

Genomics of Pulmonary Hypertension

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Background - Pulmonary hypertension (PH), defined by mean pulmonary artery pressure >20 mmHg, is a common physiologic manifestation of many diseases. Pulmonary arterial hypertension (PAH) represents a smaller subgroup of patients who have PH, and PAH causes significant cardiorespiratory morbidity and premature mortality. PH can manifest across the lifespan, with similar incidence for both pediatric- and adult-onset disease. However, pediatric-onset disease is particularly challenging because it is frequently associated with a more severe clinical course and comorbidities including lung and heart developmental anomalies. For PH Group 1/pulmonary arterial hypertension, causal genetic variants can be identified in ~13% of adults and ~43% of children.

Clinical implications - Education about the option for genetic testing is strongly recommended for all pediatric and adult HPAH/IPAH patients. Both gene panel and exome/genome sequencing tests can be useful in diagnosis, but exome/genome sequencing provides a comprehensive dataset for reanalysis over time for cases without an initial diagnosis. Knowledge of genetic diagnoses can immediately impact clinical management of PH, including multimodal medical treatment, surgical intervention, transplantation decisions, and screening for associated conditions.

Conclusions - There is a need for large, diverse, international consortia with ever-improving analytical pipelines to confirm previously implicated genes, identify additional genes/variants, assess penetrance, and clinically characterize each genetic subtype for natural history, prognosis and response to therapies to inform more precise clinical management.

INTRODUCTION

Pulmonary hypertension (PH), defined by mean pulmonary artery pressure >20 mm Hg, is a common physiologic manifestation of many diseases. Pulmonary arterial hypertension (PAH) represents a smaller subgroup of patients who have PH, and PAH causes significant cardiorespiratory morbidity and premature mortality. PH can manifest across the lifespan with an estimated incidence of 28.7 cases/100 000 individuals/year and prevalence of 127.3 cases/100 000 individuals.¹ The World Symposium on PH² and World Health Organization¹ define 5 main PH Groups, with the majority of adult cases classified as Group 2/left heart disease (34%) and childhood cases classified as Group 1/PAH (65%).¹ The diseases are 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. This review will focus on the genomics of PH. Most genetic studies to date have been carried out in cohorts of European-centric, adult-onset Group 1/PAH because of the accessibility of cases and relative homogeneity of heritable and idiopathic PAH compared to other subtypes. We highlight studies with increased diversity of PAH subtype, age of PAH onset, and genetic ancestry where applicable. Emerging data from genetic studies of pediatric-onset PH indicate that the genetic basis is different from that of adults. Thus, we also highlight differences between adult-onset PH and pediatric-onset PH.

GENOMIC CAUSES OF PAH

Recent analyses of relatively large PAH cohorts have further defined the frequency of individuals with deleterious variants in established PAH risk genes and the variant types (Table 1).^{3,4} *BMPR2* (bone morphogenetic protein receptor 2) mutations are observed in the majority of heritable PAH cases across genetic ancestries,³⁻⁷ but only 10% to 20% of previously classified idiopathic PAH (IPAH) cases, and rarely for PAH associated with other diseases (autoimmune connective tissue diseases, congenital heart disease [CHD], portopulmonary disease, and others)³ or PAH induced by diet and toxins.³ *BMPR2* carriers have younger mean age of onset and are less responsive to vasodilators compared to noncarriers.^{3,8,9} The pathogenetic mechanism of *BMPR2* variants in adult-onset disease is haploinsufficiency due to likely gene-disrupting (including stop-gain, frameshift, splicing, and exon deletion)

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Table 1. Allele Frequencies and Associated Variant Types for PAH Causal Genes in the National Biological Sample and Data Repository for PAH (PAH Biobank, n = 2572 cases^a); 90% of Cases are Adult-Onset

Gene	Gene name	# cases (%)	Variant type ^b
<i>BMPR2</i>	Bone morphogenetic protein receptor 2	180 (7%)	LGD, missense
<i>ABCC8</i>	ATP binding cassette subfamily C member 8	29 (1.1%)	LGD, missense
<i>GDF2</i>	Growth and differentiation factor 2	28 (1.1%)	LGD, missense
<i>TBX4</i>	T-box transcription factor 4	23 (0.89%)	LGD, missense
<i>ACVRL1</i>	Activin receptor (type II) like 1	16 (0.62%)	LGD, missense
<i>SMAD9</i>	SMAD family member 9	13 (0.51%)	LGD, missense
<i>KCNA5</i>	Potassium voltage-gated channel subfamily A member 5	13 (0.51%)	LGD, missense
<i>SOX17</i>	SRY-box transcription factor 17	10 (0.39%)	LGD, missense
<i>CAV1</i>	Caveolin1	10 (0.39%)	LGD, missense
<i>KDR</i>	Kinase insert domain receptor	7 (0.27%)	LGD, missense
<i>ATP13A3</i>	ATPase 13A3	7 (0.27%)	LGD, missense
<i>ENG</i>	Endoglin	6 (0.23%)	LGD, missense
<i>EIF2AK4</i>	Eukaryotic initiation translation factor 2 alpha kinase 4	5 (0.19%)	LGD, missense
<i>KCNK3</i>	Potassium two pore domain channel subfamily K member 3	3 (0.12%)	missense

Abbreviations: LGD, likely gene-disrupting; PAH, pulmonary arterial hypertension.

^aPAH cases include 48% PAH associated with other diseases (autoimmune connective tissue diseases, congenital heart disease, portopulmonary disease, and others), 43% idiopathic disease, 4% heritable PAH, 5% other.

^bVariants filtered by gnomAD AF ≤ 0.0001 and variant type LGD or damaging missense defined by REVEL score > 0.5 . Bold typeface indicates primary variant type.

variants. Among children with PAH, there is an enrichment of predicted deleterious missense variants, suggesting that dysfunctional *BMPR2* may be more harmful than inactivation or deletion of a normal copy of the gene.^{3,10} *ACVRL1* (activin A receptor type II-like 1) and *ENG* (endoglin), both encoding protein receptor components of the *BMPR2* complex, contribute to ~0.85% of PAH cases,³ especially adult-onset PAH associated with hereditary hemorrhagic telangiectasia. Variants in *SMAD9* (mothers against decapentaplegic 9), encoding a downstream signaling molecule, contribute rarely. The newest PAH causal gene identified in the TGF- β pathway is *GDF2* (growth and differentiation factor 2), encoding *BMP9*, a circulating cytokine and ligand of coreceptor complex *BMPR2/ACVRL1*. Genome-wide significance was demonstrated in both European⁴ and Asian¹¹ cohorts with replication in the PAH Biobank cohort.³ Similar to other PAH risk genes in the TGF- β pathway, the mode of inheritance was autosomal dominant. Variants in *GDF2* contribute to ~1% of PAH (mostly IPAH) cases in European-enriched cohorts^{3,4} and

more frequently in Chinese patients (~6.7%).¹¹ Most of the PAH-associated *GDF2* variants are missense variants, a variant type that could not be rigorously assessed in smaller-sized cohorts.

Outside of the TGF- β /BMP pathway, channelopathy gene *ABCC8* (ATP-binding cassette subfamily member 8) and transcription factor *TBX4* (T-box transcription factor 4) are the most common causes of PAH, accounting for ~1% of cases each (Table 1). More than 40 *ABCC8* missense variants have been reported for PAH cases with IPAH, heritable PAH, and PAH associated with other diseases,^{3,12,13} and at least some of the variants have demonstrated reduced channel function.¹² While the genetic evidence for *ABCC8* in PAH is well-documented, more experimental evidence is needed to elucidate the pathogenetic mechanism. *TBX4* was originally identified as a PAH causal gene in a cohort of children with PAH, some of whom had contiguous gene deletions and a more complex phenotype including intellectual delay and/or structural heart defects.^{14,15} Subsequent studies revealed an enrichment

of likely gene-disrupting and missense *TBX4* variants in pediatric-onset PAH, primarily IPAH and PAH associated with CHD, with rare adult-onset cases caused by *TBX4*.^{3,6,16} Originally described as a determinant of pattern formation including limb development,¹⁷ the association of *TBX4* with PAH, cardiac defects^{18,19} and, more recently, a variety of developmental lung disorders^{19,20} indicates an expanding role for *TBX4* in development.

Other established PAH causal genes contribute importantly but rarely to PAH. Evidence for these genes stems from family studies with corroboration in additional sporadic cases or large cohort studies. Biallelic variants in *EIF2AK4* (eukaryotic initiation translation factor) cause rare forms of PAH, once known as pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis, and now classified as PAH with overt features of venous/capillary involvement.^{21,22} Loss of function missense variants identified 2 channelopathy genes, *KCNK3* (potassium two pore domain channel),²³⁻²⁶ a regulator of pulmonary vascular tone, and *CAV1* (caveolin-1),²⁷⁻²⁹ encoding a structural

Table 2. Clinical Characteristics and Hemodynamic Parameters of Pediatric-Onset vs Adult-Onset PAH Cases at Diagnosis. Data From PAH Biobank (n = 2572 Cases). Pediatric-Onset, <18 Years of Age at Diagnosis. Mean \pm SD

PAH Group (n)	Age at diagnosis (y)	Female:Male ratio	mPAP (mm Hg)	mPCWP (mm Hg)	CO (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, DS, and other rare genetic syndromes
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 ^a	< 0.0001 ^b	< 0.0001 ^b	< 0.0001 ^b	< 0.0001 ^b	

Abbreviations: DS, Down Syndrome; CHD, congenital heart disease; CO, cardiac output; HTN, systemic hypertension; mPAP, mean pulmonary artery pressure; mPCWP, mean pulmonary capillary wedge pressure; PAH, pulmonary arterial hypertension; PVR, pulmonary vascular resistance.

^aFisher exact test.

^bStudent *t* test, 2-tailed.

and signaling component of lipid rafts abundant in pulmonary endothelial membranes. Variants in these channel genes are associated with heritable PAH and IPAH. More recently, exome sequencing of large cohorts identified variants in pro-angiogenic gene *KDR* (kinase insert domain receptor),^{22,30,31} developmental transcription factor *SOX17*^{3,14,16,32} and channel gene *ATP13A3* (ATPase 13A3).^{3,4} *KDR* is highly expressed in lung and encodes the receptor for vascular endothelial growth factor type 2. Vascular endothelial growth factor signaling plays an essential role in embryonic lung development and structural maintenance of adult lung.³³ Loss of function variants in *KDR* are associated with low diffusion capacity for carbon monoxide in IPAH cases.³⁰

Recent associations of variants in aquaporin 1 (*AQP1*),⁴ fibulin-2 (*FBLN2*),³¹ gamma-glutamyl carboxylase (*GGCX*),³ kallikrein 1 (*KLK1*),³ and platelet-derived growth factor D (*PDGFD*)^{31,34} have been reported but

require independent confirmation. Finally, de novo variant (DNV) analysis of a PAH parent-child trio cohort (n = 124 trios) identified an important role of DNVs across all genes in pediatric-onset PAH,³¹ but larger cohorts are needed to confirm the role of individual genes (see below).

PEDIATRIC PH

PH differs from adult-onset disease in several important aspects including sex bias, associated clinical features, etiology, and response to therapy. Data from the National Biological Sample and Data Repository for PAH (PAH Biobank)^{3,31} indicate a markedly lower female sex bias among children with PAH (Table 2), suggesting less dependence on sex-specific factors. Children present with higher pulmonary artery pressure, decreased cardiac output, and higher pulmonary vascular resistance compared to adults (Table 2). Common comorbidities in pediatric PAH include CHD and developmental syndromes in contrast to the age-related cardiopulmo-

nary and metabolic diseases commonly associated with adult-onset PAH. Data from the Pediatric PH Network Registry³⁵ underscore the role of congenital and developmental diseases in pediatric PH, with >75% of Group 1 and Group 3 cases associated with CHD, bronchopulmonary dysplasia, congenital diaphragmatic hernia (CDH), or other rare developmental syndromes or anomalies (Table 3). Relative to adult-onset PH, pediatric PH has been vastly understudied, and little is known regarding the natural history, mechanisms of disease, and treatment of PH in children. The standard of care for pediatric PH patients is primarily based on extrapolations from adult data with only 1 pharmaceutical therapy approved by the Federal Drug Administration for use in children because of the lack of safety and efficacy data.

Emerging data from genetic studies of Group 1 PAH indicate that the genetic basis in children is different from that of adults.³⁶ We recently combined data from our Columbia University Irving Medical Center PAH cohort^{16,32} and the PAH Biobank^{3,31} to compare the genetic contribution of inherited and DNVs in pediatric- vs adult-onset PAH, including PAH associated with other diseases. We identified a greater genetic burden of rare pathogenic/likely pathogenic variants among pediatric-onset PAH cases (~43%) compared to adult-onset cases (~13%) (Figure 1).³⁷ DNVs are the most frequent genetic etiology of PAH in children, likely contributing to ~15% of all cases. While a few DNVs have been identified in known PAH risk genes –

Table 3. Frequent Occurrence of Developmental Comorbidities in Pediatric PH. Data From the Pediatric Pulmonary Hypertension Network (n = 1475 Cases)³⁶

PH Group	Associated comorbidity (% of subgroup)
Group 1 (n = 663)	60% CHD
	14.5% rare genetic syndromes
Group 3 (n = 720)	44% BPD
	36% CDH
	10% other developmental anomaly ^a

Abbreviations: BPD, bronchopulmonary disease; CDH, chronic diaphragmatic hernia; CHD, congenital heart disease; PH, pulmonary hypertension.

^a10.7% Down, 1.4% DiGeorge, 0.6% Trisomy 18, 0.5% Noonan, 0.5% CHARGE, 0.8% others.

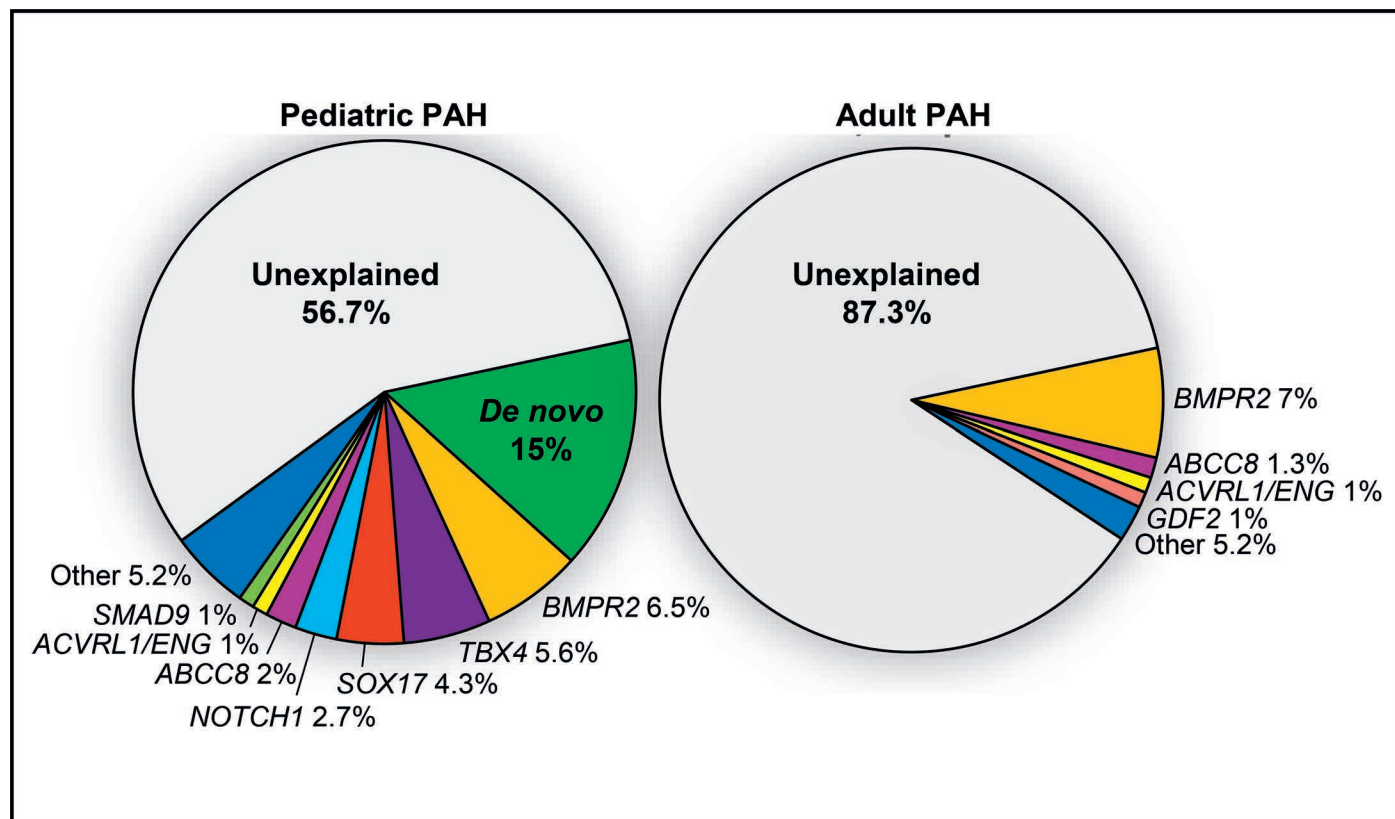


Figure 1: Relative contribution of de novo variants and 18 PAH risk genes in a cohort of 443 pediatric and 2628 adult PAH cases from Columbia University Irving Medical Center and the PAH Biobank. Risk genes included *BMPR2*, *ABCC8*, *ACVRL1*, *ATP13A3*, *BMPR1A*, *BMPR1B*, *CAV1*, *EIF2AK4*, *ENG*, *GDF2*, *KCNA5*, *KCNK3*, *KDR*, *NOTCH1*, *SMAD1*, *SMAD4*, and *TBX4*. PAH cases included IPAH, APAH, FPAH, and other rarer cases.

Table 4. Novel Genes With de novo LGD or D-Mis Variants in Pediatric-Onset PAH (n = 124 trios)

Gene symbol	Variant type	Protein change	REVEL	CADD	Allele frequency (gnomAD) ^a	E14.5 heart expression rank %	E16.5 lung expression rank %	pLI ^b	PAH class
<i>AMOT</i>	LGD	p.(Leu320 Cysfs*55)	.	31	.	95	68	0.21	IPAH
<i>CSNK2A2</i>	D-Mis	p.(His184Leu)	0.50	25	.	77	55	1	IPAH
<i>HNRNPF</i>	LGD	p.(Tyr210Leufs*14)	.	29	.	98	85	0.86	NA
<i>HSPA4</i>	D-Mis	p.(Pro684Arg)	0.62	30	4.1e-6	96	43	0.03	APAH-CHD
<i>KDM3B</i>	D-Mis	p.(Pro1100Ser)	0.66	29	.	87	89	1	IPAH
<i>KEAP1</i>	LGD	p.(Tyr584*)	.	35	.	82	79	0.25	IPAH
<i>MECOM</i>	D-Mis	p.(Phe762Ser)	0.76	32	.	60	82	1	IPAH
<i>ZMYM2</i>	LGD	p.(Arg540*)	.	36	.	77	93	0.97	IPAH

Abbreviations; *AMOT*, angiominotin; APAH-CHD, pulmonary arterial hypertension associated with congenital heart disease; *CSNK2A2*, casein kinase II, alpha 2; D-Mis, deleterious missense with REVEL score >0.5; *HNRNPF*, heterogeneous nuclear ribonucleoprotein F; *HSPA4*, heat shock protein family A (HSP70) member 4; IPAH, idiopathic pulmonary arterial hypertension; *KDM3B*, lysine demethylase 3B; *KEAP1*, Kelch-like ECH-associated protein 1; LGD, likely gene-disrupting; *MECOM*, MDS1 and EVI1 complex locus; *ZMYM2*, zinc finger protein 620.

^aAllele frequency "." absent from gnomAD.

^bpLI > 0.5, constrained gene.

ACVRL1, *BMPR2*, *TBX4* – the vast majority of the genes are novel candidate genes. Three of the genes are known CHD genes (*NOTCH1*, *PTPN11*, *PSMD12*), and we previously reported

rare inherited variants in PAH associated with CHD cases for *NOTCH1* (n = 5) and *PTPN11* (n = 1). *NOTCH1* is the most commonly associated gene for the congenital heart defect of tetral-

ogy of Fallot,³⁸ and the *NOTCH1* DNV carrier had a diagnosis of PAH associated with 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 DNVs identified in both of these genes are known causal Noonan syndrome variants,³⁹ and at least 3 cases of fatal pediatric PAH with Noonan syndrome have been previously reported.^{40,41} Of note, 37% of the candidate genes identified by DNV analysis are causal genes for rare developmental syndromes.⁴² Novel genes with plausible roles in lung/vascular development but not previously implicated in PH are listed in Table 4. Notably, variants in the novel genes have not been observed in adult-onset cases and likely are specific to pediatric PH.

The frequent presentation of pediatric PH with other congenital and early-onset comorbidities suggests that the causal genes in children have roles in cardiopulmonary development. Decreased lung vascular and alveolar growth predispose to vascular injury during susceptible periods of growth and adaptation. Histopathological studies have identified abnormal lung development and lung hypoplasia as common features of PAH, CHD, CDH, and Down syndrome.^{43,44} Two established PAH genes, *TBX4* and *SOX17*, are highly expressed in embryonic tissues and have roles in lung and vasculature development. Rare DNVs or heritable variants in these genes account for 5.6% (*TBX4*) and 4.3% (*SOX17*, usually associated with CHD) of pediatric PAH cases but less than 1% (*TBX4*) or rarely (*SOX17*) of adult-onset PAH. Although *rare* variants in *SOX17* are an infrequent cause of PAH in adults, *common* variants in *SOX17* contribute to adult PAH.⁴⁵ Thus, different classes of variants in the same genes may contribute to and inform PAH across the lifespan.

While most PAH risk genes exhibit an autosomal dominant mode of inheritance in adult-onset PAH, there is increasing evidence of codominant inheritance in pediatric-onset PAH. For example, biallelic variants of *ATP13A3*, encoding an ATP-driven pump involved in polyamine homeostasis, were recently identified in 5 children from 3 families diagnosed under the age of 3 years with severe PAH largely refractory to treatment and associated

with high mortality.⁴⁶ These data suggest that *ATP13A3* exhibits a dose-dependent effect in which 2 variant alleles cause severe, early-onset PAH. Together, the data from pediatric cohorts indicate that there is a greater genetic burden, differences in causal variant type and class, and increased occurrence of biallelic inheritance compared with adult-onset disease. Thus, studies of children will likely identify a greater number and broader spectrum of PH risk genes, and may also lead to insights in adult PH.

DNVs have emerged as an important class of genetic factors underlying early-onset, rare, and lethal developmental disorders^{47,48} because of strong negative selection decreasing reproductive fitness.⁴⁹ These genes tend to be *constrained* genes that are intolerant to loss of function alleles,^{50,51} involved in coordinated organogenesis, and include transcription factors, RNA-binding proteins, protein kinases, and chromatin modification. While we have demonstrated a significant contribution of DNVs in pediatric PAH,^{16,31} our studies have been underpowered to definitively implicate which of the large numbers of genes identified are truly associated with PH. We recently identified *LONP1* as a CDH causal gene in the DHREAMS pediatric CDH cohort.⁵² Using 827 child-parent trios, we identified CDH cases with rare deleterious *de novo* missense variants implicating *LONP1* at a false discovery rate <0.05. Nearly 3% of the CDH cases had likely gene-disrupting or deleterious missense variants. We further demonstrated that heterozygous individuals with rare variants in *LONP1* had PH with higher mortality and greater need for extracorporeal membrane oxygenation compared to noncarriers. *LONP1* is a nuclear-encoded mitochondrial protease. Using a novel conditional knockout mouse model, we showed that inactivation of *LONP1* in embryonic lung epithelium only *with an intact diaphragm* leads to disrupted lung development and 100% neonatal lethality (Figure 2). These data implicate a primary developmental lung defect independent of the CDH. The potential role of *LONP1* in PH in general is unknown and will

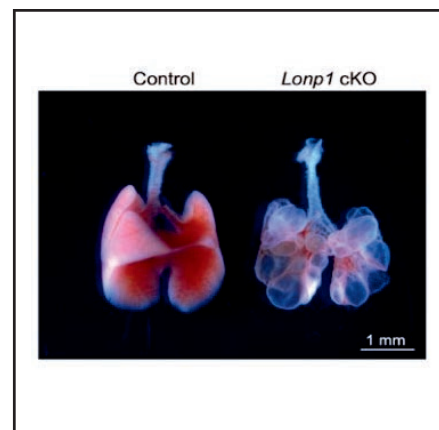


Figure 2: Embryonic epithelial-specific inactivation of *LONP1* causes immature, fluid-filled lungs in mice and neonatal lethality with intact diaphragm.

be part of future studies. Studies of CDH and CHD can complement and inform studies of pediatric PH, but independent PH cohorts are necessary for identifying PH risk genes because PH is not always associated with CDH/CHD and such cases may be harder to treat.

PENETRANCE

Genetic linkage and candidate gene studies indicate an autosomal dominant mode of inheritance for PAH risk, and most causal genetic factors for PAH are typically autosomal dominantly inherited, such as *BMPT2*.^{53,54} However, many individuals who carry monogenic risk variants in *BMPT2* and other causal genes never develop PAH. This issue of incomplete penetrance suggests that additional genetic, epigenetic, environmental factors, and gene x environment interactions contribute to risk for PAH. Exome and genome sequencing studies have identified a subset of PAH cases with deleterious variants in more than one risk gene^{3,4,31} but the relative contribution of each risk allele to the development of PAH is unknown, including the potential for gene-gene interactions. Tests of oligogene or multiple-gene models will require hundreds of thousands of cases, clearly much greater than the number of cases in current cohorts. Similarly, identification of modifier genes affecting PAH penetrance is an area of great interest but will require larger cohorts.

GENETIC ANCESTRY

Most of the large genetic studies conducted to date have used 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^{3,16} but the frequency of *ACVRL1* variants alone may be closer to 13% among Asian children.⁵⁵ As mentioned, *GDF2* variants might be a more frequent cause of PAH among Asians compared to Europeans.^{3,4,11} Data from the PAH Biobank indicate that *GDF2* variants may contribute more frequently to PAH cases of Asian (3/98 cases, 3.1%), Hispanic (8/309, 2.6%), and African (4/283, 1.4%) ancestries compared to Europeans (13/1852, 0.7%).³ Further study is required to determine whether these differences are true genetic ancestry effects or random differences due to relatively small sample size. A PAH case study of a 5-year-old boy of Hispanic ancestry identified a homozygous *GDF2* likely gene-disrupting variant, and the unaffected parents were heterozygous for the variant.⁵⁶ Interestingly, the gnomAD population database (gnomADv2.1.1, n = 141 456 samples)⁵¹ contains only 2 heterozygous counts of this allele, both of Latino ancestry, suggesting that this might be an ancestry-specific allele. Clearly, larger studies of PH with greater diversity are needed to define population-specific risk gene allele frequencies as well as ancestral-specific genetic factors.

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.^{57,58} For example, West and colleagues performed RNA sequencing of blood lymphocytes derived from *BMPR2* variant carriers with and without PAH to identify transcriptional patterns

relevant to disease penetrance.⁵⁹ More recently, *FHIT* was identified as a potentially clinically relevant *BMPR2* modifier gene through an siRNA screen of *BMPR2* signaling regulatory genes combined with publicly-available PAH RNA expression data. Subsequently, the authors showed that pharmaceutical upregulation of *FHIT* prevented and reversed experimental PH in a rat model.⁶⁰ Stearman et al combined gene expression data with pathway analyses to identify a transcriptional framework for PAH-affected lungs.⁶¹ Similarly, Hemnes and colleagues used transcriptomics to identify RNA expression patterns predictive of vasodilator responsiveness among PAH patients.⁶² Rhodes and colleagues used 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.⁶³ Notably, increased levels of polyamine metabolites were among the prognostic metabolites identified, and PAH causal gene, *ATP13A3*, encodes a key regulator of polyamine metabolism. These studies highlight the promise of other omics in predictions of PAH risk, diagnosis, classification, drug responsiveness, and prognosis.

GENETIC TESTING

Our data indicate that the diagnostic yield of genetic testing is especially high for pediatric PAH, approaching 50%, and education about the option for genetic testing is strongly recommended for all pediatric PAH and for adult patients with heritable PAH and IPAH. For children, analysis of child-parent trios can increase the diagnostic yield of exome sequencing up to 15% based on analysis of DNVs. Knowledge of genetic diagnoses can immediately impact clinical management of PH, including multimodal medical treatment, surgical intervention, transplantation decisions, and screening for associated conditions. A genetic diagnosis can lead to early treatment of associated medical conditions, cascade genetic testing of family members to identify those at risk for

developing PAH, and can clarify reproductive risks to inform family planning decisions. Biallelic mutations in *EIF2AK4* are diagnostic for pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis,⁶⁴ which can be difficult to diagnose clinically without a lung biopsy, and patients can be listed for transplant earlier in the course of disease, which may improve outcomes. *ACVRL1/ENG* variant carriers with PAH associated with hereditary hemorrhagic telangiectasia are prone to arteriovenous malformations in brain, intestine, liver, and lung;⁶⁵ these patients require periodic MRI surveillance. *TBX4* variant carriers, especially children, are prone to other lung, cardiac, or skeletal defects¹⁹ and should be assessed by imaging studies and physical exam of the hands, hips, knees, and feet. In addition, a diagnosis of *TBX4* variants in newborns with persistent PH indicates increased risk for developing PAH later in childhood,¹⁹ and these patients should be screened annually by echocardiography. Rare biallelic forms of very-early-onset severe PAH have recently been identified for *ATP13A3*,⁴⁶ *GDF2*,⁵⁶ and *KCNK3*.²⁴ Such cases may be largely refractory to treatment and with high mortality, requiring early referral for surgery for a Potts shunt or lung transplantation.

While panel testing is often used for clinical diagnostic testing, the gene-sets included in panels are highly variable and can be limited in scope, so the gene list should be carefully reviewed. Decreasing cost and increasing availability of clinical exome and genome sequencing services⁶⁶ will soon allow genomic sequencing tests to become the gold standard for genetic testing, either for adult patients with familial disease and without identified mutations from gene panels and in children regardless of family history. Exome/genome sequencing data provides a permanent dataset that can be reassessed over time as new risk genes are identified. Periodic reanalysis is highly recommended for cases without a diagnosis.

To support families with genetic diagnoses, gene-specific family support groups and virtual or in-person family meetings can be organized to update

families on new findings related to their conditions and build communities for each of the rare subtypes. For example, TBX4Life is a recently organized and active family-based effort to raise awareness, educate families, and identify additional *TBX4* variant carriers to enhance further research.

SUMMARY

The genetic landscape of PH continues to emerge, primarily through genetic studies of PAH. Currently, the diagnostic yield for PAH is ~13% for adults and 43% for children. DNVs account for ~15% of pediatric-onset cases, but larger pediatric cohorts are needed to confirm the role of individual genes and identify new genes. *LONP1* is a new CDH causal gene associated with PH, but the role of *LONP1* in PH, in general, will require assessment in PH cohorts. Genetic sequencing tests are recommended for clinical diagnoses, are readily available, and are usually covered by insurance in the United States. Exome or genome sequencing allows for periodic reanalysis of cases with no initial genetic diagnosis. Clearly, there is a need for large, diverse, international consortia with ever-improving analytical pipelines to confirm known candidate genes, identify additional genes and variants, assess penetrance, the role of genetic ancestry, and characterize each genetic subtype including natural history, prognosis, and response to therapies to inform more precise clinical management.

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