

Pulmonary Hypertension in the RASopathies

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Abstract

The RASopathies are a class of developmental disorders caused by a genetic mutation in the Ras signaling pathway and associated mitogen-activated protein kinases that control the cell cycle, differentiation and senescence. These diseases encompass a diverse set of clinical syndromes including neurofibromatosis type 1 and Noonan syndrome. Although the pathophysiological manifestations of these conditions are diverse, they share some common phenotypic features. The prevalence of pulmonary hypertension in the RASopathies is not well established as compared to cardiac and neurocognitive impairments. This paper reviews the cases of pulmonary hypertension in these member syndromes. Due to the aggressive and often fatal nature of pulmonary hypertension, a diagnosis of a RASopathy should also include screening for pulmonary hypertension.

Keywords: Pulmonary hypertension; RASopathies; Congenital heart disease; Noonan's syndrome; von Recklinghausen neurofibromatosis

Introduction

The RASopathies are a class of developmental disorders caused by a genetic mutation in the Ras signaling pathway and associated Mitogen-Activated Protein (MAP) kinases that control the cell cycle, differentiation and senescence [1]. These diseases encompass a diverse set of clinical syndromes including: capillary malformation-AV malformation syndrome, autoimmune lymphoproliferative syndrome, Costello syndrome, hereditary gingival fibromatosis type 1, Legius syndrome, LEOPARD syndrome, cardiofaciocutaneous syndrome, neurofibromatosis type 1 (NF-1; also called von Recklinghausen neurofibromatosis), and Noonan syndrome [2]. While some of these conditions had been recognized as early as the 1800s, [3] the identification of a common etiological signaling pathway and the idea that these diseases form a class of RASopathy malformations is a much more recent event [4]. In 1990, NF-1 was the first member of this group of diseases whose genetic etiology was identified [5] and a major breakthrough came in mid 2000 as the alterations in the Ras-Raf-MEK-ERK pathway were characterized and associated mutations identified [4]. RASopathies encompass one of the largest malformation syndrome groups with a frequency ap-

proaching 1:1000[2]. The Ras signaling pathway has long been studied for its association with cancer and the Ras gene can function as an oncogene [6].

Although the pathophysiological manifestations of these conditions are diverse, they share some common phenotypic features and have collectively been termed "Neuro-Cardio-Facial-Cutaneous" (NCFC) syndromes [7]. Patients exhibiting these syndromes tend to present with a combination of cranio-facial abnormalities, cardiac malformations, and short stature. Also common among these patients are cutaneous, musculoskeletal and genital abnormalities [8]. While most of these syndromes exhibit some degree of common phenotypic presentation, they each have unique features and can be considered a spectrum. Although they may have a common etiology, these conditions are often difficult to accurately diagnose without genetic testing.

Multiple malformation syndromes are commonly associated with cardiac abnormalities. In Noonan Syndrome (NS), valvular pulmonary stenosis is the most common defect but Atrial Septal Defect (ASD), Ventricular Septal Defect (VSD), and Patent Ductus Arteriosus (PDA) have all been reported in NS as well as all the other RASopathies. Pulmonary Arterial Hypertension (PAH) is frequently seen in patients with a large VSD or PDA but is also important in ASD. Malformations involving cardiac valves can have drastic effects on patients' health and quality of life and often are the primary complication in

these patients. However, less commonly studied are the effects of these syndromes on the respiratory system and, in particular, Pulmonary Hypertension (PH). The difficulty in studying PH in these patients is that it can often develop secondary to cardiac malformations or other lung diseases. Since PH is not a disease of the pulmonary vasculature in these patients, it is treated as a symptom of another pathological process. In addition, PH in patients with RASopathies presents very similar to other thoracic complaints and is often overlooked in favor of its more frequent cardiac etiologies.

In 1951, Dresdale coined the term primary pulmonary hypertension to describe hypertensive vasculopathy occurring exclusively in the pulmonary vasculature without a definable cause. In 2003, following the Third World Symposium on Pulmonary Arterial Hypertension held in Venice, Italy, the term “primary pulmonary hypertension” was replaced with the term “idiopathic pulmonary arterial hypertension” and two additional categories, namely “familial pulmonary arterial hypertension” and “pulmonary arterial hypertension”. The etiology of PH has been classified into 5 major groups: (1) pulmonary arterial hypertension, (2) left heart disease, (3) lung disease/hypoxia, (4) thromboembolic disease and (5) multifactorial [11]. PAH is a disease of the pulmonary vasculature and is not secondary to another disease process. Whatever the cause, severe PAH is a rare but very serious condition.

PH can occur as a result of disease causing increased resistance in the pulmonary arterial resistance vessels (arterioles) or passively in disease causing increased pressure in the pulmonary veins (such as increased left atrial pressure). Patients can present with decreased exercise tolerance and symptoms of heart failure. Physical examination reveals increased jugular venous pressure, a reduced carotid pulse, and a palpable right ventricular impulse. Most patients have an increased S2 heart sound. Imaging studies including ECG and echocardiography have distinct presentation and diagnosis is confirmed via catheterization with pulmonary arterial pressures greater than 25 mm Hg at rest. Autopsy results of long standing PH show common histopathologic changes characterized by medial hypertrophy, eccentric and concentric intimal fibrosis, recanalized thrombi appearing as fibrous webs, and plexiform lesions [9].

With the increasing knowledge in genetics now available, the mutation in the bone morphogenetic protein receptor type II (BMPR2) gene on chromosome 2 and 33 has been identified as a common cause of familial PAH. PAH has also been reported in a number of rare genetic and metabolic diseases. A rapidly developing PH in von Gierke’s disease, or glycogen storage disease type 1a, has also been reported, perhaps due to an abnormal production of serotonin [10,11] or to the possible risk from a porto-caval shunt. There have also been several cases of Gaucher’s disease with unexplained PH and in one a polymorphism in exon 13 of BMPR2 was found. Hereditary Hemorrhagic Telangiectasia (HHT or Osler-Weber-Rendu disease) is another genetic disorder with associated PAH. Mutations in genes encoding endoglin (ENG) and activin A receptor-like kinase type I (ALK-1) are associated with HHT1 and HHT2, respectively. It is not surprising therefore to learn that germline mutations in the RAS-MAPK pathway could

also lead to PAH. In this review, we will report on the association of the RASopathies with PAH.

Neurofibromatosis Type 1 (NF-1; von Recklinghausen disease)

NF-1 was the first of the RASopathies to be formally described (1882) and has the second highest incidence of 1:3000 [12]. It is inherited as an autosomal dominant trait and phenotypically manifests as a heterozygous mutation of the NF-1 gene, a tumor suppressor that regulates the RAS gene [13] located on chromosome 17 [14]. NF-1 is characterized by a number of distinct clinical features -- in particular, café-au-lait spots, plexiform neurofibromas, intertriginous freckling, iris Lisch nodules, osseous dysplasia, optic pathway glioma and other features [2]. Cardiac malformations are quite rare when compared to other RASopathies. Its clinical presentation is very similar to Legius syndrome (also a RASopathy), which is caused by a mutation in the *SPRED1* gene and is often misdiagnosed as NF-1 mainly because both of these diseases present with café-au-lait spots [15]. An under-recognized pathology in these patients is the presence of vascular lesions due to vasculopathy, with an estimated incidence of 6.4%. However, this might be an underestimation, as lesions are only diagnosed following development of symptoms and many small lesions may be clinically occult [16].

PH in cases of NF-1 has been reported in medical literature as early as the 1980s. However, advancements in the understanding of the pathology of NF-1 have caused a shift in the proposed etiology. Thoracic complications of NF-1, in particular the association with pulmonary interstitial fibrosis, was initially described by Massaro and Katz in 1966 who showed that in 76 patients with roentgenographic changes, most showed a diffuse, mottled infiltrate resulting in what they termed a “honey-comb lung” on chest radiographs. They went further to describe interstitial fibrosis by lung biopsy in several of these patients that showed a normal chest radiograph and showed that chest films may not be the most accurate method of diagnosing interstitial fibrosis in these patients [17,18]. A case study by Porterfield *et al.* in 1986 [19] linked the occurrence of PH to interstitial fibrosis in NF-1 patients, describing the case of a 56 year old female. The patient presented with dyspnea and exercise intolerance and, following examination, the diagnosis of PH was made. Based on the presence of increased interstitial markings they concluded that the PH was secondary to interstitial fibrosis based on the work of Massaro and Katz. By contrast, in 1981 Riccardi did not observe pulmonary parenchymal disease in over 200 patients with NF-1 [20]. In 1986, Burkhalter *et al.* showed that less than 7% of their patients with NF-1 showed diffuse interstitial lung disease but it has been suggested that since this study did not control for smoking, the incidence of fibrosis may, in fact, be much lower [21]. More recently in 2005, Ryu *et al.* examined 156 adult patients with NF-1 over a 6 year period and showed that bilateral interstitial infiltrates were noted in only three patients (1.9%), all of whom had potential causes other than NF-1 for their conditions, including smoking-related disease, rheumatoid lung disease, recurring pneumonias and a history of ARDS [22].

A shift in the understanding of PH pathology occurred in 1999 when Samuels *et al.* published the case of a 51 year old male with NF-1 who was diagnosed with PH with symptoms suspected to be secondary to thromboembolic disease [23]. Diagnostic work-up ruled out this cause and biopsies for interstitial fibrosis were negative. This patient's echocardiography showed right ventricular hypertrophy with atrial and ventricular dilation and PH with an established pulmonary artery pressure of 80-90 mm Hg. The pathologic report on endarterectomy showed extensive irregular thickening of the intima by fibrous tissue. This was the first published account of pulmonary vasculopathy involvement in NF-1. Shortly thereafter, in 2001, Aoki *et al.* published two cases of PH in two Japanese women (aged 19 and 70) whose etiology could not be attributed to secondary causes or interstitial fibrosis and the authors hypothesized it to be caused by vasculopathy [24]. Cardiac catheterization pressures in the 19-year-old female revealed a pulmonary artery pressure of 84/31 mm Hg with a systemic arterial pressure of 102/65. The patient was treated with nifedipine to reduce total pulmonary resistance without significantly affected systemic blood pressure. Her symptoms worsened after discharge and she was admitted with pulmonary artery pressures of 117/54 mm Hg on cardiac catheterization. This time she was treated with IV epoprostenol and her dyspnea and chest discomfort improved. She received oral beraprost and IV epoprostenol and returned home. She died at the age of 20. The second case was a female who died with PH at 70 years of age. On her first admission at 66 years of age, she had pulmonary arterial pressures of 70/22 mmHg by cardiac catheterization with systemic blood pressure of 132/78 Hg and she was diagnosed with primary PH and placed on isosorbide dinitrate, diuretics, and home oxygen. On second admission, oral beraprost was started but her symptoms did not improve. By her third admission at 69 years of age, cardiac catheterization pressures demonstrated pulmonary artery pressures of 96/30 mm Hg and systemic blood pressure of 132/78 mm Hg. She improved within 3 months of receiving continuous epoprostenol infusion therapy.

In 2007 Simeoni *et al.* published a similar case of PH with pulmonary artery intimal fibrosis [25] in a 51 year old female with progressive dyspnea on exertion with hypertension since 20 years of age. On examination, she had a systemic blood pressure of 180/105, oxygen saturation of 90%, and cardiac catheterization pulmonary artery pressures of 108/39 with a mean of 65. Based on the diagnosis of severe PH, the patient was started on diuretics and bosentan. When her dyspnea worsened, sildenafil therapy was initiated. To exclude other causes of PAH, BMPR2 analysis was conducted and found to be negative. Pulmonary artery biopsy revealed medial hypertrophy and marked intimal proliferation in this patient with NF-1. The authors concluded that all patients with NF-1 require trans-thoracic echocardiogram for detecting PAH.

The largest series so far reported is from a 2007 published report by Stewart *et al.* who described four new patients with NF-1 who developed PAH and reviewed an additional four previously reported patients. They described NF-1 as a distinct cause of PH which should be reclassified as *NF-1-associated PAH* [26] under the *Revised Clinical Classification of Pulmo-*

nary Hypertension [27]. While earlier studies suggested that NF-1 is associated with vasculopathies, this study elaborated on the pulmonary arterial bed susceptibility to vasculopathy based on radiography and CT studies. Descriptions of the patients (gender, age at presenting complaint, cardiac catheterization pressure, vital status, echocardiographic results, imaging, BMPR2 testing, treatment, biopsy samples, and cause of death)" are summarized by Stewart *et al.*

Following diagnosis of patients with PAH, CT studies showed a mosaic vasculature pattern indicative of an intrinsic vasculopathy. All patients in this study presented with sub-acute progressive dyspnea and previous diagnosis of NF-1. In all cases, all other causes of dyspnea were ruled out and upon diagnosis of PH all extrinsic causes were eliminated. *BMPR2* gene testing was negative in the seven patients tested. Upon conclusion of the study, all four patients died due to respiratory failure. In one patient studied postmortem, the lung pathology was consistent with PAH, demonstrating marked medial hypertrophy and intimal proliferation with characteristic plexiform lesions. In another patient, lung biopsy showed medial hypertrophy and marked intimal thickening.

It is not surprising that NF-1 could be associated with PAH since vasculopathies are well known associations. NF-1 vasculopathy is usually associated with smooth muscle hyperplasia and intimal proliferation leading to luminal occlusion. The renal arteries are muscular arteries and are not similar to the peripheral pulmonary artery resistance vessels which are much smaller and more distal. Renal vascular disease leading to systemic hypertension in NF-1 is the most common cause of pediatric renal artery stenosis. The vascular lesions in PAH are remarkably similar to that found in the affected renal arteries. NF-1 encodes neurofibromin, a tumor suppressor, which serves as a negative regulator in the Ras signal transduction pathway. Mutations in the NF-1 gene result in inactivated forms of neurofibromatosis and the resulting increased cellular proliferation is a major contributor to the high rate of tumor formation. This same mechanism contributes to the vasculopathy associated with NF-1.

More recently, Montani *et al.* further examined the relationship between PAH and vasculopathy in NF-1 patients and expanded upon the work of Stewart *et al.* [16]. In a study of 8 patients, Montani *et al.* concluded that NF-1 associated with PAH was predominately observed in females, with a median age of diagnosis of 62 compared to 21 years in Idiopathic PAH. The mean delay between diagnosis of NF-1 and development of PAH was about 44.5 years. Two more cases of the vasculopathy etiology were further described in a study by Malviya *et al.* in 2012. [28]

Noonan's syndrome (NS)

As with most RASopathies, Noonan syndrome is an autosomal dominant condition. About 50% of patients with NS have a mutation in the *PTPN11* gene [29]. It is among the most common of the malformation syndromes with a frequency estimated at 1:1000-1:2500, approaching that of Down syndrome. With approximately 50-80% of patients having a cardiac pathology, [30] it is the most common genetic syndrome

associated with cardiac malformation [31]. Phenotype characteristics of NS include distinctive facial features, short stature, web neck, barrel chest and cardiac anomalies (Pulmonary Stenosis > 50%) [8, 29, 30].

The role of PH in this patient population is, as yet, poorly studied with few cases present in the literature. In 1979, Holt *et al.* reported that four adult patients with NS also presented with cyanotic congenital heart disease and described some possible pulmonary hypertensive characteristics [32]. In 1989, Tinker *et al.* similarly described the case of a 19-year-old woman with Ullrich-Noonan syndrome diagnosed at two years of age who had a six-month history of progressive dyspnea and cyanosis with clinical features suggestive of severe PH. Her father and one sister also had NS. A cross sectional echocardiography showed pronounced right ventricular pressure overload with right ventricular hypertrophy and a cardiac catheterization confirmed severe PH of 140/50. Attempts to reduce pulmonary artery pressure with calcium channel blockers were unsuccessful and the patient died two weeks later. At postmortem examination there were two slit-like defects in the inter-atrial septum (1.1 x 0.3 cm and 0.9 x 0.2 cm). The pulmonary vessels showed medial hypertrophy and intimal proliferation with numerous plexiform lesions, which confirmed primary PH [33]. A case study published in 1982 by Okahata *et al.* in Japan and a review compiled by Noonan in 2005 [29] mentions PH observations in several patients but case reports are not provided.

We would like to report two additional patients with NS and PAH. Case one was a female diagnosed at 2 months of age with an ASD and treated with digoxin secondary to cardiac symptoms. At 20 months of age, a large ASD was surgically repaired. A diagnosis of failure to thrive was made and at age 3.5 a diagnosis of NS was made. There was moderate PH present at the time of ASD diagnosis. By age 7 years, her pulmonary hypertrophy had progressed and Dr. Robyn Barst at Columbia University treated her with IV prostacycline. She showed some short-term modest improvement but her symptoms progressed and she underwent a lung transplant at age 10 years. The explanted lungs showed the typical findings of PAH. She had some evidence of rejection early on but she then did well for the next 8 years. Unfortunately, she developed kidney failure from immunosuppressive therapy and her condition rapidly worsened. Peritoneal dialysis was started at age 18 years but her condition worsened and after a complicated prolonged hospitalization she died at almost 19 years of age.

Our second case is of a 9 lb. 12 oz. female noted to have non-specific dysmorphic features. Her respiratory distress attributed to a PDA resolved and she was discharged and followed with the diagnosis of a hemodynamically insignificant ASD with mild asthma. She developed pneumonia at age 9 years of age. A diagnosis of NS was made at age 13 years based on high palate, webbed neck, ptosis, low hairline, pectus excavatum, and a variant of von Willebrand's disease. She developed symptoms of shortness of breath and findings suggestive of PAH. Her pulmonary symptoms progressed and she died awaiting a lung transplant. Postmortem findings revealed a small ASD 4 x 9 mm considered hemodynamically insignificant. Histologic studies of the lung showed the expected findings of PAH with medial hypertrophy, intimal proliferation with plexiform and

angiomatosis. The terminal event was an extensive acute pulmonary hemorrhage.

Cardiofaciocutaneous Syndrome (CFC)

Cardiofaciocutaneous (CFC) syndrome is a rare, sporadic condition that shares phenotypic features with NS and Costello syndrome as well as NF-12. It presents with multiple congenital anomalies and mental retardation and is characterized by failure to thrive, relative macrocephaly, distinctive facial features, cutaneous involvement, and congenital heart defect (most commonly pulmonic stenosis and hypertrophic cardiomyopathy) [34,35]. It is caused by gain-of-function mutations in four different genes: *braf*, *kras*, *mek1* and *mek2* [34]. This syndrome has less than 60 documented cases and therefore case literature is very limited. However, in a review of autopsies conducted on 3 patients with CFC, one patient presented with a positive indication of PH. This patient was a 21-year-old male who died suddenly. In early childhood, mild PS was diagnosed but shortly before his sudden death, hypertrophic cardiomyopathy was diagnosed with confirmation at autopsy. In addition, the mitral valve was thickened and the AV valve was dysplastic with malformed leaflets. An unexpected finding was the presence of pulmonary vascular changes consisting of marked intimal thickening and lumen narrowing consistent with grade 3 PAH [35].

LEOPARD Syndrome (Cardiocutaneous Syndrome)

LEOPARD syndrome is a condition and mnemonic first described by Goran *et al.* in 1969 [36] with the letters referring to L – lentiginos, E – electrocardiographic conduction defects, O – ocular hypertelorism, P – pulmonary stenosis, A – abnormal genitalia, R – retardation of growth, D – deafness [36-38]. Related to Noonan syndrome, it is caused by a mutation in the PTPN11 gene with 5 allelic version of this condition described [39]. However, unlike Noonan syndrome, this condition is very rare, with about 200 cases reported so far [39, 40].

In 1981, Bleiden *et al.* first described a case of primary PH (IPH) in a 6 year old girl with LEOPARD syndrome [41]. The patient was evaluated due to a three-month history of episodic dyspnea and a presentation of a phenotype characteristic for this syndrome. Two years previously, medical visits had shown a cardiac murmur and physical exams described a dyspneic child without signs of congestive heart failure. While cardiac examination revealed signs diagnostic of PH, including RV hypertrophy, axis deviation and prominent main pulmonary artery, an echocardiography showed normal left heart. Cardiac catheterization was used to confirm the diagnosis of PH with a main pulmonary artery pressure of 130/80mmHg, wedge pressure of 8mmHg, and aortic pressure of 110/70mmHg. At 8 years of age, the patient died from a syncopal episode and autopsy findings ruled out a primary cardiac anomaly but found RV hypertrophy and dilation. Histological examination showed typical features of severe PAH, including medial hypertrophy, intimal fibrosis, fibrinoid vascular necrosis, necrosis arteritis and plexiform lesions.

A second case of PH in LEOPARD syndrome was published by Peter and Kemp in 1990 [42] involving 19 year old female. She was diagnosed with LEOPARD syndrome at 2 years of age and first presented with dyspnea at age 7. PH was confirmed with cardiac catheterization, which found a pulmonary artery pressure of 40/20 mmHg. This mild PAH resolved following the treatment of the sub-aortic membrane. At age 16, the PAH diagnosis was reconfirmed following an echocardiogram and measurement of right ventricular systolic time. At age 19, she presented with severe PAH with a pressure >64mmHg and six days following admission she died from a combination of bilateral pneumonia, CHF, dyspnea and cyanosis. The post-mortem showed extensive pulmonary hypertensive vascular changes.

Costello Syndrome (Faciocutaneoskeletal Syndrome)

Costello syndrome is also a rare member of the RASopathies linked to mutation of the HRAS gene [43] with less than 300 cases having been reported. A characteristic phenotype includes coarse facial features, intellectual disability, cardiac malformations (cardiac hypertrophy), failure to thrive, and short stature [44].

In 2008, O'Shea *et al.* reported a case of persistent PH in a newborn leading to respiratory distress and necessitating emergency intubation [45]. At 4 months of age, the patient was readmitted due to a history of rapid onset respiratory distress secondary to lung collapse and consolidation at which time Costello syndrome was also diagnosed. The patient was discharged with a permanent tracheostomy following difficulty in extubation. In a study delineating Costello syndrome from CFC, Gripp *et al.* reported the case of an infant diagnosed with reactive PH [46] but the authors suggested that it was secondary to cardiomyopathy. However, an infant sharing a phenotype similar to both Costello and CFC was diagnosed with PAH secondary to an arteriopathy.

Legius Syndrome

This syndrome has recently gained a separate classification from that of NF-1, which it closely resembles. In 2007 Brems *et al.* identified a series of loss of function mutations in the gene SPRED1 which resulted in a phenotype resembling a milder form of NF-1 [47]. Patients with Legius syndrome, much like NF-1, present with café-au-lait spots, axillary freckling, mild cognitive impairment, macrocephaly and similar facial features. However, unlike patients with NF-1, Legius syndrome patients lack neurofibromas, iris Lisch nodules and CNS tumors [2].

As of 2011, approximately 140 cases of Legius syndrome have been reported and classified and, not surprisingly, a comprehensive description of this syndrome is still in its early stages [48]. No cases reports thus far have been published linking Legius syndrome to PH.

Arteriovenous Malformation (AVM)

This RASopathy is characterized by an abnormal connection between arteries and veins that bypass the capillary system.

This abnormal vascular structure can be clinically silent or have drastic effects on the cardiovascular system. This disease has been recognized since the 1800's and has been linked to a mutation in the RASA1 gene [49]. These anomalies can occur anywhere in the body but can have dramatic effects when they occur intracranially where they have been associated with stroke, hemorrhages and high output cardiac failure as well as other local and systemic neurologic features [50]. AVMs have also been described in the pulmonary vasculature (PAVM) although they are rare with an incidence estimated to be 2-3:100,000 and can be complicated by PH.

Conclusions

The grouping of the RASopathy syndromes is a relatively new system and studies of the collective group are limited. While the major syndromes (NF-1 and Noonan) are relatively common and well established in the medical literature, the others are quite rare, often with less than 200 cases confirmed. The prevalence of pulmonary pathologies, and PH in particular, in the RASopathies is not well established as compared to cardiac and neurocognitive impairments. In fact, this topic has generated relatively few reviews of the literature. However after examining the available case reports in detail, it appears that individual cases of PH have been reported in all member syndromes, especially in NF-1 where it appears to be much more common and well characterized. The clinical manifestation of RASopathic disease appears as a spectrum, with significant phenotypical overlap between related syndromes. It is our opinion that the incidence of PH in RASopathies is greater in NF-1 as compared to Noonan. Due to the aggressive and often fatal nature of PH, a diagnosis of a RASopathy should also include screening for PH.

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Author Contributions

The authors of this manuscript made substantial contributions to research design and concept; analysis and interpretation of data; drafting the paper and revising it critically; approval of the submitted and final versions.

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