive RV dilatation or dysfunction occurs (Baumgartner et al., 2021; Stout et al., 2019). Although cardiac complications are common findings in the adult population, these heart diseases are usually stable and non-progressive after the surgical procedure (Smpokou et al., 2012).

Some adults with RASopathies were rarely found to have cardiac abnormalities other than structural CHDs, such as aortic root aneurysm, dilation of the ascending aorta, aortic dissection and giant aneurysms of the sinuses of Valsalva, in particular in Noonan syndrome~~NS~~ patients with *PTPN11* mutations (Morgan et al., 1989; Power et al., 2006; Purnell et al., 2005; Shachter et al., 1984). In a retrospective study, Cornwall et al reported that aortic root aneurysms (defined as z-score ≥2) were prevalent in Noonan syndrome~~NS~~ patients (~20%), often presenting during childhood, detected by routine screening and progressing over time (Cornwall et al., 2014). These findings imply that some individuals with Noonan syndrome may have connective tissue disorder-like vascular changes in adulthood, suggesting that all adults with Noonan syndrome should have lifelong cardiac follow-up.

Coronary artery ~~CA~~ dilation, either isolated or with HCM, has also been reported in patients with RASopathies (Calcagni et al., 2016, 2020; Pacileo et al., 2006). In the setting of HCM, coronary artery ~~CA~~ ectasia likely reflects the consequences of increased myocardial mass, left ventricle outflow tract obstruction and diastolic dysfunction (Limongelli et al., 2007). Conversely, in patients without HCM or any other coexistent cardiovascular defects, ~~CA~~coronary artery ~~ectasia~~ ectasia could be related to the RAS-MAPK system dysregulation itself (Calcagni et al., 2020). Although the clinical significance and long-term outcome of this finding remain to be clarified, clinicians should be aware of the increased cardiovascular risk in these patients, and careful coronary multimodality imaging, including coronary CT angiography or MRI angiography, is mandatory to monitor whether this anomaly may progress. Especially in adulthood, it is essential to prevent risk factors for myocardial infarction, such as systemic hypertension and hypercholesterolemia, which could accelerate atherosclerotic coronary artery~~CA~~ disease. In such cases, use of antiplatelet or anticoagulant to prevent coronary artery ~~CA~~ thrombosis might be considered.

Of note, non-cardiac comorbidities may influence the cardiac surgery outcome, such as lymphatic abnormalities resulting in chylothorax in up to 10% (Hemmati et al., 2019) and bleeding diathesis, widely ranging in prevalence from 50% to 89% when considering either a history of bleeding and/or abnormal hemostatic lab results (Briggs & Dickerman, 2012; Artoni et al., 2014). Indeed, a wide spectrum of bleeding abnormalities including coagulation factor deficiency and

platelet dysfunction has been described in patients with RASopathies, leading to possible bleeding complications during and after surgical procedures (Di Candia et al., 2021; Ruiz-Llobet et al., 2020). Thus, it is essential to investigate the coagulation system in these patients.

2. HYPERTROPHIC CARDIOMYOPATHY IN RASOPATHIES

2.1 Cardiovascular anomalies and genotype-phenotype correlation

In recent longitudinal cohorts of pediatric patients with HCM, RASopathies represent a common underlying etiology (approx. 20% of cases), with the highest prevalence of HCM in infancy (up to 42% of cases) (Alexander et al., 2018; Norrish et al., 2019) and a significant morbidity and mortality among affected individuals (Lioncino et al., 2022).

The occurrence of HCM is heterogeneous among the different RASopathies. The prevalence is highest in NSML, where HCM is diagnosed in up to 80% of patients, generally occurring during infancy (Limongelli et al., 2007). On the other hand, it occurs less frequently in the other RASopathies: 65% in Costello syndrome~~CS~~, 40% in CFCS, 20-25% in Noonan syndrome~~NS~~ (Monda, Rubino, et al., 2021).

It has been suggested that the pathophysiology of HCM is related to a hyperactivation of the RAS-MAPK cascade, responsible for cardiomyocyte hypertrophy and myocardial disarray. However, this pathophysiological mechanism cannot be generalized to all RASopathies. For example, variants in *PTPN11* associated with Noonan syndrome~~NS~~ are different from those related to NSML (Gelb & Tartaglia, 2011). While Noonan syndrome~~NS~~-related variants behave as a gain-of-function alleles with increased basal phosphatase activity (Keilhack et al., 2005), NSML-related variants are responsible for catalytic impairment (Lauriol & Kontaridis, 2011). Thus, in *PTPN11* Noonan syndrome~~NS~~-related variants the mechanism of HCM development is the upregulation of MAPK signaling, while *PTPN11* hypomorphic mutants associated with NSML cause enhanced signal flow through the PI3K-AKT-mTOR pathway. The elucidation of the pathophysiology of RASopathy-related HCM has significant clinical relevance for the possible development of targeted therapies.

2.2 Clinical features and diagnosis

HCM in RASopathies has higher risk of death and transplantation when compared to non-syndromic forms. When presenting below the 6 months of age with symptoms of heart failure, there is a higher risk of mortality, reaching early 22% at 1 year (6-fold higher than non-syndromic forms). While in surviving subjects without symptoms of heart failure, sudden cardiac death (SCD) is more frequent among adolescents and young adults (Alexander et al., 2018).

HCM in RASopathies is characterized by a more severe left ventricular hypertrophy (LVH) and a higher prevalence and severity of left ventricular outflow tract obstruction (LVOTO) compared with non-syndromic forms (Cerrato et al., 2008). Several factors contribute to generating LVOTO, including systolic anterior motion (SAM) of the MV, the displacement of papillary muscles, the anomalous insertion of mitral chordae, and an accessory fibrous connective tissue that can cause subaortic stenosis. These complex mechanisms for LVOTO result in a high risk for reintervention and death (Calcagni et al., 2017). Biventricular hypertrophy, due to the coexistence of HCM and PVS, is relatively common and may represent a specific red flag for RASopathies (Limongelli et al., 2020). Coronary artery ~~CA~~ abnormalities are commonly identified (up to 30%) and contribute to myocardial ischemia, worsening the imbalance between myocardial oxygen supply and demand (Calcagni et al., 2020). In less than 6% of cases, MV abnormalities cause severe mitral regurgitation, making more prone to symptoms for heart failure (Marino et al., 1995). Decreased height-for-age and lower left ventricular fractional shortening z-score are independent predictors of mortality in patients with Noonan syndrome~~NS~~ with HCM.

Several ECG abnormalities have been reported, with signs of LVH and diffuse repolarization abnormalities representing the most common findings. In addition, extreme right axis deviation (a “superior” QRS axis) represents a specific disease marker, commonly identified in patients with Noonan syndrome~~NS~~ with biventricular hypertrophy (Limongelli et al., 2020; Rapezzi et al., 2013). Other ECG abnormalities that could be encountered are pseudo-infarction q waves and prolonged QT interval (Limongelli et al., 2008). Atrial tachyarrhythmias are commonly experienced by patients with Costello syndrome~~CS~~ (in more than 50%), but the natural history is usually benign, with a high rate of responsiveness to medical therapy and spontaneous regression within the first year of life (Levin et al., 2018). However, atrial tachycardia is not an exclusive feature of the Costello Syndrome (Lin et al., 2011). Non-reentrant atrial tachycardias (such as multifocal atrial tachycardia and ectopic atrial tachycardia) have also been reported in patients with Noonan syndrome (with *RAF1*, *SOS1* and *PTPN11* mutated genes). Furthermore, patients with mutation of *PTPN11* gene in the spectrum of NSML may present with atrial disorders (Levin et al., 2018). Even rare, these atrial arrhythmias may appear in early infancy or in the first 1-2 months of life. These

forms present with a high ventricular rate and are often a challenge to be controlled by the medical treatment.

Atrial tachycardia in RASopathy patients may occur in the presence or absence of HCM. In addition, these atrial arrhythmias could cause tachycardia-induced cardiomyopathy with a reduced cardiac function or they may a consequence of cardiomyopathy itself. Patients without HCM frequently experience a hyperdynamic left ventricle which probably may be related to the increased intracellular calcium. Disorders of intracellular calcium homeostasis have also been reported in RASopathies and may influence the management of antiarrhythmic therapy (Wehrens et al., 2004).

A recent study investigated the morphology of the ventricular septum determined by echocardiography, comparing patients with NSML and Noonan syndrome ~~NS~~ patients. In this study, a sigmoid septum and a ventricular septal bulge were observed predominantly in NSML patients, whereas biconvex septa were more common in Noonan syndrome~~NS~~ patients. Furthermore, each cardiac phenotype showed association with specific genotypes and the clearest genotype-cardiac phenotype association occurred in patients carrying variants affecting specific exons of *PTPN11* (Kauffman et al., 2021). A more recent study confirmed the sigmoid-shaped ventricular septum morphology in a small subset of patients of its cohort of 116 cases, occurring in different RASopathies and associated with pathogenic variants involving multiple genes (Delogu et al., 2022). Whether ventricular septum morphology represents a distinct cardiac phenotype in RASopathies with correlations between echocardiographic features and the involved gene/variant remains to be addressed with further research.

The diagnostic algorithm for the diagnosis of HCM-related RASopathy is described in **Figure 2**. In summary, in patients fulfilling the diagnostic criteria for HCM (maximal left ventricular wall thickness ≥15 mm or ≥13 mm, without or with family history for HCM in adults, respectively, or ≥2 z-score in children), attention should be paid to identifying diagnostic clues suggestive for RASopathies (Authors/Task Force members et al., 2014; Limongelli et al., 2022; Ommen et al., 2020). In these patients, genetic testing for the identification of the disease-causing mutation is required for the diagnosis.

2.3 Management options and outcomes

The diagnosis of HCM represents a major prognostic determinant in patients with RASopathies since the severity of the cardiac phenotype is associated with a low survival rate and high risk of death (Calcagni et al., 2017).

The risk for SCD appears to be significantly lower compared with patients with sarcomeric variants, but risk stratification for SCD in patients with RASopathies is challenging (Monda, Lioncino, Rubino, et al., 2022). In non-syndromic HCM, a previous history of sudden cardiac arrest, sustained or non-sustained ventricular tachycardia, unexplained syncope, and massive LVH have been suggested as risk factors for SCD, and in their presence, implantable cardioverter defibrillation (ICD) implantation may be considered (Monda, Lioncino, Rubino, et al., 2022; Ommen et al., 2020). The relevance of these clinical features in RASopathy patients need to be confirmed.

Medical therapy in patients with RASopathy-related HCM is mainly focused on managing symptoms associated with LVOTO (Limongelli et al., 2022) (**Figure 3**). Non-vasodilating beta-blockers represent the first line and should be titrated to the maximum tolerated dose to obtain a LVOT gradient target <50 mmHg (i.e., the threshold for invasive strategy) (Authors/Task Force members et al., 2014; Monda, Lioncino, Palmiero, et al., 2022; Ommen et al., 2020). Non-vasodilating calcium antagonists should be considered when beta-blockers are contraindicated or not tolerated. However, their use should be carefully monitored since a rare association with severe bradycardia or heart failure worsening in infants treated with verapamil has been reported (Moran & Colan, 1998). Disopyramide may be considered in addition to beta-blockers to reduce the degree of obstruction and improve symptoms. This drug has proved to be effective also in Noonan syndrome~~NS~~, but the magnitude of reduction should be tempered because the effect is temporary (O'Connor et al., 2018). Surgical myectomy is the treatment of choice for patients with LVOTO who remain symptomatic despite optimal medical therapy. Patients with biventricular obstruction with severe PVS usually manifest severe heart failure and symptoms refractory to medical therapy. Pulmonary valvuloplasty is often ineffective in patients with RASopathies, and surgical repair is generally required.

Orthotopic heart transplantation is rarely required in patients with RASopathies. It should be considered in patients with severe heart failure and refractoriness to medical therapy, intractable ventricular arrhythmias, cardiogenic shock requiring inotropes, severe diastolic dysfunction or in patients with severe LVOTO when surgical myectomy is not effective or feasible (Limongelli et al., 2022; Monda, Lioncino, et al., 2021). The evaluation for indication to transplant should assess the cardiac and non-cardiac risk (Gajarski et al., 2009). Knowledge of specific mutation should be of particular value in risk assessment: *PTPN11 p.Gln510Glu* mutation should be considered for an earlier evaluation for transplant. Also, *PTPN11*- and *RIT1*-associated Noonan syndrome ~~NS~~ patients have a known coagulopathy risk. Other mutations carry a higher risk for malignancies. This information should be taken into account when assessing the individual risk prior to transplant

listing. Growth issues and gastrostomy feeding are also commonly encountered in post-transplant management (McCallen et al., 2019). Treatment of RASopathies with therapies targeting the RAS/MAPK cascade (in Noonan syndrome) or the PI3K/AKT/mTor pathway (in NSML) are limited to case reports suggesting a beneficial effect of these therapeutic approaches in improving clinical status and resulting in LVH regression (Andelfinger et al., 2019; Marin et al., 2011; Nakano et al., 2022; Mussa et al., 2021). MEK inhibition, specifically, has also been reported as a treatment for arrythmia and for lymphatic dysplasia, each of which can be isolated or comorbid conditions in children with RASopathies and cardiomyopathy, further supporting the efficacy of targeted therapy in RASopathy-associated conditions (Meisner et al., 2021; Dori et al., 2020; Nakano et al., 2022). However, the absence of clinical trials or large studies evaluating the risk and benefits of these drugs limits their use in clinical practice.

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CONCLUSIONS AND PERSPECTIVES

As this review demonstrates, the cardiovascular phenotype associated with RASopathies has expanded far beyond the original descriptions of pulmonary valve stenosis by Dr. Jaqueline Noonan in 1968 and hypertrophic cardiomyopathy by Hirsch *et al* in 1975 (Noonan, 1968; Hirsch et al., 1975). Yet, we still can appreciate the importance of these two cardiac findings with respect to disease burden and morbidity among individuals with RASopathy disorders. Our understanding of the phenotypes associated with RAS pathway gene variants has continued to expand at a rapid pace with a great deal of interest in the associated cardiovascular phenotypes based on the specific gene (Pierpont & Digilio, 2018). The common and overlapping cardiovascular phenotypes among all of the RASopathies underscores the recognized common pathophysiology of this group of conditions which generally speaking results in activating RAS/MAPK signal transduction. Still, there are clearly systemic—morphologic and other organ system—differences that are clear when one compares genotype groups. For example, patients with *PTPN11*-associated Noonan syndrome are distinguishable from patients with *RAF1*-associated Noonan syndrome, and their risk for cardiovascular disease also diverge slightly, with *PTPN11* conferring higher risk for pulmonary valve stenosis and less risk for hypertrophic cardiomyopathy, the converse being true for *RAF1*. Determination and understanding of genotype is not only important for counselling a family of an

infant with a new diagnosis of a RASopathy condition but is also critical for their clinical prognosis with respect to cardiac disease, neurodevelopment and other organ system involvement over the lifetime of the patient.

Equally important is our better understanding of the prevalence of RASopathy disorders in patients with these common cardiac phenotypes, individually and in various combinations: pulmonary valve stenosis, infantile hypertrophic cardiomyopathy, polyvalvular dysplasia, and incidentally detected coronary artery ectasia. While pediatric cardiologists have, as a specialty, become quite knowledgeable about common syndromic forms of congenital heart disease and the relevance of genetic diagnosis in patients with certain types of congenital heart defects and cardiomyopathy, much more is still to be learned about how to use genetic diagnosis to improve clinical outcomes. While barriers still exist to collecting genetic information from medical records datasets, future research will depend on the ability to determine hospital and surgical outcomes based on genetic etiology of diseases such as RASopathies. This data collection and analysis is necessary for understanding outcomes for individuals with RASopathies and providing evidence-based precision care. Better understanding of new cardiovascular phenotypes is another area that warrants further investigation. While treatments of pulmonary valve stenosis or hypertrophic cardiomyopathy are well studied, and clinical guidelines established, mildly dysplastic heart valves and coronary ectasia/aneurysm attributable to RAS pathway variants are two examples of cardiovascular disease for which there are no standards of care for monitoring or treatment. The prevalence and associated morbidity of these findings is entirely unknown.

Efforts to improve our understanding of genotype-cardiac phenotype correlations in RASopathies will be critical for optimal medical and surgical management. Genotype can for example to some degree predict risk for associated bleeding disorders, lymphatic dysplasia, malignancy and other comorbidities that can have significant impact on outcome of a cardiac procedure, and on quality of life for the individual. While the collective literature on RASopathies and the associated cardiovascular features is expansive, large systematic population-based and long-term outcomes research are lacking, and especially needed to truly understand how genotype can best inform clinical care in patients with RASopathy-associated cardiovascular disease.

Of great interest is the application of FDA-approved and investigational RAS/MAPK pathway inhibitors, such as trametinib and sirolimus, in the treatment of hypertrophic cardiomyopathy and other morbid complications of RASopathies, such as lymphatic disease and malignancy.

Understanding of the influence of various gain-of function variants in the RAS/MAPK pathway will be critical to understand the utility and efficacy of these treatments in children with

Noonan syndrome and related RASopathies. While only a handful of publications exist that describe isolated experiences with these pharmacologic agents, they are being used widely throughout the Unites States, Canada and Europe under investigational/compassionate use or off-label. Real-world collection of this collective experience is likely to shape the next decade of clinical research in RASopathy conditions and will be a paradigm of personalized medicine for monogenic disease in the modern era.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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FIGURE LEGENDS

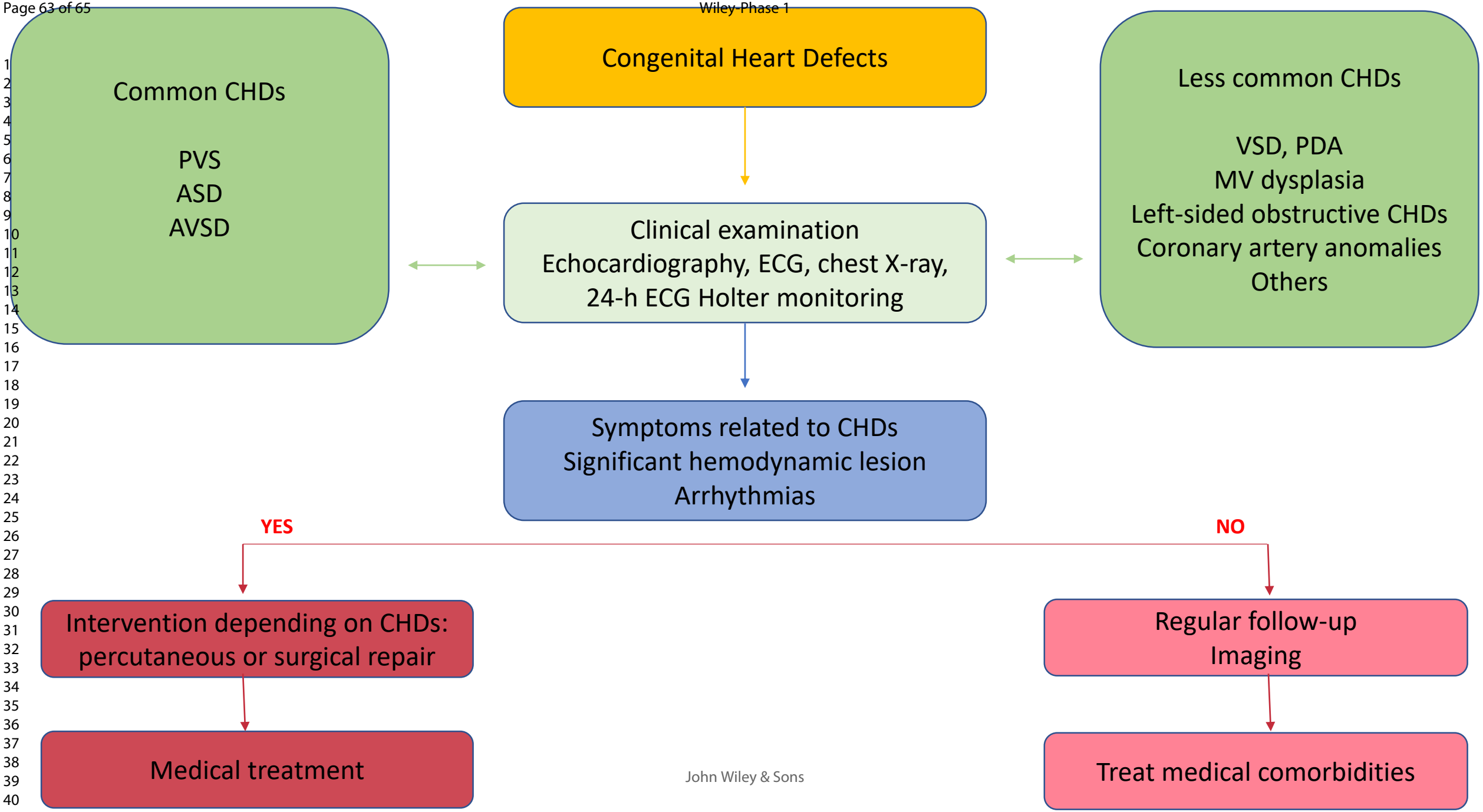
Figure 1. Diagnosis–treatment flow-chart for congenital heart defects associated with RASopathies.

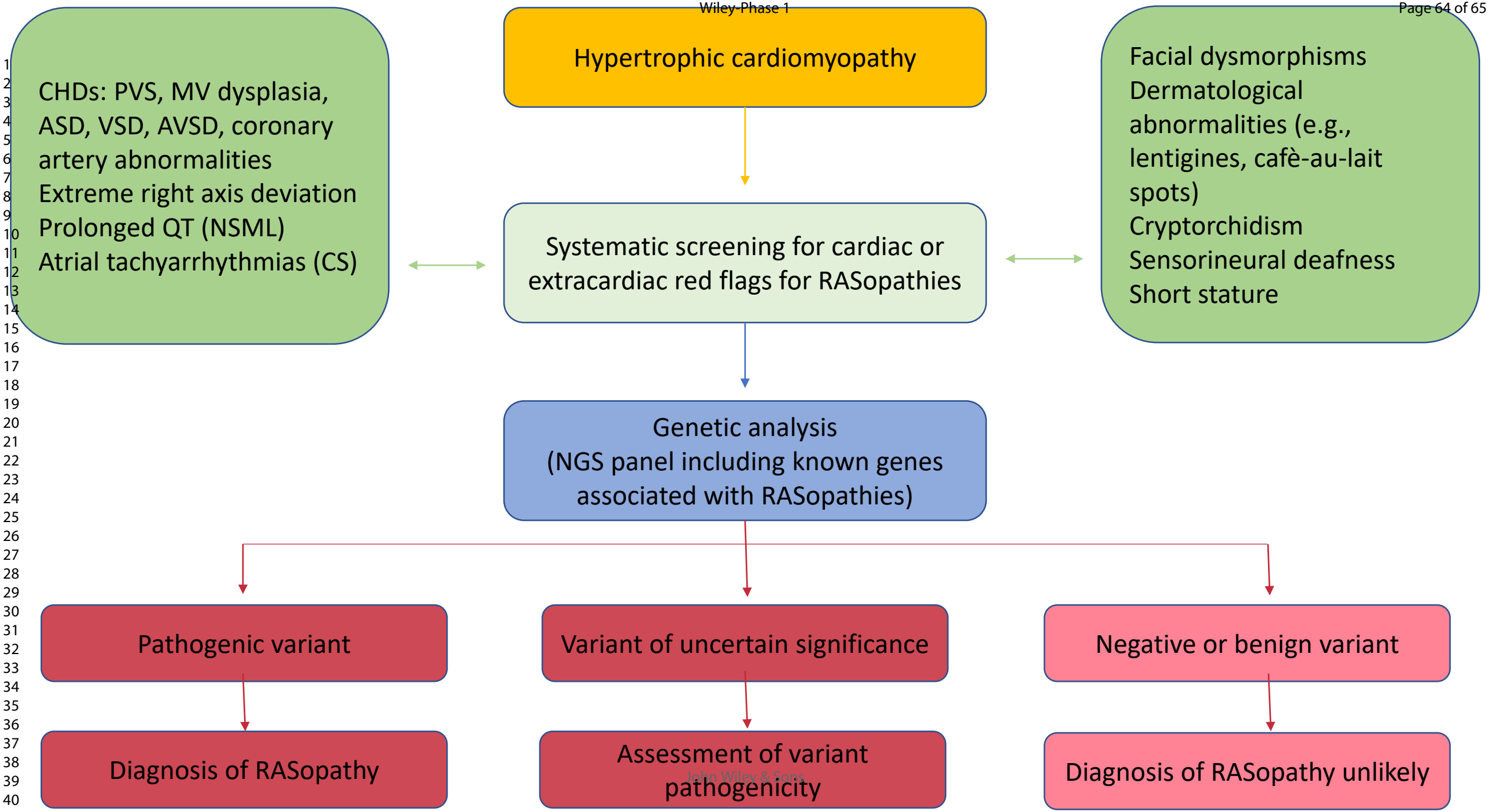
Abbreviations: CHDs, congenital heart defects; PVS, pulmonary valve stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; MV, mitral valve; ECG, electrocardiogram.

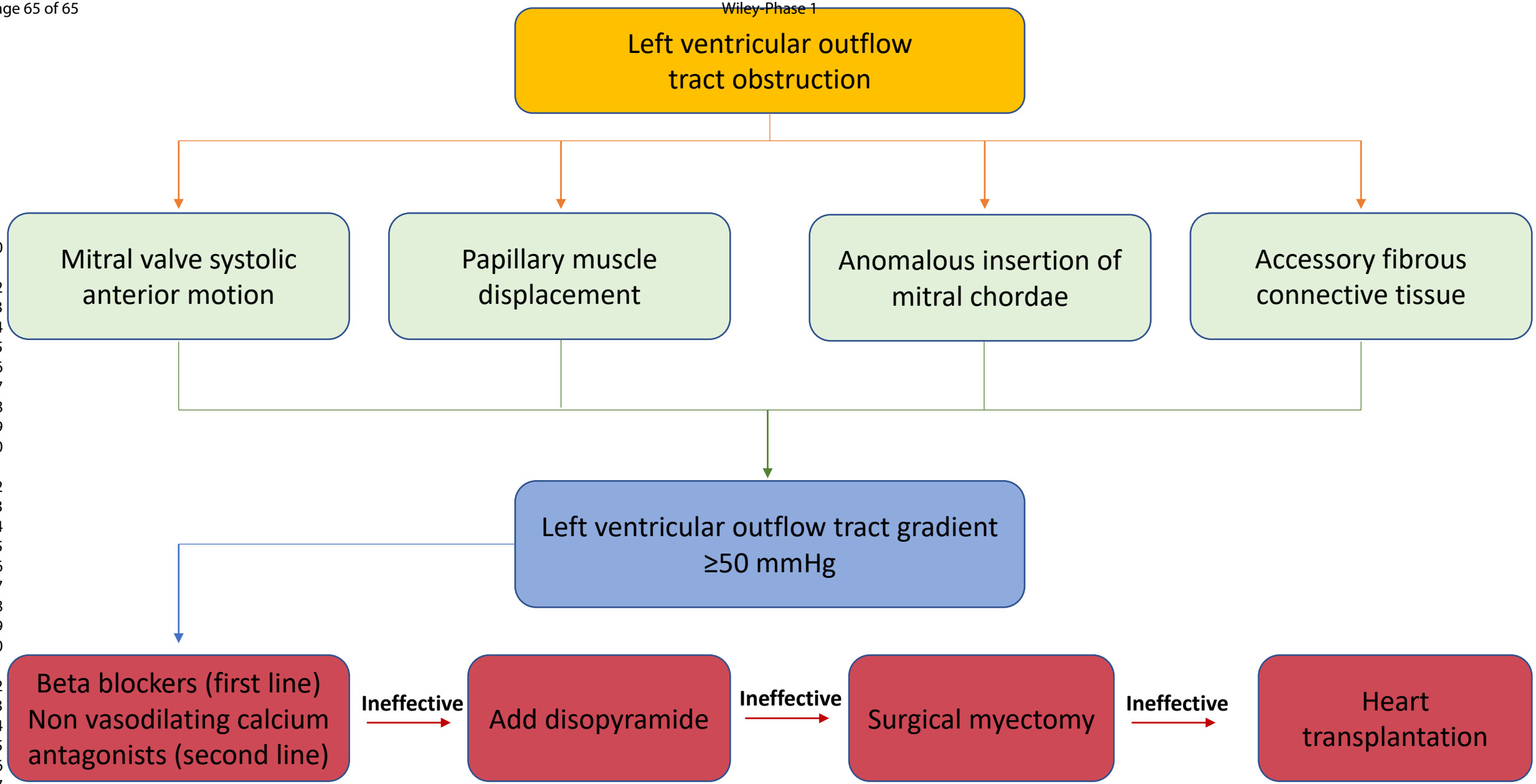
Figure 2. Diagnostic flow-chart for hypertrophic cardiomyopathy associated with RASopathies.

Abbreviations: ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHDs, congenital heart defects; CS, Costello syndrome; MV, mitral valve; NGS, next-generation sequencing; NSML, Noonan syndrome with multiple lentigines; PVS, pulmonary valve stenosis; VSD, ventricular septal defect.

Figure 3. Determinants and management of left ventricular outflow tract obstruction in hypertrophic cardiomyopathy associated with RASopathies.


Congenital Heart Defects
Common CHDs
PVS
ASD
AVSD
Clinical examination
Echocardiography, ECG, chest X-ray,
24-h ECG Holter monitoring
Less common CHDs
VSD, PDA
MV dysplasia
Left-sided obstructive CHDs
Coronary artery anomalies
Others
Symptoms related to CHDs
Significant hemodynamic lesion
Arrhythmias
YES
NO
Intervention depending on CHDs:
percutaneous or surgical repair
Medical treatment
Regular follow-up
Imaging
Treat medical comorbidities




Left ventricular outflow tract obstruction
Mitral valve systolic anterior motion
Papillary muscle displacement
Anomalous insertion of mitral chordae
Accessory fibrous connective tissue
Left ventricular outflow tract gradient ≥50 mmHg
Beta blockers (first line) Non vasodilating calcium antagonists (second line)
Ineffective
Add disopyramide
Ineffective
Surgical myectomy
Ineffective
Heart transplantation