

Review

Genetics and Genomics of Pediatric Pulmonary Arterial Hypertension

Carrie L. Welch¹ and Wendy K. Chung^{2*}

¹ Department of Pediatrics, Columbia University Irving Medical Center, 1150 St. Nicholas Avenue, New York, New York 10032; cbw13@columbia.edu

² Department of Pediatrics, Columbia University Irving Medical Center, 1150 St. Nicholas Avenue, New York, New York 10032; Department of Medicine, Columbia University Irving Medical Center, 622 W 168th St, New York, New York; wkc15@columbia.edu

* Correspondence: wkc15@columbia.edu; Tel.: (212)851-5313

Abstract: Pulmonary arterial hypertension is a rare disease with high mortality despite recent therapeutic advances. The disease is caused by both genetic and environmental factors, and likely gene x environment interactions. While PAH can manifest across the lifespan, pediatric-onset disease is particularly challenging because it is frequently associated with a more severe clinical course and comorbidities including lung/heart developmental anomalies. In light of these differences, it is perhaps not surprising that emerging data from genetic studies of pediatric-onset PAH indicate that the genetic basis is different than that of adults. There is a greater genetic burden in children, with rare genetic factors contributing to at least 36% of pediatric-onset idiopathic PAH (IPAH) compared to ~11% of adult-onset IPAH. *De novo* variants are frequently associated with PAH in children, and contribute to at least 15% of all pediatric cases. The standard of medical care for pediatric PAH patients is based on extrapolations from adult data. However, the increased etiologic heterogeneity, poorer prognosis and increased genetic burden for pediatric-onset PAH calls for a dedicated pediatric research agenda to improve molecular diagnosis and clinical management. A genomics-first approach will improve the understanding of pediatric PAH and how it is related to other rare pediatric genetic disorders.

Keywords: genomics; pediatrics; lung disease; pulmonary arterial hypertension

1. Introduction

Pulmonary arterial hypertension (PAH) is a rare disease with an estimated prevalence of 4.8-8.1 cases/million for pediatric-onset [1] and 15-50 cases/million for adult-onset disease [2]. Pathogenic changes in the pulmonary vasculature - including endothelial dysfunction, aberrant cell proliferation and vasoconstriction - give rise to the clinical consequences of increased pulmonary vascular pressures, increased vascular resistance, heart failure and premature death[3]. The disease is caused by genetic, epigenetic and environmental factors, as well as gene x environment interactions wherein genetic contributions to disease risk are modified by environmental exposures. Causal genetic factors for PAH are typically autosomal dominantly inherited for genes such as *BMPR2*, the major gene causing familial forms of PAH[4, 5]. Environmental risk factors include hypoxia and exposure to drugs and toxins. Most of our understanding of PAH etiology and treatment is based upon studies in adults[6, 7]. However, emerging clinical and genetic data indicate that there are fundamental differences between pediatric and adult onset disease.

Pediatric PAH differs from adult-onset disease in several important aspects including sex bias, clinical presentation, etiology, and response to therapy [6-8]. The frequency of PAH is ~3-4-fold higher in females relative to males for adult-onset disease. However, data from the National Biological Sample and Data Repository for PAH (aka PAH Biobank, Table 1)[9, 10], and other studies[11, 12], indicate that the frequency of pediatric-onset PAH is similar for females and males, suggesting less dependence on sex-specific factors in children. Children present with increased severity of disease - elevated mean pulmonary artery pressure (mPAP), decreased cardiac output, and increased pulmonary vascular resistance - compared to adults at diagnosis (Table 1) [9, 10]. The clinical manifestations likely reflect the complex etiology of disease in children. While prenatal and early postnatal influences on

lung growth and development can contribute to the development of PAH across the lifespan, early developmental influences play a particularly prominent role in pediatric-onset PAH in which patients frequently have complex comorbidities such as congenital heart disease (APAH-CHD), Down syndrome, congenital diaphragmatic hernia (CDH), and other developmental lung diseases including persistent pulmonary hypertension of the newborn (PPHN)[6, 13, 14]. Histopathological studies have identified abnormal lung development and lung hypoplasia as common features of PAH, CHD, CDH and Down syndrome [13, 15]. While the mechanism(s) for impaired lung development are not known, altered expression of angiogenic and anti-angiogenic genes likely contribute[16-19]. Decreased lung vascular and alveolar growth predispose to vascular injury during susceptible periods of growth and adaptation. The presentation of pediatric PAH with developmental comorbidities contributes to poor outcomes in these children[6, 14]. Few pharmaceutical therapies are approved for use in children due to the lack of safety and efficacy data[7]. However, a retrospective study of pediatric PAH patients suggested that PAH patients with Down syndrome may be less responsive to PAH treatments than non-Down syndrome patients[20]. Clearly, pediatric-focused studies are needed to increase our understanding about the natural history, pathogenic mechanisms, and treatment of PAH in children.

Table 1. Clinical characteristics and hemodynamic parameters of child- vs adult-onset PAH cases at diagnosis.

Data are from the PAH Biobank (n=2,572). Child-onset, <18 years of age at diagnosis. Mean \pm SD.

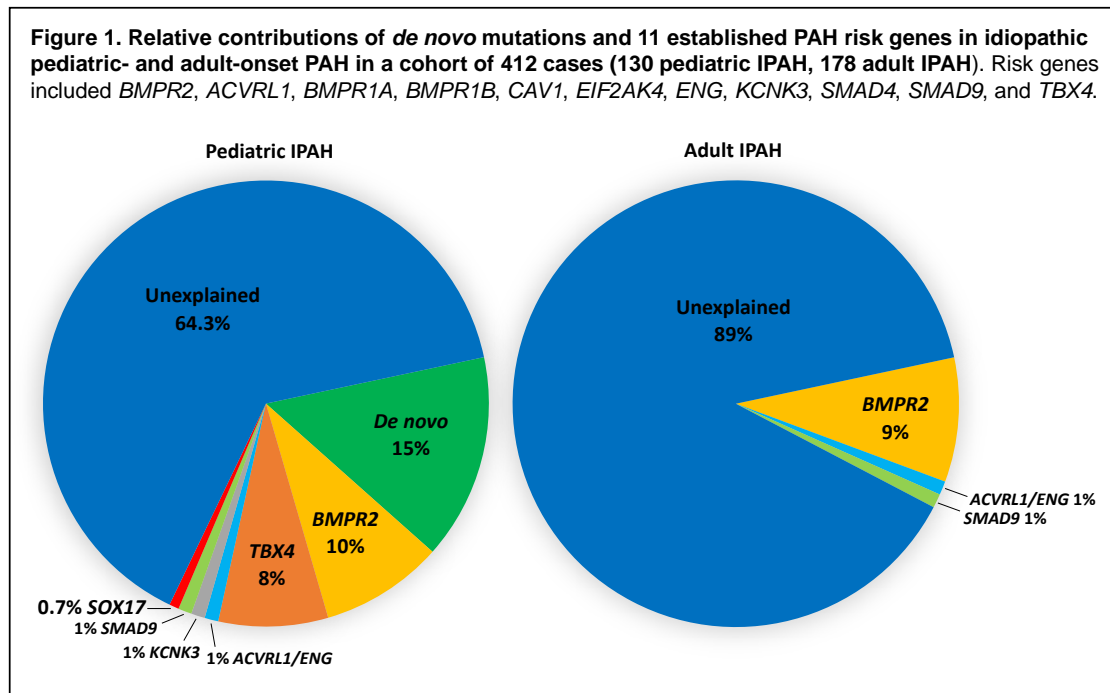
Group (n)	Age at dx (y)	F:M ratio	mPAP (mm Hg)	mPCWP (mm Hg)	CO Fisk (L/min)	PVR (Woods units)	Common comorbidities
Child (226)	7.7 \pm 5.4	1.65:1	55.1 \pm 18.6	9.0 \pm 3.0	3.2 \pm 1.6	18.1 \pm 11.7	CHD, CDH, DS, lung growth/development
Adult (2,345)	51.6 \pm 14.7	4.02:1	49.6 \pm 13.9	10.2 \pm 4.2	4.6 \pm 1.7	10.0 \pm 5.9	HTN, hypothyroidism, other pulmonary & metabolic diseases
P-value	<0.0001*	<0.0001**	<0.0001*	<0.0001*	<0.0001*	<0.0001*	

Abbreviations: dx, diagnosis; F:M, female:male; MPAP, mean pulmonary artery pressure; MCWP, mean pulmonary capillary wedge pressure; CO, cardiac output; PVR, pulmonary vascular resistance; CHD, congenital heart disease; CDH, congenital diaphragmatic hernia; DS, Down syndrome; HTN, systemic hypertension.

*Student's t-test, 2-tailed. **Fisher exact test.

2. Genetics of pediatric PAH – current knowledge

Emerging data from genetic studies of pediatric-onset PAH indicate that the genetic basis in children is different from that of adults [9, 10, 12]. There is a greater genetic burden in children, with rare genetic factors contributing to at least 36% of pediatric-onset idiopathic PAH (IPAH) compared to ~11% of adult-onset IPAH (Figure 1). *De novo* variants are frequent in children, likely contributing to ~15% of both IPAH (Figure 1) and all PAH[9, 12]. Among rare inherited variants, variants in *BMPR2* are causal in ~9% of both pediatric- and adult-onset IPAH but rarely explain PAH associated with other diseases (APAH)[10, 21] and have not been observed in PPHN[22]. Notably, two of the other known causal genes with the highest frequencies of rare deleterious variants among pediatric cases – *TBX4* and *SOX17* – are highly expressed in embryonic tissues and have prominent roles in lung and vasculature development[23-25].



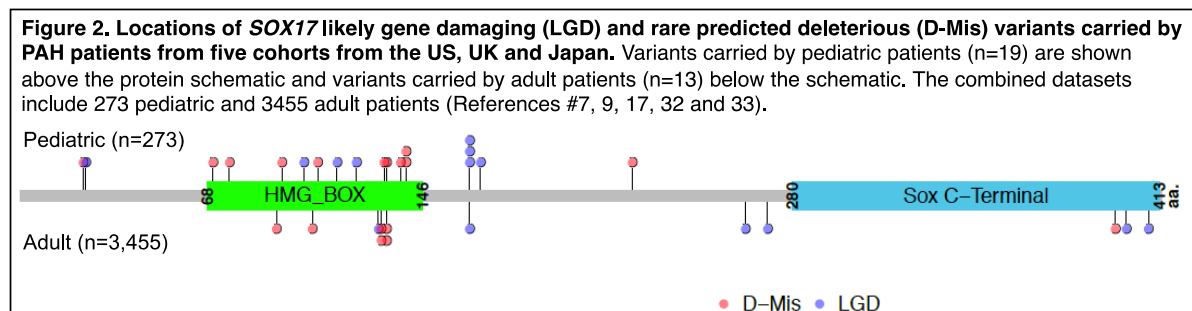
2.1 *TBX4*

Unlike *BMPR2* and other known causal PAH genes, *TBX4* is not expressed in pulmonary arterial endothelial cells or smooth muscle cells. *TBX4* is a transcription factor in the T-box gene family that is co-expressed with *TBX5* throughout the mesenchyme of developing lung and trachea[23]. Lung-specific *Tbx4/Tbx5* deficient mice exhibit impaired lung branching and hypoplasia during gestation, and early postnatal death due to severe respiratory disease[23]. *TBX4* is also expressed in the developing atrium of the heart and limb buds[26]. In humans, rare but recurrent microdeletions of chromosome 17q23, including *TBX4*, have been observed in children with complex phenotypes including PAH, heart and skeletal defects, and neurodevelopmental delay[27-29]. More recently, *TBX4*-specific likely gene-disrupting (LGD) and damaging missense variants have been associated with PAH with or without small patella syndrome (OMIM #147891), most frequently in pediatric cases[10, 12, 21, 30, 31]. In two independent cohorts[10, 12], rare deleterious variants in *TBX4* showed significant enrichment among pediatric- compared to adult-onset IPAH cases (CUIMC cohort: 10/130 vs 0/178; PAH Biobank: 12/155 vs 1/257, respectively). In the PAH Biobank, ten additional *TBX4* variants were identified for other PAH subtypes, including three APAH-CHD cases with heart defects. In a cohort of 256 APAH-CHD cases (144 pediatric- and 112 adult-onset), we identified *TBX4* variants in seven cases with age-of-onset from newborn-11 years, one associated with alveolar hypoplasia[21]. Skeletal and other developmental defects are not routinely assessed as part of a PAH diagnosis. To this end, Galambos et al carried out detailed clinical and histopathologic characterization of 19 pediatric PAH cases with *TBX4* variants: 6 microdeletions, 12 LGD and 1 missense. Seven infants had evidence of abnormal distal lung development and there was a high frequency of heart and skeletal developmental anomalies; neurodevelopmental delay was also observed among those patients with microdeletions, likely due to haploinsufficiency of other adjacent genes. Ten newborns presented with PPHN which resolved but recurred later in infancy or childhood[32]. Together, the data suggest that rare *TBX4* variants contribute to 7.7% of pediatric IPAH and 4.9% of pediatric APAH-CHD cases. Notably, *TBX4* variants have not been observed in CHD alone[33]. Genetic diagnosis of rare deleterious *TBX4* variants or *TBX4*-containing microdeletions in pediatric PAH predicts a more complex developmental phenotype and potential recurrence of PAH following neonatal PPHN.

2.2 *SOX17*

SOX17 is a member of the conserved family of SRY-related HMG box transcription factors, originally identified as key regulators of male sex determination but now recognized to have critical

roles in embryogenesis[24, 25]. *SOX17* is specifically required for endoderm formation and vascular morphogenesis[24, 34, 35], and germline deletion of *Sox17* results in embryonic lethality by E10.5[24]. In the developing murine lung, *Sox17* is expressed in mesenchymal progenitor cells and is then restricted to endothelial cells of the pulmonary vasculature[36]. Conditional deletion of *Sox17* in mesenchymal progenitor cells causes abnormal pulmonary vascular morphogenesis resulting in postnatal cardiopulmonary dysfunction and juvenile death [36]. Endothelial-specific inactivation of *Sox17* in mice leads to impaired arterial specification and embryonic death or, with conditional postnatal inactivation, arterial-venous malformations [34]. Transcriptional activation of *Sox17* via hypoxia-induced factor 1 α , leads to upregulation of cyclin-E1 and endothelial regeneration in response to lung injury [37]. We identified *SOX17* as a candidate risk gene for PAH using exome sequencing data in a cohort of 256 APAH-CHD patients [21]. Thirteen cases with rare predicted deleterious *SOX17* variants were identified, seven LGD and six missense variants located primarily within the conserved HMG box domain (Figure 2). Fifty-six percent of the overall cohort were pediatric cases but nine of thirteen cases with rare deleterious variants in *SOX17* were pediatric cases with mean age-of-onset of 14 years. A recurrent frameshift variant, p.(Leu167Trpfs*213), was identified in three APAH-CHD cases with age-of-onset ranging from 7 months-5 years. We[10, 21], and others[38, 39], have identified *SOX17* variants in IPAH cases but with lower frequency in adults. Combined data from five cohorts ([10, 12, 21, 38, 39] indicate that *SOX17* variants contribute to 7% of all pediatric-onset PAH cases compared to 0.4% of adult-onset cases (Figure 2). Protein modeling indicates that at least three of the APAH-CHD case missense variants localize to the transcription factor DNA binding pocket[21], and missense variants in this region have been shown to impair both direct DNA binding and *SOX17*/ β -catenin nucleoprotein complexes at target gene promoters[40, 41]. These data suggest that haploinsufficiency with complete or partial loss of function alleles is the likely mechanism of *SOX17* risk in PAH.



Some variants in *SOX17* downstream target genes may be predicted to mimic the consequences of *SOX17* LGD variants or haploinsufficiency. We identified 163 rare predicted deleterious variants in 149 putative *SOX17* target genes, most with prominent expression in pulmonary artery endothelial cells and/or developing heart[21]. For the 32 LGD and 131 missense variants, we observed a moderate but significant enrichment of rare missense variants in cases compared to controls. Approximately one-third of these genes had top quartile gene expression in both pulmonary artery endothelial cells and developing heart. Pathway analysis indicated that the genes have likely roles in developmental biology, small molecule transport/homeostasis, and extracellular matrix interactions (Table 2). While these results are intriguing, they require confirmation in larger cohorts to determine which specific *SOX17*-regulated genes/pathways contribute to PAH risk.

Table 2. Biological pathway analysis of SOX17 target genes harboring PAH-CHD patient rare deleterious variants. Data obtained using Reactome 2016. Pathways with FDR-adjusted p-value ≤ 0.05 are listed.

Term	Reactome ID	# Genes in overlap	p-value	Adjusted p-value	Genes
Developmental biology	R-HAS-1266738	16/786	6.8e-05	0.03	<i>KLB, ROBO2, LAMA1, EGF, ANK3, LAMC, SLC2A4, MED6, SPRED2, MEIS1, NRFA2, PCMC4, NF1, EP300, TCF4, EPHB4</i>
Transmembrane transport of small molecules	R-HAS-382561	13/594	1.7e-04	0.03	<i>RYR2, ABCC4, ABCC1, SLC1A3, SL#3A4, SLC8A1, CLCN5, SLCA9, ATPB7, ASPH, WNK1, NUP35, EMB</i>
Non-integrin membrane ECM interactions	R-HAS-3000171	4/42	1.7e-04	0.03	<i>LAMA1, LAMA4, LAMC1, THBS1</i>
Ion homeostasis	R-HAS-5578775	4/51	1.7e-04	0.03	<i>RYR2, ASPH, TPR3, SLC8A1</i>

*SYN, synonymous; LGD, likely gene disrupting; MIS, missense; D-MIS, predicted deleterious missense based on REVEL score >0.5 .

2.3 De novo variants

De novo variants have emerged as an important class of genetic factors underlying rare diseases, especially early-onset severe conditions[14, 33, 42-44], due to strong negative selection decreasing reproductive fitness[45]. We recently assessed the role of rare deleterious *de novo* variants in pediatric PAH using a cohort of 124 parent-child trios (56% IPAH, 38% APAH-CHD, 6% other PAH)[9]. We observed a 2.5-fold enrichment of *de novo* variants among all PAH cases compared to the expected rate, almost entirely due to genes that are highly expressed in developing lung or heart (Table 3). Similar

Table 3. Burden of de novo variants in 5,756 genes highly-expressed in developing lung (murine E16.5 lung stromal cells) and/or developing heart (murine E14.5 heart) in pediatric-onset PAH (n=124 child/parent trios).

Variant type*	Observed in trios (n=124)	Expected by chance	Enrichment	p-value	Estimated # of true risk variants
SYN	18	14.0	1.3	0.28	---
LGD	11	4.7	2.4	0.06	---
MIS	40	31.7	1.3	0.15	---
D-MIS	19	7.2	2.6	2.0e-04	12
LGD + D-MIS	30	11.8	2.5	7.0e-06	18

*SYN, synonymous; LGD, likely gene disrupting; MIS, missense; D-MIS, predicted deleterious missense based on REVEL score >0.5 .

enrichment was observed for IPAH cases alone, but the study was underpowered to detect a significant burden of *de novo* variants among APAH-CHD cases. *De novo* variants were identified in three known PAH risk genes (4 variants in *TBX4*, 2 in *BMPR2*, 1 in *ACVRL1*) and 23 additional genes with high expression in developing lung and/or heart but little to no previous association with PAH. Based on the enrichment rate, we estimate that ~18 of the identified variants are likely to be implicated in pediatric PAH. The identified genes fit a general pattern for developmental disorders - genes intolerant to LGD variants (pLI >0.5 for 40% of the PAH genes) and with known functions important for coordinated organogenesis including transcription factors, RNA binding proteins, protein kinases and chromatin modification. Three of the genes are known CHD risk genes (*NOTCH1*, *PTPN11* and *RAF1*). *NOTCH1* is the most commonly associated gene for the congenital heart defect of tetralogy of Fallot,[46] and the *NOTCH1* *de novo* variant carrier had a diagnosis of APAH-CHD with tetralogy of Fallot. Rare variants in *PTPN11* and *RAF1* are causal for Noonan syndrome which has a high frequency of congenital heart defects. The *de novo* variants identified in both of these genes are known causal Noonan syndrome variants[47], and three cases of fatal pediatric PAH with Noonan syndrome have been previously reported[48, 49]. We previously reported rare inherited variants in *NOTCH1* (n=5), *PTPN11* (n=1) and *RAF1* (n=2) carried by APAH-CHD cases[21]. Aside from known PAH and CHD genes, at least eight of the other genes with identified *de novo* variants have known or plausible roles in lung/vascular

development (Table 4). For example, *AMOT* (angiomin) encodes an angiostatin-binding protein involved in embryonic endothelial cell migration and tube formation as well as endothelial cell tight junctions and angiogenesis[50-52]. *HSPA4* (heat shock protein A4) encodes a chaperone that, together with *HSPA4L*, functions in embryonic lung maturation and dual deletion of *Hspa4/Hspa4l* results in intrauterine pulmonary hypoplasia and early neonatal death[53]. *KEAP1* (Kelch-like ECH associated protein 1) regulates oxidative stress and apoptosis through interactions with *NRF2* in murine vascular cells[54], and endothelial-specific deletion of *NRF2* reduces endothelial sprouting *in vivo*[55] and increases susceptibility to bronchopulmonary dysplasia and other respiratory diseases[56]. An *NRF2* activator is currently being investigated in a Phase 2 clinical trial for PAH (ichgcp.net/clinical-trials-registry/NCT02036970). Finally, one-third of all of the *de novo* variants identified in the trio analysis are in causal genes for developmental syndromes and at least one-third of the *de novo* variant carriers were diagnosed with growth/developmental phenotypes [9]. The genes identified in this study require replication in a larger pediatric cohort. Due to the low background rate of rare deleterious *de novo* variants[57], the statistical evidence for a candidate risk gene is effectively equivalent to multiplicity. That is, genes with ≥ 2 rare deleterious *de novo* variants are unlikely to be mutated by chance and should be considered candidate risk genes. The genes and variants identified in the pediatric trio analysis have not been observed in adult-onset cases and likely will be specific to pediatric PAH. Thus, it is imperative that larger pediatric-focused PAH cohorts are studied to advance our knowledge of the causal genes specific to pediatric-onset PAH.

Table 4. Novel genes with rare deleterious *de novo* variants in pediatric-onset PAH (n=124 trios).

Gene symbol	Variant type	Protein change	REVEL score	CADD score	Allele frequency (gnomAD)	E16.5 lung expression rank	E14.5 heart expression rank
<i>AMOT</i>	LGD	p.(Leu320Cysfs*55)	.	31	.	68	95
<i>CSNK2A2</i>	D-MIS	p.(His184Leu)	0.50	25	.	55	77
<i>HNRNPF</i>	LGD	p.(Tyr210Leufs*14)	.	29	.	85	98
<i>HSPA4</i>	D-MIS	p.(pro684Arg)	0.62	30	4.1e-06	43	96
<i>KDM3B</i>	D-MIS	p.(Pro1100Ser)	0.66	29	.	89	87
<i>KEAP1</i>	LGD	p.(Tyr584*)	.	35	.	79	82
<i>MECOM</i>	D-MIS	p.(Phe762Ser)	0.76	32	.	82	60
<i>ZMYM2</i>	LGD	p.(Arg540*)	.	36	.	93	77

AMOT, angiomin; *CSNK2A2*, casein kinase II, alpha 2; *HNRNPF*, heterogeneous nuclear ribonucleoprotein F; *HSPA4*, heat shock protein A (HSP70), member 4; *KDM3B*, lysine demethylase 3B; *KEAP1*, Kelch-like ECH-associated protein 1; *MECOM*, MDS1 and EVI1 complex locus; *ZMYM2*, zinc finger protein 620. LGD, likely gene disrupting; MIS, missense; D-MIS, predicted deleterious missense based on REVEL score >0.5. Allele frequency “.” absent from gnomAD.

2.4 Genetic ancestry

Most of the large genetic studies conducted to date have utilized cohorts of predominantly European ancestry. However, the role of specific genes in PAH may be heterogeneous across genetic ancestries, and the results of these studies may not be generalizable to all other populations. For example, the frequency of *ACVRL1* and *ENG* variants combined is ~1% among pediatric IPAH cases of European ancestry [10, 12] but the frequency of *ACVRL1* alone may be closer to 13% among Asian children[58]. *GDF2/BMP9* was recently identified as a novel PAH risk gene with genome-wide significance in both European[38] and Asian[59] cohorts with replication in the PAH Biobank cohort[10]. Similar to other PAH risk genes, the mode of inheritance was autosomal dominant. The frequency of *GDF2/BMP9* variants among children was 2.1% (2/94 cases) in the PAH Biobank and 5.2% (3/57 cases) in the Asian cohort, suggesting that *GDF2/BMP9* variants might be a more frequent cause of PAH among Asian children. Further study is required to determine whether this difference is a true genetic ancestry effect or random variation due to relatively small sample size or differences in bioinformatic pipelines. A PAH case study of a five-year-old boy of Hispanic ancestry identified a homozygous *GDF2/BMP9* LGD variant, NM_016204.1:c.76C>T; p.(Gln26Ter)[60]. The unaffected

parents were heterozygous for the variant. Interestingly, the gnomAD population database (gnomADv2.1.1, n=141,456 samples)[61] contains only two heterozygous counts of this allele, both of Latino ancestry, suggesting that this might be an ancestry-specific allele. Clearly, larger studies of children with greater diversity are needed to define population-specific risk gene allele frequencies as well as ancestral-specific genetic factors.

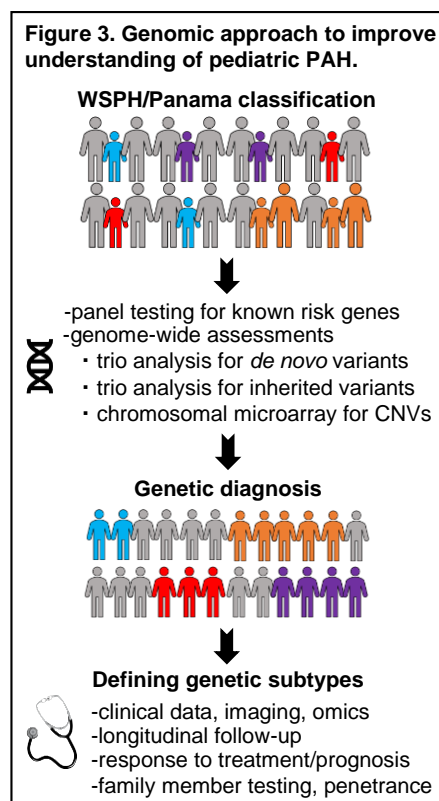
2.5 The role of other “omics” in PAH

In addition to DNA sequencing to identify genetic etiologies of PAH, other “omics” including RNA sequencing, metabolomics and proteomics can provide valuable predictions of who is at risk for disease, define endophenotypes, and guide effective therapies[62, 63]. For example, West and colleagues performed RNA sequencing of blood lymphocytes derived from *BMP2* variant carriers with and without PAH to identify transcriptional patterns relevant to disease penetrance[64]. More recently, *FHIT* was identified as a potentially clinically relevant *BMP2* modifier gene through an siRNA screen of *BMP2* signaling regulatory genes combined with publicly-available PAH RNA expression data. Subsequently, the authors showed that pharmaceutical upregulation of *FHIT* prevented and reversed experimental pulmonary hypertension in a rat model[65]. Rhodes and colleagues utilized metabolomics to identify circulating metabolites that distinguish PAH cases from healthy controls, predict outcomes among PAH cases, and to monitor metabolite levels over time to determine whether correction could affect outcomes[66]. Stearman et al combined gene expression data with pathway analyses to identify a transcriptional framework for PAH affected lungs[67]. Similarly, Hemnes and colleagues used transcriptomics to identify RNA expression patterns predictive of vasodilator responsiveness among PAH patients[68]. These studies highlight the promise of other omics in predictions of PAH risk, diagnosis, classification, drug responsiveness and prognosis. However, such studies have not been conducted in children. Detailed omic phenotyping requires biologic sampling which can be difficult in pediatric patients, especially for the very young or those with complex medical conditions. We propose a pilot genomics-first approach followed by detailed phenotyping of patients grouped by genetic diagnosis to enrich the biologic sampling and assess utility before performing larger studies across all pediatric PAH patients.

2. A genomics first approach for pediatric PAH

Identification of molecular subtypes of PAH has been proposed as a means to improve risk stratification, treatment and outcomes. Obtaining a genetic diagnosis in children requires more extensive genetic testing than in adults (Figure 3). If testing for a panel of genes known to be associated with PAH is not diagnostic, children should be evaluated genome-wide for rare *de novo* and inherited variants with trio (parental and child) exome sequencing/chromosome microarray or genome sequencing. With knowledge of the causal gene, the natural history, penetrance, and response to treatment can be refined for that specific genetic subtype of PAH to allow for more precise care for each genetically defined group. Individuals across genes in the same biological pathway can then be compared to assess similarities and differences.

Collaboration across national and international clinical PH sites will be necessary to yield sufficient sample sizes due to the extremely small number of pediatric PAH patients at single PH sites, heterogeneity of risk genes for PAH, and need for ancestral diversity. PPHNet is an example of a pediatric-specific PAH consortium with ongoing recruitment across 13 North American clinical



sites[69-73]. PVDomics[74], a US multicenter study launched in 2014, and PAH-ICON (pahicon.com), a new international effort, represent additional large-scale PAH cohorts. PAH was defined as mPAP ≥ 25 mmHg, but was recently updated to include mPAP 20-25 mmHg in both children and adults[7]. Due to variability in pulmonary hemodynamics during the post-natal transition period, pediatric PAH is defined by elevated mPAP *after 3 months of age* in combination with pulmonary vascular resistance as indexed to body surface area, PVRI ≥ 3 Woods units/m²[7]. Clinical classification of PAH subtypes aims to improve clinical management and enhance research efforts, and is typically based on the World Symposium on Pulmonary Hypertension (WSPH) system, updated during the 2018 Nice session[75]. In children, use of a pediatric-specific classification system developed in Panama by the Pulmonary Vascular Research Institute (PVRI) Pediatric Task Force[76], provides more definitive classification of developmental and complex phenotypes.

Biological trios, comprised of two unaffected parents and an affected child, are preferred over singleton cases for pediatric studies of PAH in order to identify both inherited and *de novo* variants causal for disease. However, as the number of ethnically-matched genomic data in public databases increases, the need for trios will decrease. DNA can be reliably obtained from small samples of blood or saliva. We have developed methods in a large national autism study to collect saliva from pediatric patients and their parents in their homes, with instructional videos to support clinical sites with remote biospecimen collection[77, 78]. Biological samples can be shipped to a central biorepository for DNA extraction and then processed and sequenced using a single genetic platform. Following extensive quality control, filtering and annotation, sequencing data is screened for rare deleterious variants in known candidate genes or undergoes trio analysis for *de novo* variants or association analysis for inherited variants[9, 10].

Once genetic subtypes are defined, demographic data, clinical phenotypes and imaging data (pulmonary vascular angiography, chest x-ray or CT scans, chest/cardiac MRI, and lung biopsies) can be compared among cases with variants in the same gene/pathway. Relevant cases can be recalled or remotely interviewed for targeted clinical assessments to determine if there are similarities among cases with rare variants in the same gene/pathway.

To increase rigor and assess the full phenotypic spectrum of the new genetic subtypes, additional cases can be identified using GeneMatcher, clinical diagnostic laboratories, and large sequencing centers. Longitudinal phenotypes of the genetic subtypes can be assessed retrospectively and prospectively, including death/transplant, response to medication, other medical diagnoses, and changes in lung function. For inherited variants, cascade genetic testing of family members with clinical evaluation of “unaffected” individuals who carry the relevant genetic variant can inform penetrance by age and sex. To support families with genetic diagnoses, “virtual” family meetings can be organized to update families on new findings related to their conditions and build communities for each of the rare endophenotypes.

3. Summary

Pediatric PAH differs from adult-onset PAH in many important aspects including clinical presentation, etiology, genetic burden and specific genes involved. In many young children, PAH is a developmental disease with a complex phenotype. *TBX4* and *SOX17* are developmental transcription factors, and variants in these genes occur much more frequently in pediatric- compared to adult-onset disease. *De novo* variants likely contribute to at least 15% of pediatric-onset PAH but the specific genes require confirmation in larger pediatric cohorts. Many of these genes likely contribute to developmental phenotypes and complex medical conditions. A genomics-first approach to pediatric PAH starts with a genetic diagnosis followed by phenotypic characterization of cases with variants in the same genes/pathways. Large, diverse pediatric populations are needed to confirm the candidate genes identified thus far, characterize the rare endophenotypes and natural history and assess the efficacy of therapies to inform more precise clinical management. In addition, questions related to which children are at risk for developing PAH - especially children with CHD, CDH, bronchopulmonary dysplasia and Down syndrome - may be answered. Identification of the

genes, pathways, and networks in children could provide novel target for therapy not only for children but for all patients with and at high risk for PAH.

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