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Pulmonary Hypertension in Patients with 9q34.3 microdeletion-associated Kleefstra Syndrome

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Abstract

Kleefstra Syndrome is a rare genetic disorder caused by mutations in *EHMT1*, Euchromatin Histone Methyl Transferase 1, or deletions encompassing *EHMT1* on 9q34.3. Congenital heart defects are among the major findings in patients with 9q34.3 microdeletion/Kleefstra Syndrome along with recognizable facial appearance, developmental delay/intellectual disability including severely delayed or absent speech, hypotonia, seizures, behavioral and sleep abnormalities.

Pulmonary hypertension (PH) is a rare condition associated with increased pulmonary artery and right heart pressures that can lead to right heart failure and death if untreated. PH can be idiopathic (IPAH), heritable (HPAH), or associated (APAH) with co-morbid conditions including congenital heart disease (CHD), lung diseases and other metabolic disorders. Genetic factors play important roles in heritable and idiopathic PH development and are particularly relevant but more diverse in etiology in children. PH is also reported in some chromosomal disorders such as Down syndrome in which congenital heart defects are common; however, PH has rarely been reported in patients with 9q34.3 microdeletion/Kleefstra Syndrome. Here, we present 3 patients with 9q34.3 microdeletions with CHD and PH along with review of 5 similar cases reported in the literature and discuss the potential association of PH with Kleefstra syndrome.

Keywords

Kleefstra Syndrome; 9q34.3 microdeletion; Pulmonary Hypertension (PH); Microarray; Congenital Heart Disease (CHD)

INTRODUCTION

Kleefstra Syndrome (OMIM# 610253) is a rare genetic disorder caused by mutations of *EHMT1*, Euchromatin Histone Methyl Transferase 1, or deletions encompassing *EHMT1* on 9q34.3. Kleefstra Syndrome is characterized by distinctive facial features, developmental

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CONFLICTS OF INTEREST

Authors declare no conflict of interest.

delay/intellectual disability including severely delayed or absent speech, hypotonia, seizures, behavioral and sleep abnormalities, congenital heart defects, recurrent infections, eye problems, and minor genital anomalies in males (GeneReviews®, Last Update: May 7, 2015, Accessed on 1/15/2018).

Pulmonary Hypertension (PH) has been classified into 5 groups, based on the etiology; however many pediatric patients, including those with genetic disorders have components of Group I, II and III pulmonary hypertension (Cerro et al., 2011; Simonneau et al., 2013). Group I pulmonary arterial hypertension (PAH) includes idiopathic (IPAH; Group 1.1), heritable (HPAH; Group 1.2), drug/toxin induced (Group 1.3), or associated (APAH) with other co-morbid conditions (Group 1.4) such as congenital heart disease. The other groups include PH from left heart disease or pulmonary venous hypertension (Group II), lung disease (Group III), thromboembolic PH (Group IV) and other (including metabolic disorders) (Group V). In children greater than 3 months, PH is defined as a mean PA pressure (mPAP) ≥ 25 mmHg and PAH is defined as mPAP ≥ 25 mmHg with pulmonary arterial wedge pressures (PAWP) < 15 mmHg and pulmonary vascular resistance index (PVRI) > 3 Woods Units (WU) $\times m^2$. All of these are determined through right heart catheterization but right heart pressures can be estimated based on echocardiographic criteria (Abman et al., 2015; Hoeper et al., 2013). While heritable PH accounts for 5–10% of all PH cases and usually follows an autosomal dominant inheritance pattern with incomplete penetrance, genetic factors can also play a role in idiopathic PH and associated PH (Ma & Chung, 2017). Congenital heart diseases (CHD) are the most frequently associated co-morbid conditions in associated PH (Group 1.4.4), particularly in children (Li et al., 2017). Congenital heart defects are common in patients with Kleeftstra syndrome; however, PH has not been recognized as a consistent feature. Here, we present three patients with 9q34.3 microdeletions associated with Kleeftstra Syndrome with PH and review 5 other reported patients with Kleeftstra syndrome and PH from the literature.

CLINICAL REPORT

This study was approved by the Institutional Review Board of Columbia University and informed consent was obtained from the families. The genetic and clinical findings of the patients are summarized in Table 1.

Patient 1 is an 18-month old male born at term via vaginal delivery with a prenatal diagnosis of 9q34.3 microdeletion-related Kleeftstra Syndrome identified by chromosome microarray (3.45 Mb deletion). Genetic testing was performed upon detection of complex CHD on prenatal ultrasonographic evaluation. Postnatal echocardiogram showed a double outlet right ventricle variant with a large ventricular septal defect (VSD) and a hypoplastic arch with coarctation of the aorta (CoA). In addition to his cardiac findings, he also had microcephaly, hypotonia, dysmorphic features (hypertelorism, epicanthal folds, broad nasal bridge, anteverted nares, and short neck), frequent respiratory infections, obstructive sleep apnea (on chronic CPAP), and developmental delay. Although he had frequent respiratory infections, initial immunologic workup was not suggestive of evident immunodeficiency. Additional workup was recommended but not completed. He underwent surgery with a VSD closure and an arch reconstruction when he was one month old. Moderate mitral and aortic stenosis

developed after surgery. At 9 months of age, he was diagnosed with severe PH on right heart catheterization under general anesthesia. His pulmonary artery pressure was 53/20 with mPAP of 35mmHg and an arterial blood pressure of 70/45 indicating greater than half-systemic right sided pressures. PAWP was 7mmHg, indicating that the elevated pressures were not secondary to left sided heart disease and calculated PVRi was severely elevated at $8.5 \text{ WU} \times \text{m}^2$ which decreased to $7.2 \text{ WU} \times \text{m}^2$ on acute vasodilator testing. This was unexpected given his age and type of congenital heart disease. He was started on sildenafil with some improvement in symptoms. He died at the age of 10 months due to a cyanotic and bradycardic arrest at home from which he was not able to be resuscitated.

Patient 2 is a 7-month old female born at term via vaginal delivery. Upon cardiac evaluation in the neonatal intensive care unit (NICU), she was found to have bidirectional patent ductus arteriosus (PDA), a moderate VSD and atrial septal defect (ASD), and PH based on an elevated tricuspid regurgitation gradient, low VSD gradient and ventricular septal flattening in systole. She was noted to have significant developmental delay, congenital microcephaly, and marked hypotonia. Follow-up echocardiogram at 2 months of age suggested that the VSD and PDA did not have significant enough left to right shunts to account for the PH. She had a cardiac catheterization at 3 months which confirmed the diagnosis of PH with pulmonary artery pressure of 52/20 with mPAP of 37mmHg. Arterial blood pressure was 69/32, PCWP 8mmHg, and PVRi $3 \text{ WU} \times \text{m}^2$ with a decrease to $1.9 \text{ WU} \times \text{m}^2$ on vasodilator testing. Multiple congenital anomalies prompted a chromosomal microarray study that identified a 2.9 Mb deletion at 9q34.3 consistent with Kleefstra Syndrome. She underwent surgery for her congenital heart defects at 5 months of age. Post-operatively, she was started on oral sildenafil for the PAH. On follow up, she has shown improvement in respiratory symptoms and improved growth.

Patient 3 is a 3-year old female born at term via C-section for cord prolapse with a prenatal history of intrauterine growth restriction, left ventricular brain cyst ($2.1 \times 1 \text{ cm}$), oral cyst, and joint contractures. Echocardiogram on the third day of life revealed VSD, small secundum ASD, and small to moderate PDA. On fifteenth day of life, PH was diagnosed based on an echocardiogram that demonstrated a hypertrophied right ventricle with systolic septal flattening, dilated pulmonary arteries, and right to left flow through a moderate perimembranous VSD, all consistent with suprasystemic right ventricular pressures. She developed bacterial endocarditis with vegetations on the aortic valve and she underwent surgical repair of the VSD and ASD and resection of the valve vegetation when she was 6 months old. Her PH has been intermittent after repair, with surges to systemic levels in association with respiratory infections, and down to completely normal levels when she is well. Karyotype and chromosome microarray analyses showed an unbalanced translocation between the long arm of chromosome 9 and short arm of chromosome X along with a 1.24 Mb deletion of 9q34.3 and a 13.24 Mb duplication of Xp22.2 translocated onto 9q34.3. Additionally, she is small, has feeding difficulties, is developmentally delayed, hypotonic, and has dysmorphic features (mild hypertelorism, slightly downslanting palpebral fissures, slightly anteverted nares, midline sublingual cystic mass, short neck, mild shield shape of chest, swan neck deformity of bilateral second and third fingers, mild arthrogryposis of the wrists, sacral dimple). She has a history of frequent respiratory infections, obstructive sleep

apnea, seizures and hydronephrosis. She is taking sildenafil, and her PH has been under control with improvement in somatic growth and developmental milestones.

The annotation of deletions of the patients to hg19 genome assembly is illustrated in Supplementary Figure 1. The commonly deleted region in all three patients is 1.24 Mb in size and contains 47 protein-coding RefSeq genes. The pLI (Lek et al., 2016), haploinsufficiency rank (Huang, Lee, Marcotte, & Hurles, 2010), and mouse lung expression rank (Galvis et al., 2015) scores of the genes in the commonly deleted region are shown in Supplementary Table 1.

Review of the literature identified five other individuals with PH and 9q34.3 deletions, one of which resolved (Table 2) (Ayyash et al., 1997; Iwakoshi et al., 2004; Neas et al., 2005). The breakpoints of deletions of those patients are not known due to the limitations of the diagnostic methods at the time of the studies. The most common CHD was a VSD in four of the five patients with a left-to-right shunt in two of them. Sleep apnea was reported in the one patient whose PH resolved. Two patients died early due to respiratory infections.

DISCUSSION

We report infantile onset pulmonary hypertension (PH) and CHD despite early CHD repair in three unrelated children with Kleefstra syndrome with deletions at 9q34.3 and define a 1.24 Mb critical region containing 47 protein-coding RefSeq genes including *EHMT1*.

The majority of recognized Kleefstra Syndrome patients (75%) are attributed to microdeletions of 9q34.3 (GeneReviews®, Last Update: May 7, 2015, Accessed on 1/15/2018). While CHDs are among the major medical problems associated with Kleefstra Syndrome, PH has not been recognized as a consistent feature with either *EHMT1* mutations or 9q34.3 microdeletion associated Kleefstra Syndrome.

All of our patients had CHD associated PH (Table 1). The amount of left to right blood flow or length of time before corrective surgery cannot account for the PH in our patients. We hypothesize that one of the genes in the critical region has a developmental effect on the pulmonary vascular development and leads to congenital PAH. It is unclear if haploinsufficiency of *EHMT1* that is responsible for the CHD or another gene in the 9q34.3 deletion is responsible for the PH.

Pulmonary hypertension is more commonly reported in patients with other chromosomal disorders such as Down syndrome (Sharma, Khera, Sondhi, & Devgan, 2013) compared to the general population and compared with children with isolated congenital heart defects (Jortveit et al., 2016). PH can also be observed in patients with Down syndrome in the absence of congenital heart defects (Weijerman et al., 2010). Obstructive sleep apnea is another common feature of Down syndrome and can contribute to PH (de Miguel-Diez, Villa-Asensi, & Alvarez-Sala, 2003; Fan, Ahn, Roth, Li, & Vaughn, 2017; Goffinski et al., 2015). Two of our patients and one in the literature had obstructive sleep apnea, which may contribute to the PH development (Fukushima et al., 2010).

In conclusion, there are now eight patients who have Kleefstra Syndrome with 9q34.3 deletions with CHD associated PH, often symptomatic within infancy and unlikely to be secondary to excessive pulmonary blood flow. CHDs are observed in almost half of the patients with Kleefstra Syndrome, but PH has not been clinically recognized as an associated feature. We have also observed sleep apnea in several of the Kleefstra syndrome patients, and this could contribute to the pathogenesis. Physicians caring for patients with Kleefstra syndrome should be aware of the association with PH and evaluate respiratory symptoms with an echocardiogram to evaluate elevated right sided pressures.

Future studies are needed to determine the frequency, association with CHD and other contributing factors (i.e. sleep apnea) to PH in patients with Kleefstra Syndrome. It is not yet clear what the natural history of the PH is and if certain therapeutic strategies are more effective. Finally, by characterizing Kleefstra patients with smaller deletions and *EHMT1* mutations it should be possible to identify the gene responsible for PH in Kleefstra syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Genetic and clinical findings of patients with 9q34.3 deletion

	Patient 1	Patient 2	Patient 3
Age	18 months	7 months	3 years
Gender	Male	Female	Female
Molecular Abnormality	arr[hg19]9q34.3(137,757,311-141,020,389)x1	arr[hg19]9q34.3(138,157,660-141,073,897)x1	arr[hg19]9q34.3(139,776,707-141,020,389)x1, Xp22.33p22.2(169,921-13,409,172)x3 46, XX, der(9)t(X;9)(p22.2; q334.3)
Deletion Size	3.45 Mb	2.9 Mb	1.24 Mb
WHO Functional Class	WHO Functional Class II	WHO Functional Class II	WHO Functional Class II
Cardiac Findings	DORV VSD CoA MS AS	Moderate VSD Moderate ASD Bidirectional PDA	VSD ASD PFO Small to moderate PDA
Additional Findings	Microcephaly Hypotonia Developmental delay Dysmorphic features Sleep apnea Frequent respiratory infections	Microcephaly Hypotonia Developmental delay	Hypotonia Developmental delay Dysmorphic features Sleep apnea Frequent respiratory infections Feeding difficulties

Abbreviations; AS: Aortic Stenosis, ASD: Atrial Septal Defect, CoA: Coarctation of Aorta, DORV: Double Outlet Right Ventricle, LV: Left Ventricle, Mb: Megabase, MS: Mitral Stenosis, MV: Mitral Valve, PAH: Pulmonary arterial hypertension, VSD: Ventricular Septal Defect, PDA: Patent Ductus Arteriosus, RV: Right Ventricle, RA: Right Atrium, PFO: Patent Foramen Ovale

Table 2

9q34.3 deletion patients with reported PH in the literature.

Case	This study Patient 1	This study Patient 2	This Study Patient 3	Ayyash et al., 1997	Iwako shi et al., 2004-1	Iwako shi et al., 2004-2	Neas et al., 2005-1	Neas et al., 2005-2
Genetic Abnormality (Method)	9q34.3 deletion (Microarray)	9q34.3 deletion (Microarray)	9q34.3 deletion (Microarray) and t(Xp:9p) (Karyotype)	9q34.3 deletion (Karyotype)	9q34.3 deletion (FISH)	9q34.3 deletion (CGH, FISH)	9q34.3 deletion (FISH)	9q34.3 deletion (FISH)
PH	+	+	+	+	+	+	+	* +
Heart Defects	DORV VSD CoA MS AS	VSD ASD PDA	VSD ASD PDA PFO	VSD RV hypertrophy	VSD	VSD	ASD PDA BAV	VSD
Sleep Apnea	+	-	+	n/a	-	-	n/a	+

* PAH was present at birth but resolved at 4 weeks.

Abbreviations; AS: Aortic Stenosis, ASD: Atrial Septal Defect, BAV: Bicuspid Aortic Valve, CoA: Coarctation of Aorta, CGH: Comparative Genomic Hybridization, DORV: Double Outlet Right Ventricle, FISH: Fluorescence In Situ Hybridization, LV: Left Ventricle, MS: Mitral Stenosis, MV: Mitral Valve, PAH: Pulmonary arterial hypertension PDA: Patent Ductus Arteriosus, PFO: Patent Foramen Ovale, RV: Right Ventricle, VSD: Ventricular Septal Defect