Nonreentrant atrial tachycardia occurs independently of hypertrophic cardiomyopathy in RASopathy patients

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Abstract

Multifocal atrial tachycardia (MAT) has a well-known association with Costello syndrome, but is rarely described with related RAS/MAPK pathway disorders (RASopathies). We report 11 patients

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with RASopathies (Costello, Noonan, and Noonan syndrome with multiple lentigines [formerly LEOPARD syndrome]) and nonreentrant atrial tachycardias (MAT and ectopic atrial tachycardia) demonstrating overlap in cardiac arrhythmia phenotype. Similar overlap is seen in RASopathies with respect to skeletal, musculoskeletal and cutaneous abnormalities, dysmorphic facial features, and neurodevelopmental deficits. Nonreentrant atrial tachycardias may cause cardiac compromise if sinus rhythm is not restored expeditiously. Typical first-line supraventricular tachycardia anti-arrhythmics (propranolol and digoxin) were generally not effective in restoring or maintaining sinus rhythm in this cohort, while flecainide or amiodarone alone or in concert with propranolol were effective anti-arrhythmic agents for acute and chronic use. Atrial tachycardia resolved in all patients. However, a 4-month-old boy from the cohort was found asystolic (with concurrent cellulitis) and a second patient underwent cardiac transplant for heart failure complicated by recalcitrant atrial arrhythmia. While propranolol alone frequently failed to convert or maintain sinus rhythm, fleccainide or amiodarone, occasionally in combination with propranolol, was effective for RASopathy patient treatment for nonreentrant atrial arrhythmia. Our analysis shows that RASopathy patients may have nonreentrant atrial tachycardia with and without associated cardiac hypertrophy. While nonreentrant arrhythmia has been traditionally associated with Costello syndrome, this work provides an expanded view of RASopathy cardiac arrhythmia phenotype as we demonstrate mutant proteins throughout this signaling pathway can also give rise to ectopic and/or MAT.

Keywords

Calcium; Costello syndrome; ectopic atrial tachycardia; multifocal atrial tachycardia; Noonan syndrome; Noonan syndrome with multiple lentigines; RAS/MAPK signaling pathway

1| INTRODUCTION

The RAS/MAPK signaling pathway performs critical cellular differentiation and maintenance functions in a variety of organ systems (Figure 1) (Rauen, 2013; Tidyman & Rauen, 2009). Activating mutations in the RAS/MAPK signaling pathway result in a family of related disorders termed "RASopathies" that include Costello syndrome (OMIM #218040), Noonan syndrome (#163950), Noonan syndrome with multiple lentigines (formerly known as LEOPARD syndrome) (#151100), and Cardiofaciocutaneous syndrome (#115050). This group of disorders can have overlapping skeletal, dermatologic, musculoskeletal, and neurocognitive features. Additionally, individuals with RASopathies frequently express similar cardiac phenotypes, encompassing structural and electrophysiologic abnormalities. Common cardiac abnormalities seen in RASopathies include hypertrophic cardiomyopathy (HCM), valve abnormalities, particularly pulmonic valve stenosis, and dysplasia, as well as an increased incidence of atrial and ventricular septal defects among others (Lin et al., 2011; Prendiville et al., 2014). While the frequency and type of structural cardiovascular abnormalities in RASopathies have been detailed elsewhere (Lin et al., 2011; Pandit et al., 2007; Pierpont et al., 2014; Prendiville et al., 2014; Rauen et al., 2010; van Berlo, Maillet, & Molkentin, 2013), cardiac electrophysiological abnormalities in these syndromes have been less well characterized (Dodo, Gow, Hamilton, & Freedom, 1995), with the exception of individuals with Costello syndrome who have been

described with multifocal atrial tachycardia (MAT). Although much progress has been made in identifying the genetic causes of heritable arrhythmias, the genetic basis of nonreentrant atrial arrhythmias in infants and children is largely unknown. An attempt to identify genes that are known to cause triggered activity, and are not already known to cause channelopathy (long QT syndrome [LQTS], catecholaminergic polymorphic ventricular tachycardia [CPVT], or Brugada Syndrome) or familial atrial fibrillation yields a surprisingly small number of genes. Such genes include guanine nucleotide-binding protein inhibiting polypeptide (*GNAI2*, OMIM #192605); and trans-2,3-enoyl-CoA reductase-like protein (*TERCL*, OMIM #617242), which appears to regulate two proteins known to cause CPVT, the ryanodine receptor (*RYR2*, OMIM# 180902) and calsequestrin (*CASQ2*, OMIM#114251) . One gene associated with both ectopic and MAT includes troponin I interacting kinase (*TNNI3K*, OMIM#613932), a kinase that is also thought to affect calcium handling, and is associated with a cardiomyopathy phenotype in some instances (Theis et al., 2014). The only other two genes associated with these arrhythmias identified in OMIM are those in the RAS/MAPK pathway (*HRAS, KRAS, NRAS*, OMIM #190020, OMIM #218040).

The purpose of this study is to detail the cardiac electrophysiologic phenotypes and to characterize arrhythmia clinical course including treatment seen in individuals with RASopathies. We demonstrate that nonreentrant atrial tachycardias, MAT and ectopic atrial tachycardia (EAT), can arise from gain-of-function mutations in multiple genes giving rise to RAS/MAPK pathway dysregulation. This represents an expanded description of RASopathy cardiac phenotype as nonreentrant arrhythmia had previously only been reported in Costello syndrome patients. We further show that arrhythmia can occur in the presence or absence of cardiac hypertrophy, suggesting that the atrial arrhythmia seen in these patients is not simply resulting from hypertrophic cardiomyopathy pressure load. These findings provide important clinical information for the diagnosis and management of RASopathy patients, and may provide insights into nonreentrant atrial arrhythmia mechanisms.

2| MATERIALS AND METHODS

We retrospectively collected and reviewed the clinical, cardiac, and molecular genetic features of patients with a RASopathy who presented with arrhythmia and/or cardiomyopathy, and were cared for at six institutions (the Children's Hospital of Philadelphia, A.I. du Pont Hospital for Children, Maine Medical Center, Cincinnati Children's Hospital Medical Center, Children's Hospital Colorado, and Sanford Children's Hospital) between 2008 and 2013. Two-dimensional echocardiography was performed using standard pediatric echocardiographic methods and commercially available machines. Segmental anatomy was confirmed and quantitative measures for left ventricular (LV) end diastolic dimension (LVeDD), LV end-systolic dimension (LVeSD), interventricular septal dimension (IVS) were obtained from m-mode imaging in the parasternal short axis. These values were normalized using gender, age, and body surface area resulting in a *z*-score. Echocardiography images and reports were reviewed to evaluate for evidence of congenital heart defects, hypertrophic cardiomyopathy, and quantitative wall dimensions. *Z*-score were extracted and displayed in table format. Standard 12 or 15 lead surface electrocardiograms with rhythm strips were obtained from each patient and reviewed to confirm arrhythmia

diagnosis. Of the nonreentrant tachycardias, we defined MAT (also sometimes described as chaotic atrial rhythm, CAR) as a tachyarrhythmia with the following characteristics: (a) multiple (at least three) distinct P-wave morphologies; (b) irregular P-P intervals; (c) isoelectric baseline between P-waves; and (d) ventricular rate >100 beats/min (Bradley, Fischbach, Law, Serwer, & Dick, 2001). EAT is characterized by (a) a P-wave morphology during tachycardia that is unique from the sinus P-wave, (b) the P wave of the first beat is identical to that of subsequent beats in tachycardia; (c) the cycle length of the first few beats may progressively shorten; (d) atrioventricular block can occur in the presence of continuing atrial arrhythmia; and (e) ventricular rate >100 beats/min (Bradley et al., 2001; Salerno, Anderson, Sharkey, & Iber, 1987) .

All patients had a clinical and molecular diagnosis of a RASopathy syndrome. Molecular genetic testing was performed as clinically indicated at the commercial molecular diagnostic labs routinely used by each patient's institution.

3 | RESULTS

Table 1 summarizes the clinical, molecular and cardiac details of 11 patients with RASopathies, including two patients with Costello syndrome, Noonan syndrome with multiple lentigines (LEOPARD syndrome) (two patients), and Noonan syndrome (seven patients).

3.1 | Molecular testing

RASopathy phenotypes are seen in patients with missense mutations in *HRAS, BRAF, MEK1/2, KRAS, RAF1, PTPN11 (SHP2), SOS1, SHOC2,* and *CBL,* and since the diagnosis of the patients in this series, *RIT1*. These mutations confer a dominant inheritance pattern, resulting in gain-of-function mutations or activation of the RAS/MAPK pathway (Figure 1) (Pandit et al., 2007; Rauen, 2013; Rodriguez-Viciana et al., 2006; Tartaglia, Cotter, Zampino, Gelb, & Rauen, 2003; Tidyman & Rauen, 2009; Wu et al., 2011), with the exception of Noonan syndrome with multiple lentigines in which there are dominant negative effects from loss-of-function mutations. Each confirmed mutation is listed in Table 1.

3.2 | Noncardiac clinical features

Table 1 summarizes noncardiac and fetal findings where available. Patients were ascertained based on their clinical features. While each syndrome has characteristic clinical features, overlap of the clinical features among syndromes is well-recognized, making it difficult to predict the molecular defect. This is particularly true among Noonan syndrome patients, where mutations in different genes give rise to similar phenotypes (*PTPN11, SOS1, RAF1*). While cardiac findings featured

3.3 | Cardiac clinical features

Arrhythmias were documented from the fetal period to as late as 4 years of age (mean 222 ± 95 days) (Table 1). Presenting rhythms included MAT and EAT. Among those patients with MAT, EAT was also noted later in their clinical course. Four of these patients

demonstrated supraventricular (reentrant) tachycardia in addition to their nonreentrant atrial tachycardia. Ventricular ectopy was present in some individuals.

Figure 2 shows representative rhythm strips from a patient in MAT (Patient 3, Figure 2a) and EAT in a different patient (Patient 8, Figure 2b). Identification of multiple P-wave morphologies was facilitated by recording the trace at twice the normal sweep speed (50 mm/s; Figure 2a). By contrast, EAT tracings demonstrate typical findings of two different P-wave morphologies (one representing sinus rhythm) with irregular RR intervals (Figure 2b). In contrast to reentrant supraventricular tachycardia which demonstrates abrupt initiation and termination of the abnormal rhythm, intermittent sinus beats can be seen interspersed between tachycardia runs in Figure 2b, which is characteristic of EAT.

Each patient had an echocardiogram at the presentation of arrhythmia. Signs of cardiomyopathy were present in seven patients, while four patients showed no overt evidence of HCM. HCM is typically defined by abnormal wall thickness (Elliott & McKenna, 2004; Maron, 2006). Figure 3 provides parasternal short axis images at end-diastole and end-systole for one patient showing tachycardia without evidence of cardiomyopathy (Figure 3a,b, Patient 4) as well as images from one patient who had both atrial tachycardia and hypertrophic cardiomyopathy (Figure 3c–f, Patient 8). Echocardiographic images from Patient 4 show normal septal (labeled) and posterior left ventricular free wall thickness (Figure 3a,b). By contrast, short axis images from Patient 8 show massive septal hypertrophy that nearly obliterates the left ventricular cavity at end systole (Figure 3d). Note that during ventricular filling (end-diastole, Figure 3c) the left ventricular volume available for filling is greatly reduced compared to the filling area of the nonhypertrophic heart (Figure 3a). Four chamber imaging of Patient 8 demonstrates the ventricular septum protruding into the left ventricular outflow tract (Figure 3e,f) and companion Doppler flow imaging shows mid left ventricular cavity flow acceleration which defines a subset hypertrophic cardiomyopathy.

Quantitative echocardiographic data were available for eleven patients (Table 1) including LVeDD (z-score range: −3.84 to 2.77), LVeSD (z-score range: −5.02 to 5.32), and LV shortening fraction (range: 38.9 to 66%). Left ventricular mass z-score was available for 10 of 11 patients and ranged from −3.27 to +9.42. No patient had complex congenital heart disease, but septal defects were detected in three patients. In addition, six patients demonstrated valvar pulmonary stenosis, which was severe in one patient. No other hemodynamically significant congenital heart defects were diagnosed.

3.4 | Arrhythmia treatment

Patients presenting in the neonatal period often had mild hemodynamic compromise and poor feeding over time prior to restoration of sinus rhythm. Initial medical therapies included propranolol in 10 patients. Treatment with propranolol alone and/or with digoxin failed to maintain sinus rhythm in all but one patient (Table 1). Flecainide alone or in combination with propranolol was the most common chronic anti-arrhythmic treatment chosen. Amiodarone alone was used successfully for two patients, although one patient subsequently transitioned to propranolol and verapamil due to amiodarone toxicity. Anti-arrhythmic agents were successfully weaned in all patients, except for one who died from

complications of a rhabdomyosarcoma and a second who underwent cardiac transplantation, in part because of functional hemodynamic instability that was compounded by rhythm disturbance despite flecainide treatment. In the 10 patients whose clinical arrhythmias resolved, medications were discontinued at a mean age of 28.0 months (range 6.5–49 months). A third child from our cohort died from complications of cardiac surgery (septal myomectomy). This child's arrhythmia resolved prior to surgery, so this case was not considered an anti-arrhythmic treatment failure. A 4-month-old child with cellulitis apparently died of complications of systemic infection, unrelated to arrhythmia.

4 | DISCUSSION

This multi-institutional case series illustrates an association between mutations in RAS/ MAPK signaling pathway genes and nonreentrant atrial tachycardia (MAT and EAT). Importantly, rhythm disturbances occurred both in the presence and absence of cardiac hypertrophy. We observed predominantly nonreentrant atrial tachycardia in our cohort (i.e., EAT and MAT, which is also known as CAT). Reportedly, 84% of atrial arrhythmias in children arise from reentrant circuits involving accessory pathways (Ko, JK, BJ, Strasburger, and Benson DW jr., 1992). In contrast, in our cohort, we observed nonreentrant atrial arrhythmias, which had previously been identified only in Costello syndrome patients (Lin et al., 2011).

Distinguishing between reentrant and nonreentrant arrhythmias can be critically important as therapies and prognoses differ greatly between these two entities. Propranolol and digoxin continue to be the preferred first line treatment for reentrant arrhythmias. However, we found that infants with RASopathies and atrial tachycardia can be difficult to manage, partially because propranolol and digoxin fail to convert or maintain sinus rhythm in patients with MAT and EAT. By contrast, in this cohort, effective medications included flecainide alone, flecainide in combination with propranolol, or amiodarone alone. While propranolol can be used to treat EAT effectively in other patient populations, it is not immediately clear why this drug is less effective in this subgroup of patients. Importantly, arrhythmia resolved or was well-controlled in all but two cases. The former were successfully weaned off anti-arrhythmic medications after a period ranging from 6.5 to 49 months. The latter group included a 4 months old boy who was found pulseless and unresponsive during inpatient cellulitis treatment and died from an unclear etiology and another patient who underwent cardiac transplantation. This second patient had poorly tolerated break-through tachycardia in the context of worsening left ventricular outflow obstruction. While an atrial arrhythmia episode would not be predicted to cause catastrophic hemodynamic compromise, we cannot exclude the possibility that atrial arrhythmia complicated this boy's death.

While flecainide, a sodium channel blocker, has been used successfully to treat ectopic or MAT for over 20 years (Houyel, Fournier, & Davignon, 1990), the mechanism of its efficacy is not immediately obvious and remains controversial (Bannister et al., 2015; Hilliard et al., 2010; Smith & Macquaide, 2015; Watanabe et al., 2009). Previous studies suggested that MAT and EAT result from disrupted calcium homeostasis leading to triggered activity (Levine, Michael, & Guarnieri, 1985; Marchlinski & Miller, 1985). Flecainide, by contrast, is traditionally classified as a sodium channel blocker (Vaughan Williams, 1975), and

sodium channel blockers would not be expected to affect calcium homeostasis directly. Importantly, it has been argued that flecainide diminishes calcium release from the sarcoplasmic reticulum through ryanodine receptors in addition to its direct inhibitory action on sodium channels (Bannister et al., 2015; Mehra, Imtiaz, van Helden, Knollmann, & Laver, 2014; Smith & Macquaide, 2015; Watanabe et al., 2009). Again, nonreentrant arrhythmias arise secondary to dysregulated calcium release, such as inappropriate calcium release from the sarcoplasmic reticulum through the ryanodine receptor. While it is difficult to imagine how direct sodium channel blockade would diminish sarcoplasmic reticulum calcium release and treat nonreentrant arrhythmias, perhaps flecainide's direct or indirect action on the ryanodine receptor accounts for the drug's efficacy in treating MAT and EAT. The fact that flecainide, a drug used to restore calcium homeostasis, is an effective anti-arrhythmic for MAT and EAT in RASopathy patients suggests that disordered calcium release may result directly from RASopathy mutation expression.

In addition to increased arrhythmogenesis, disordered calcium handling can contribute to cardiomyopathy and heart failure (Anastasaki, Estep, Marais, Rauen, & Patton, 2009; Lan et al., 2013). Incessant atrial arrhythmias may cause cardiomyopathy and diminished cardiovascular function. Conversely, arrhythmias may be secondary to the pressure load conferred by a cardiomyopathy. Finally, the presence of cardiomyopathy prior to arrhythmia might suggest that nonreentrant atrial tachycardia is secondary to elevated end-diastolic pressure subsequent to cardiomyopathy (pressure load). In this retrospective case series, we found that 4 (36%) of 11 patients presenting with nonreentrant atrial tachycardia had no evidence of cardiomyopathy as indicated by increased wall thickness. Thus, at least some atrial tachycardia in our cohort did not arise secondary to pressure overload, again suggesting disordered calcium release arises as a result of some other mechanism.

Hypertrophic cardiomyopathy is defined by abnormal wall thickness (Elliott & McKenna, 2004; Maron, 2006; McNally, Barefield, & Puckelwartz, 2015). Seven (64%) of our 11 patients demonstrated cardiac hypertrophy, meeting American Heart Association criteria for hypertrophic cardiomyopathy. Four of the seven with hypertrophy demonstrated asymmetric septal hypertrophy. While 4 of 11 (36%) patients did not meet traditional criteria for diagnosing hypertrophic cardiomyopathy, despite repeated episodes of atrial tachycardia, they had elevated shortening fractions (Patient 1: 39%; Patient 4: 42%; Patient 7: 56%; Patient 10: 46%). This increased shortening fraction suggests that these patients have elevated intracellular calcium concentrations since there is a strong dependence between intracellular calcium concentration and myocyte contraction force (Kentish, ter Keurs, Ricciardi, Bucx, & Noble, 1986; Sun & Irving, 2010).

Thus, several observations suggest that RASopathy patients exhibit calcium dysregulation that can give rise to cardiomyopathy and/or atrial tachycardia. First, RASopathy patients demonstrate MAT and EAT thought to be initiated by triggered activity (Bradley et al., 2001; Marchlinski & Miller, 1985; McCord & Borzak, 1998). Furthermore, triggered activity is caused by disordered calcium homeostasis, that frequently results from inappropriate calcium release from ryanodine receptors (Wehrens et al., 2004). Medications diminishing ryanodine receptor calcium release, such as flecainide, have been particularly effective in treating RASopathy patients with MAT or EAT, suggesting that restoring calcium

homeostasis by decreasing calcium release results in decreased triggered activity and diminished arrhythmia. Finally, increased shortening fraction is consistent with increased calcium release from intracellular stores and those RASopathy patients not showing overt cardiomyopathy demonstrate hyper-contractile shortening fractions. These observations may provide the basis for testable hypotheses directed at understanding RASopathy cardiac pathology. Such testing could include expression of RASopathy-causing mutations in animal models or patient derived induced pluripotent stem cells (iPSC) and quantifying intracellular calcium content or numbers of calcium release events in models versus controls.

The current study is limited by its small patient numbers. Given the relatively rare occurrence of non-Noonan syndrome RASopathies, we pooled patients from a multi-institutional network. Although Cardiofaciocutaneous syndrome was not represented in this series, it is likely that nonreentrant tachycardia will be observed. Further work to verify our observations in a larger cohort should be performed, ideally in a prospective manner. Additional studies using animal models or patient-specific expression systems should be used to test the hypotheses raised here. This would be an important first step in deepening our understanding of RASopathy cardiac disease mechanisms and could ultimately lead to the identification and development of novel therapeutics for patients with RASopathies.

In conclusion, we report that patients with RAS/MAPK pathway mutations have nonreentrant atrial tachycardia that is poorly treated by propranolol or digoxin (typical first-line SVT treatments), but responds well to flecainide or amiodarone alone or in concert with propranolol. Atrial tachycardia in RASopathy patients occurred in the presence and absence of hypertrophic cardiomyopathy. Those patients with nonreentrant tachycardia without hypertrophic cardiomyopathy frequently demonstrated hyper-dynamic function as evidenced by increased shortening fraction, suggesting that they possessed increased intracellular calcium. Nonreentrant tachycardia resolved in 9 of 11 patients. In addition to providing insights that will aid management of patients with a RASopathy, the clinical findings here suggest the hypothesis that activating RAS/ MAPK pathway mutations result in intracellular calcium dysregulation that leads to either arrhythmia, cardiac hypertrophy, or both. There is a need for additional investigation and longitudinal study in this patient population to define evidence based guidelines for cardiac surveillance, but, in the interim, contributing authors have suggested collaborative expert opinion consensus statements may also be useful. Toward this end, we would suggest raising suspicion for arrhythmia and cardiomyopathy in neonatal RASopathy patients. Respiratory distress, diminished feeding tolerance, or unexplained irritability should suggest cardiac evaluation, including ECG, echocardiogram and 24 hr ambulatory ECG monitoring. In addition to typical signs and symptoms of arrhythmia or cardiomyopathy, evidence of hyperdynamic cardiac function seen on echocardiogram should also raise suspicion for arrhythmia, cardiomyopathy or nascent cardiomyopathy as this was present in some study patients with arrhythmia prior to overt chamber dimension abnormalities. This case series should increase awareness among pediatric cardiologists and geneticists about these increasingly recognized syndromes and provide clinical information assisting the diagnosis and management of cardiac disease, and, ultimately promote understanding of the molecular mechanisms underlying these diseases.

Acknowledgments

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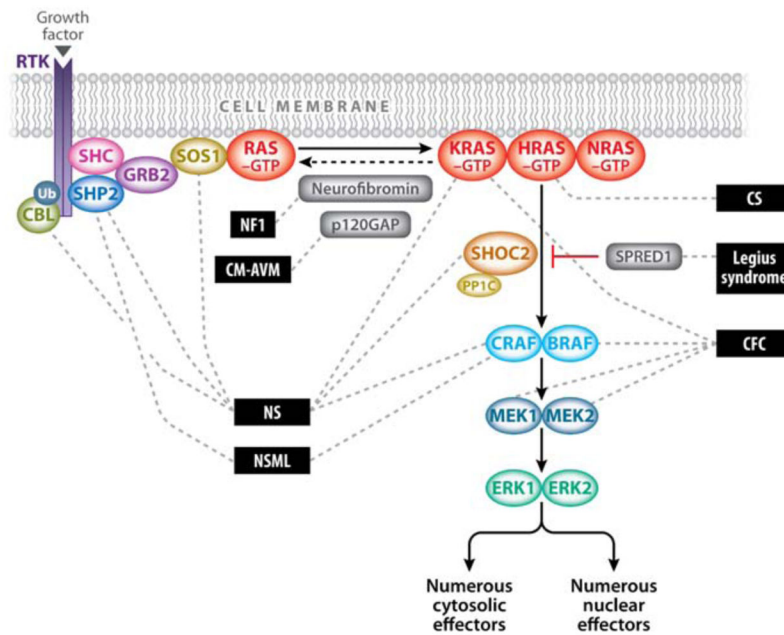
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FIGURE 1.
The RAS/mitogen-activated protein kinase (MAPK) signaling pathway and associated developmental syndromes (indicated by dashed lines). The MAPK signaling pathway of protein kinases is critically involved in cell proliferation, differentiation, motility, apoptosis, and senescence. The RAS/MAPK pathway proteins with germline mutations in their respective genes are associated with Noonan (NS), Noonan syndrome with multiple lentigines (NSML)(LEOPARD), neurofibromatosis, type 1 (NF1), capillary malformation–arteriovenous malformation (CM-AVM) due to *RAS1* mutations, Costello (CS), Cardiofaciocutaneous (CFC) and Legius syndromes. Figure reprinted from Rauen (2013) with permission from Elsevier. [Color figure can be viewed at wileyonlinelibrary.com]
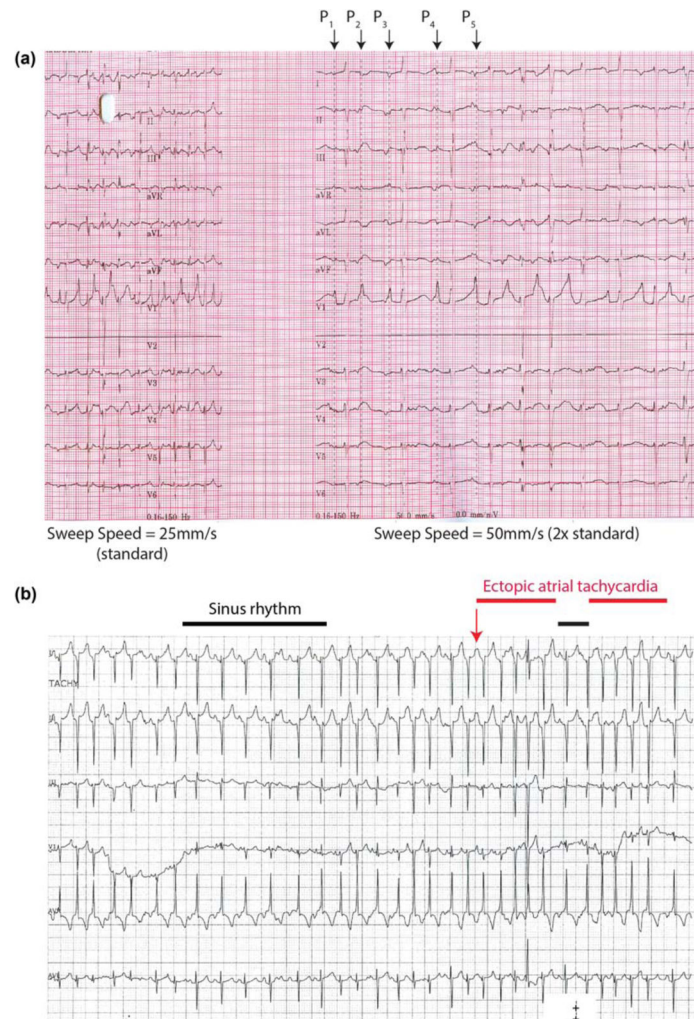
FIGURE 2.

Rhythm strips showing multi-focal atrial tachycardia and ectopic atrial tachycardia (a) 12 lead rhythm strip from Patient 3 recorded at sweep speeds of both 25 and 50 mm/s demonstrating multifocal atrial tachycardia with at least five different P-wave morphologies (labeled P1 to P5). Note how the increased sweep speed (50 mm/s) facilitates diagnosis when compared to the portion recorded at the standard sweep speed (25 mm/s). (b) Rhythm strip from patient 8 demonstrating episodes of sinus rhythm (denoted by black bar) followed by intermittent episodes of ectopic atrial tachycardia (red bar). In contrast to the tracing from (a), P-wave morphology, axis and amplitude are roughly the same. Unlike reentrant arrhythmias, patients may quickly enter and exit atrial tachycardia and have sinus beats interspersed between nonsinus beats as seen here. [Color figure can be viewed at wileyonlinelibrary.com]
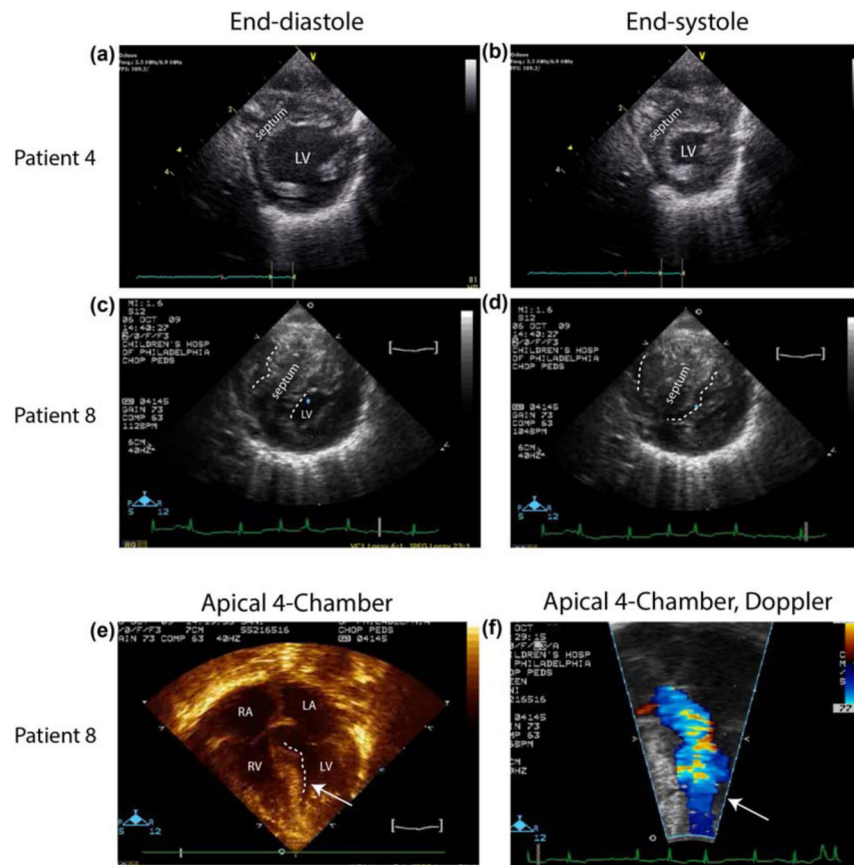
FIGURE 3.

Parasternal short axis echocardiographic images from RASopathy patients presenting with nonreentrant atrial tachycardia showing ventricular wall dimensions at both end-diastole (a, c), and end-systole (b, d), for patient 4 (a, b), patient 8 (c–f). Note that images from patient 4 show normal septal and free wall thickness, while those images from patient 8 show hypertrophied septum and myocardium, with muscle nearly obliterating the LV cavity at end systole in patient 8. Four chamber imaging from patient 8 demonstrates left ventricular outflow tract obstruction (e) and Doppler flow imaging of the identical region shows mid-cavitary flow acceleration (f). [Color figure can be viewed at wileyonlinelibrary.com]

prominently in the clinical presentation, a number of distinct phenotypic features prompted testing for RAS/MAPK disorders (Table 1). Postnatally, hyper-pigmented macules and dysmorphic craniofacial features prompted molecular testing with increased suspicion for RASopathy. Additional features suggesting RASopathies included down slanted palpebral fissures, wide nasal root with flat bridge, low set dysmorphic ears, and coarse facial features.

TABLE 1

Genetic, arrhythmia and echocardiographic findings in 11 patients with RASopathies

Pt no	1	2	3	4	5	6	7	8	9	10	11	Total
Sex	F	M	F	M	M	M	F	F	M	F	M	F 5 M 6
Genetic and molecular findings												
Syndrome	CS	CS	NS	NS	NS	NS	NS	NS-ML	NS-ML	NS	NS	CS 2 NS 7 NS-ML 2
Age at disease presentation	Birth	4 mos	Birth	Birth	Birth	Birth	Birth	Birth, after cardiac eval	Birth after cardiac eval	2 wks	Birth	
Affected Gene	HRAS	HRAS	RAF1	RAF1	RAF1	RAF1	PTPN11	PTPN11	PTPN11	SOS1	KRAS	HRAS 2 RAF1 4 PTPN11 3 SOS1 1 KRAS 1
Base Change	c.35G>C	c.34G>A	c.770C>T	c.781C>T	c.770C>T	c.770C>T	c.188A>G	c.138G>A	c.1493G>T	c.2536G>A	c.173C>T	
AA Change	p.Gly12 Ala	p.Gly12 Ser	p.Ser257 Leu	p.Pro261 Ser	p.Ser257 Leu	p.Ser257 Leu	p.Tyr63 Cys	p.Ala461 Thr	p.Arg498 Leu	p.Glu846 Lys	p.Thr58 Ile	
Arrhythmia findings												
Age at Arrhythmia presentation	Prenatal: 35 wks	Postnatal: 8 mos	Postnatal: 7 days	Postnatal: 1 day	Postnatal: 4 years 1 mo	Postnatal: 3 mos	Postnatal: 2 mos	Postnatal: 3 wks	Postnatal: 2 mos	Postnatal: 4 mos.	Postnatal: 3 wks	Mean: 222 ± 95 days
MAT	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	7 Yes 4 No
EAT	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11 Yes 0 No
SVT	No	Yes	No	No	Yes	Yes	No	No	No	No	Yes	4 Yes 7 No
Max arrhythmia rate (presenting ECG) (bpm)	250	240	246	209	220	207	227	190	220's	Atrial rate 300's, ventricular rate 170	300	Mean: 227.4 ± 37.6
Treatment failures	Amio	None	Dig, Prop	Dig, Prop, Amio	Prop, Verap, Sotalol	Prop, Amio thyroid toxicity	Prop	Prop, Amio	Esmolol	Esmolol	None	6 Prop 2 Esmolol 2 Dig 4 Amio
Arrhythmia findings												

Pt no	1	2	3	4	5	6	7	8	9	10	11	Total
Sex	F	M	F	M	M	M	F	F	M	F	M	F 5 M 6
Age at Arrhythmia presentation	Prenatal: 35 wks	Postnatal: 8 mos	Postnatal: 7 days	Postnatal: 1 day	Postnatal: 4 years 1 mo	Postnatal: 3 mos	Postnatal: 2 mos	Postnatal: 3 wks	Postnatal: 2 mos	Postnatal: 4 mos.	Postnatal: 3 wks	Mean: 222 ± 95 days
Treatment	Flec	Prop	Flec	Prop +Flec	Transplant	Propr+ Amio x 2.5 years, Prop + Verap	Propr+ Flec	Flec	Propr +Flec	Amio	Prop	3 Flec, 2 Prop, 3 Propr+Flec, 1 Amio, 1 Prop+ 1 Amio 1 Prop+ Verap
Anti-arrhythmic therapy at last FU	Yes	Yes	No	No	NA, after heart tx	Yes	No	No	Yes	No	NA	4 Yes 4 No
Duration of anti-arrhythmic therapy (mos)	24	18	24	16	49	42	36	30	34	6.5	4	Mean: 25.7 ± 14.1
Age at last FU	27 mos (deceased)	23 mos	4 yrs	3 yrs 5 mos	8 yrs 7 mos	3 yrs 4 mos	5 yrs 11 mos	4 yrs	3 yrs	3 yrs 11 mos	4 mos (deceased)	Mean: 54 ± 27.9mos
Presenting echocardiogram findings												
Cardiac hypertrophy at arrhythmia presentation	No	Conc hypertrophy	Conc hypertrophy, with thick septum	No	Mod to severe ASH	ASH	No	ASH	ASH	No	Mild septal hypertrophy	7 Yes 4 No
LVeDD Z-score	2.51	−1.34	2.77	−1	−0.54	−3.58	1.3	1.43	−3.84	−3.9	−0.63	
LVeSD Z-score	2.03	−1.76	5.32	−1.16	−4.56	−3.64	1.4	3.43	−5.02	−4.1	−2.0	
SF (%)	38.9%	45%	66%	42.11%	64%	46%	56%	55%	55%	46%	55.5%	Mean: 51.8 ± 8.8%
LV mass Z-score	−3.27	+4.22	−1.60	−1.86	+5.73	+0.95	+.25	+3.23	1.79	NA	+9.42	
Valvar PS	No	No	No	Yes	Mild	No	Mild	Mild	No	Severe	Mild	6 Yes 5 No
Other CHDs	No	Abn papillary muscle, SAM	No	Mod ASD	No	ASD, small VSD, mild MS	No	No	No	ASD	No	4 Yes 7 No
Noncardiac clinical problems												
Poor feed	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	8 Yes 3 No
Failure to gain wt	No	No	No	No	No	No	No	No	No	Yes	Yes	2 Yes 9 No
Dev. delay	No	Yes	AuSD	Speech delay	Yes	No	Mild	No	No	No	No	5 Yes 6 No
Noncardiac clinical problems												

Pt no	1	2	3	4	5	6	7	8	9	10	11	Total
Sex	F	M	F	M	M	M	F	F	M	F	M	F 5 M 6
Typical facies	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	CS-like	11 Yes ~1 No
Short stature	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	4 Yes 7 No
Hyper-pigmentation	No	No	CALM	No	No	No	Nevi	CALM	Yes, Lentigines	No	No	4 Yes 7 No
Skeletal	No	No	No	Pectus car	No	No	pectus exc	No	No	Macrocephaly	No	
Other	Embryonic RMS, pelvis			Conductive HL		SS, s/p trach, s/p G-tube	Mildly curly hair			Mild hydronephrosis		
Fetal Abnormalities												
Gest age (wks)	35	36	38	36	35	33	39.5	35	40	39	37	Mean: 36.7 ± 2.2 mos
Fetal arrhythmia	Yes	None	None	Fetal bradycardia	None	None	NA	None	Yes	No	None	3 Yes
Poly-hydramnios	Yes	Yes	Yes	No	No	Yes	NA	Yes	No	Yes	Yes	7 Yes
LGA	No	Yes	No	No	No	No	poss SGA	No	No	No	NA	1 Yes
Nuchal lucency or cystic hygroma	Yes	No	Yes	No	No	Yes	NA	Yes	No	No	NA	4 Yes
Brain abn	VM	No	Macrocelphaly	Retro-cerebellar CSF collection	No	DW	NA	WM atrophy, VM	No	NA	VM	6 Yes
Fetal heart disease	Yes Details NA	No	Small muscular VSD	Valvar PS; ASD	Mild valvar PS, abn MV	DORV, hypo MV, AV	NA	Small VSD	No	Valvar PS; ASD	No	7 Yes

AA, Amino acid; Abn, abnormal or abnormality; Amio, Amiodarone; ASD, atrial septal defect; ASH, asymmetric septal hypertrophy; AuSD, autistic spectrum disorder; CALM, cafeé au lait macule; car, carinatum; conc, concentric; CS, Costello Syndrome; decd, deceased; Dig, Digoxin; DW, Dandy-Walker malformation; DORV, double outlet right ventricle; EAT, ectopic atrial tachycardia; eval, evaluation Flec, Flecainide; exc, excavatum; eval, evaluation Flec, Flecainide; exc, excavatum; eval, evaluation; HL, hearing loss; hypo, hypoplastic; LGA, large for gestational age; LV, left ventricle; LVeDD, left ventricle end-diastolic dimension; LVeSD, Left ventricle end-systolic dimension; mod, moderate; MS, mitral stenosis; MAT, multi-focal atrial tachycardia; mos, months; NA, not available or not assessed; NS, Noonan syndrome; NS-ML, Noonan syndrome with multiple lentigines; poss, possible; Prop, Propranolol; PS, pulmonary stenosis; Pt no, patient number; RMS, rhabdomyosarcoma; SAM, systolic anterior motion; SS, subglottic stenosis; tx, transplantation; VM, ventriculomegaly; VSD, ventricular septal defect; WM, white matter; wt, weight; wks, weeks; yrs, years; mos, months.