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Genetics and PH

Program Description

The mission of *Advances in Pulmonary Hypertension* is to serve as the premiere forum for state-of-the-art information regarding diagnosis, pathophysiology, and treatment of pulmonary hypertension (PH). The 2018 Nice revision of the World Symposium on Pulmonary Hypertension (Simmonneu G, Montani D, Celermajer DS, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53(1). DOI:10.1183/13993003.01913-2018) serves as a guide to categories of PH addressed in *Advances in Pulmonary Hypertension*. While focusing on Group 1 PH (PAH: pulmonary arterial hypertension), the other categories (Group 2, PH due to left heart disease; Group 3, PH due to lung diseases and/or hypoxia; Group 4, PH due to pulmonary artery obstructions; Group 5, PH with unclear and/or multifactorial mechanisms) are also addressed. This mission is achieved by a combination of invited review articles, roundtable discussions with panels consisting of international experts in PH, and original contributions.

Objectives

- Provide up-to-date information regarding diagnosis, pathophysiology, and treatment of PH.
- Serve as a forum for presentation and discussion of important issues in the field, including new paradigms of disease understanding and investigational trial design.

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Advances in Pulmonary Hypertension: Official Journal of the Pulmonary Hypertension Association is a quarterly publication directed by an editorial board of renowned pulmonary hypertension (PH) experts with oversight by PHA's Scientific Leadership Council. The mission of *Advances in PH* is to assist physicians in their clinical decision-making by informing them of important trends affecting their practice and providing an analysis of the impact of new findings and current information in peer-reviewed publications. Each article is reviewed and approved by members of the Editorial Board.

While most articles are invited by the Editorial Board, the following submissions will be considered for publication:

- Reviews that summarize and synthesize peer-reviewed literature to date on relevant topics
- Letters to the Editor
- Clinical case studies

Submitted manuscripts are reviewed by the Editorial Board and other experts in the field. Acceptance of manuscripts is determined by factors such as quality, relevance, and perceived value to clinical decision-making.

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GENETICS MOVES TO CENTER STAGE

Our understanding of pulmonary hypertension (PH) has advanced steadily. Almost 100 years ago, light microscopy provided the earliest insights into a rare progressive and fatal disease, primary pulmonary hypertension ("PPH"). Pathologists described the histopathology of PPH, with terms like "plexogenic" and "microthromboembolic" PPH. Pathologists, who specialized in pulmonary vascular disease, identified two rare disorders, pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH), that mimicked PPH clinically, but were distinctly different under the microscope.

By the middle of the 20th century, the introduction of pulmonary artery catheterization to the study of cardiovascular diseases produced a new level of understanding of PH based upon physiologic observations of intravascular pressures and flows, with an emphasis on mechanisms that caused constriction and relaxation of pulmonary arterioles. This era supported early efforts to treat sporadic PPH and familial PPH, now known as idiopathic and heritable pulmonary arterial hypertension (PAH) with vasodilators, eventually leading to treatments which targeted three pathways integral to vasoconstriction and vasodilation of pulmonary arterioles.

Even as pathologic, physiologic, and treatment studies proceeded during the second half of the 20th century, the genetic era began. Investigators took advantage of scientific advances in genetics to discover heritable causes of PH. The first breakthrough occurred in 2000, when two research teams independently reported that mutations in the gene (*BMPR2*), encoding the bone morphogenetic protein type 2 receptor, caused familial PPH. Over the next two decades investigators linked DNA sequence variations in 16 additional genes to heritable forms of PAH, including PVOD and PCH. These discoveries shaped a new understanding of PAH.

Investigators quickly determined that *BMPR2* regulated the proliferation of vascular cells, not vasoconstriction. Recognition of the pivotal role of cellular proliferation and transforming growth factor β (TGF-β) signaling paved the way for a new approach to the treatment of PAH. In the Study of Sotatercept for the Treatment of Pulmonary Arterial Hypertension (PULSAR), sotatercept, a fusion protein that impairs activation of a TGFβ pro-proliferative pathway, produced a greater reduction in pulmonary vascular resistance than placebo. Now studies are underway to assess the efficacy and safety of sotatercept across a broad spectrum of PAH disease severity.

We have entered a new era of understanding and treating PAH, as genetics moves to center stage. This issue of *Advances in Pulmonary Hypertension* was organized to serve as a valuable resource for the PH community. The first article, authored by Drs. Carrie Welch and Wendy Chung of Columbia University, provides a comprehensive overview of the genomics of pulmonary hypertension. With this foundation in place, the second article, authored by Rachel Sullivan, MD (Stanford University) and Eric Austin, MD (Vanderbilt University) reviews the relationships between specific gene sequence variations and PAH phenotypes that clinicians are likely to encounter.

In recent years genetic counseling and genetic testing have become important affordable services for patients with PAH and their families. Sumathi Rachamadugu, MSc, MS and Melanie Emmerson, MS (Intermountain Healthcare) collaborated with Barbara Girerd, PhD (Université Paris-Saclay) and Hunter Best, PhD (University of Utah) to provide an in depth introduction pretest genetic counseling, genetic testing, and posttest genetic counseling for PAH patients and their families. The PH Professional network contribution by Athena Angelopoulos, MS and Rachel Farrell, MS (University of California San Francisco) complements the preceding article by providing additional details related to genetic counseling and testing for PAH patients and their families.

Finally, Drs. Austin, Chung, and Yu, joined us for a roundtable discussion on the genetics of pulmonary hypertension. Participants shared their personal knowledge of the history of genetic research and discovery related to PH, their advice on discussing genetic aspects of PH with patients and families, their thoughts about involving genetic counselors and ordering genetic tests, as well as how genetic test results influence treatment decisions and how knowledge of molecular pathways informs the development of new medications to treat PAH.

In closing, we thank the authors and everyone at PHA and Allen Press who worked so hard to produce this issue of *Advances*.

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REGULAR ARTICLE

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 PH[2] and World Health Organization[1] 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.

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

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

[a]PAH 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.

[b]Variants 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 European[4] and Asian[11] 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 ± SD

PAH Group (n)	Age at diagnosis (y)	Female:Male ratio	mPAP (mm Hg)	mPCWP (mm Hg)	CO fisk (L/min)	PVR (Woods units)	Common comorbidities
Child (226)	7.7 ± 5.4	1.65:1	55.1 ± 18.6	9.0 ± 3.0	3.2 ± 1.6	18.1 ± 11.7	CHD, DS, and other rare genetic syndromes
Adult (2,345)	51.6 ± 14.7	4.02:1	49.6 ± 13.9	10.2 ± 4.2	4.6 ± 1.7	10.0 ± 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.

[a]Fisher exact test.

[b]Student *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 cardiopulmonary and metabolic diseases commonly associated with adult-onset PAH. Data from the Pediatric PH Network Registry[35] 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.

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.

[a]10.7% Down, 1.4% DiGeorge, 0.6% Trisomy 18, 0.5% Noonan, 0.5% CHARGE, 0.8% others.

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 –

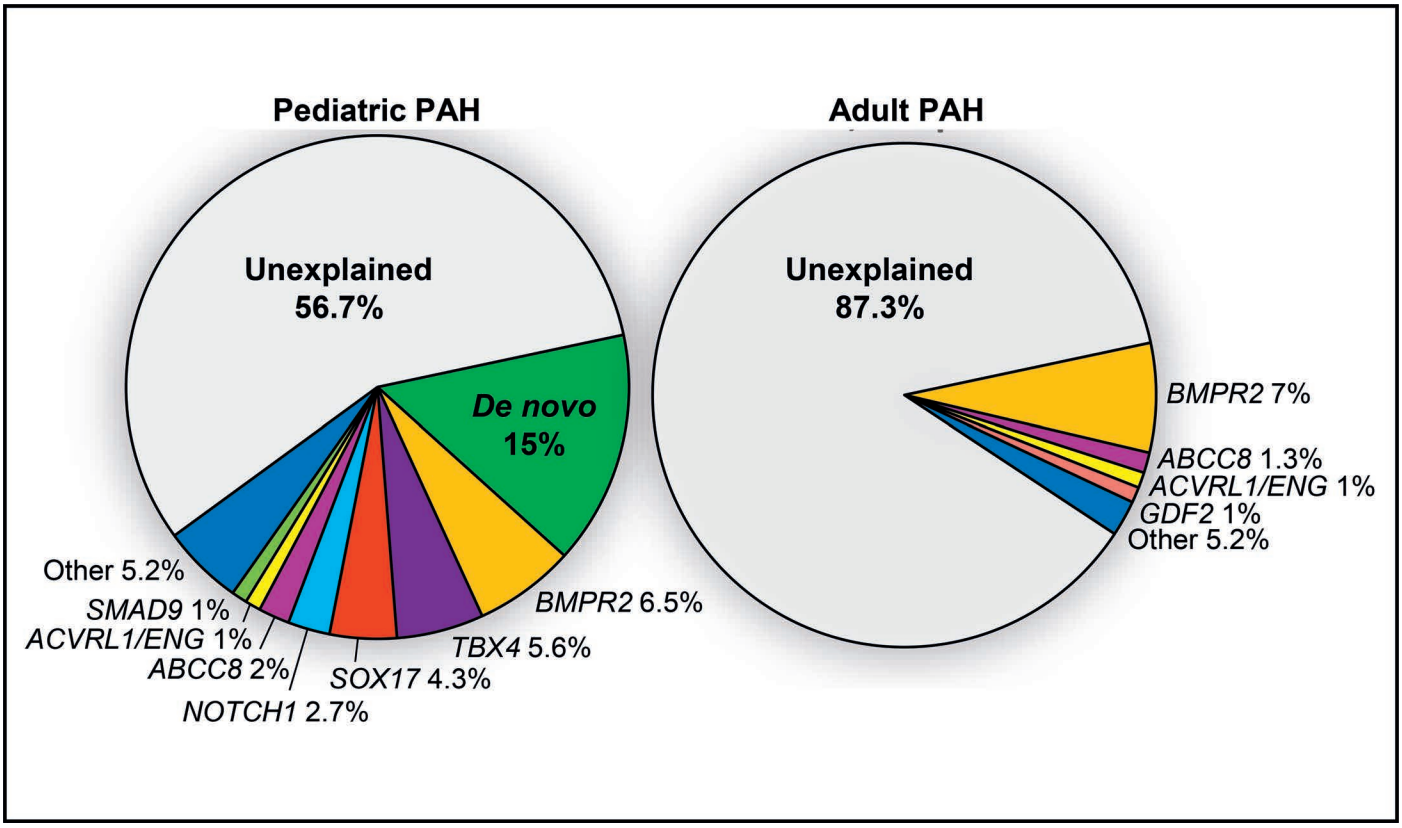

Figure 1: Relative contribution of de novo variants and 18 PAH risk genes in a cohort of 443 pediatric and 2628 adult PAH cases from Columbia University Irving Medical Center and the PAH Biobank. Risk genes included *BMPR2*, *ABCC8*, *ACVRL1*, *ATP13A3*, *BMPR1A*, *BMPR1B*, *CAV1*, *EIF2AK4*, *ENG*, *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
AMOT	LGD	p.(Leu320 Cysfs*55)	.	31	.	95	68	0.21	IPAH
CSNK2A2	D-Mis	p.(His184Leu)	0.50	25	.	77	55	1	IPAH
HNRNPF	LGD	p.(Tyr210Leufs*14)	.	29	.	98	85	0.86	NA
HSPA4	D-Mis	p.(Pro684Arg)	0.62	30	4.1e-6	96	43	0.03	APAH-CHD
KDM3B	D-Mis	p.(Pro1100Ser)	0.66	29	.	87	89	1	IPAH
KEAP1	LGD	p.(Tyr584*)	.	35	.	82	79	0.25	IPAH
MECOM	D-Mis	p.(Phe762Ser)	0.76	32	.	60	82	1	IPAH
ZMYM2	LGD	p.(Arg540*)	.	36	.	77	93	0.97	IPAH

Abbreviations; *AMOT*, angiomotin; 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.

[a]Allele frequency "." absent from gnomAD.

[b]pLI > 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 tetralogy 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 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.

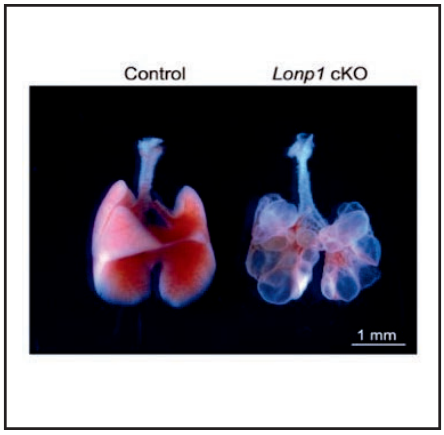

Figure 2: Embryonic epithelial-specific inactivation of *LONP1* causes immature, fluid-filled lungs in mice and neonatal lethality with intact diaphragm.

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 *BMPR2*.[53,54] However, many individuals who carry monogenic risk variants in *BMPR2* 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 defects[19] 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 services[66] 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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REGULAR ARTICLE

Genotypes and Phenotypes: A Review of Pulmonary Hypertension in Genetic Syndromes

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There has been significant advancement in the understanding of the genetics of pulmonary hypertension (PH), particularly in those with heritable or idiopathic pulmonary arterial hypertension. In addition to genetic variants with a primarily pulmonary vascular disease phenotype, the prevalence of PH in other genetic syndromes is increasingly recognized. We will review the current knowledge of PH associated with multisystem genetic syndromes. There is high prevalence of coexisting cardiac and pulmonary disease, making it challenging to discern whether PH is secondary to these processes or underlying genetic makeup. There is a paucity of data on response to PH-targeted therapy and implications on overall prognosis.

INTRODUCTION

Pulmonary hypertension (PH) is a cardiopulmonary disease with diverse etiologies that significantly impact disease progression and long-term outcomes. PH, particularly that related to left-sided heart disease, is common in adult populations.[1] Conversely, PH prevalence is lower in the pediatric population and with a different distribution of disease etiology, including a higher proportion of patients with contributory developmental factors and congenital anomalies.[2] Since the 1990s, there has been significant advancement in understanding the genetic underpinnings of PH, particularly as it pertains to those with heritable pulmonary arterial hypertension (HPAH). In fact, rare genetic variations (mutations) are now known to contribute to a considerable proportion of PH cases, including over 10% of adult idiopathic pulmonary arterial hypertension (IPAH) cases and over 35% of pediatric IPAH cases.[3] The most commonly identified pathogenic variants for IPAH and HPAH cases are within the bone morphogenetic protein receptor type II (*BMPR2*) gene, a member of the transforming growth factor-β (*TGF*-β) superfamily (recently updated by reference 2).[4] A multicenter cohort of 1550 pulmonary arterial hypertension (PAH) patients found that 29% possessed pathogenic variants in *BMPR2*. Patients with *BMPR2* mutations had a more severe phenotype with younger presentation, worse hemodynamics, lower response to acute vasodilator testing, and increased risk for death or transplant.[5] This may relate to the contribution of not only vasoconstriction, but also cell-level irregularities suggestive of alterations in cellular proliferation, migration, and apoptosis.[6] While genotype-phenotype information would be highly valuable for all relevant genes, such information is lacking due to the relative rarity of other variants.

Pediatric-onset PH has several important differences compared to adult-onset disease, which further complicates the already challenging quest to better classify disease phenotype. Pediatric-onset disease is characterized by more syncope and less right ventricular failure at time of diagnosis.[7] Important genetic differences have been identified as well. While *BMPR2* remains the most commonly identified causative gene in pediatric PH patients, variants in T-box 4–containing protein (*TBX4*) and SRY-related HMG box transcription factor (*SOX17*) are more frequently encountered compared to adult-onset disease.[3,8,9] *SOX17* notably imparts particular risk for both PAH associated with congenital heart disease (CHD) as well as HPAH.[9] And while targeted therapies have led to improved survival in pediatric patients, PH continues to impart high morbidity and mortality.[7]

In addition to the genetic variants with a primarily pulmonary vascular disease phenotype, recent pediatric registry studies have shed light on the prevalence of other genetic syndromes seen in pediatric PH cohorts. In the Spanish pediatric PH registry, 38% of patients had an underlying chromosomopathy/multiple congenital anomaly syndrome and an additional 17% of patients had trisomy 21.[10] A study from the Dutch national registry identified that outside of PH-specific genetic variants, 17% had a genetic disorder with established association with PH and an additional 23% had genetic disorders or copy number variants without established PH association.[11] The largest pediatric registry study to date is from the North American Pediatric PH Network (PPHNet) Registry, which found that 17% of the 1475 patient cohort had

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a genetic syndrome.[12] These patients' pulmonary vascular disease was classified as World Symposium on Pulmonary Hypertension (WSPH) group 1 (PAH), group 3 (associated with lung disease), or group 5 (multifactorial PH), which likely reflects the high prevalence of both CHD and/or lung disease within these diverse genetic syndromes.[12]

While the field is rapidly gaining more understanding of the clinical features of PH-associated genetic variants, there is a paucity of data about the impact of the other genetic syndromes on pulmonary vascular disease phenotype and response to therapies. This review aims to present the available data about PH associated with multisystem genetic syndromes, with focus on chromosomal abnormalities, single-gene–related syndromes, and heterogeneous disease. Because genetic syndromes with multi-organ system involvement are more commonly encountered both clinically and in the medical literature in pediatric patients, the scope of this review is inherently pediatric focused. Phenotypic descriptions for adult populations with the subsequently described genetic syndromes are described as able, based on literature review. For the sake of brevity and due to the paucity of information in many circumstances, unless treatment efficacy or PH-related prognosis is specifically mentioned, the reader can assume that there are no available data on these outcomes.

CHROMOSOMAL ABNORMALITIES

Trisomy 21

Trisomy 21 (TS21) is the most common genetic syndrome associated with PH, with 4% to 17% carrying this diagnosis in the Dutch, Spanish, and North American pediatric cohorts. Several risk factors for PH have been identified in patients with TS21. The presence of TS21 affects both alveolar and pulmonary vascular development. Since first described by Cooney and Thurlbeck in 1982,[13] histologic examination of lung tissue from TS21 patients has shown decreased alveoli, decreased airway development, thickened alveolar septa with a double capillary layer, and decreased vessel density.[13-16]

The high prevalence of cardiac and pulmonary disease in the TS21 population further enhances the risk for PH. CHD is common, occurring in 38% to 58% of patients with TS21.[17] The most commonly encountered CHDs are atrioventricular septal defect, ventricular septal defect (VSD), atrial septal defect (ASD), patent ductus arteriosus, and tetralogy of Fallot.[17-20] Type of CHD and timing of surgical intervention significantly impact the degree to which CHD may impact pulmonary vascular tone and the development of PH. Regardless, there is a higher risk of baseline PH and higher risk of postsurgical PH after cardiac repair (2.2% versus 0.7%) in TS21 patients compared to non-TS21 patients.[17,19,20] There are multiple pulmonary comorbidities that may contribute to pulmonary vascular disease in TS21, including obstructive sleep apnea, recurrent pneumonia, infection, asthma, and upper and lower airway obstruction.[21] Obstructive sleep apnea is particularly prevalent in roughly 60% of patients, including those without clinical suspicion.[22,23] Patients with TS21 and PH may be classified as multiple WSPH groups depending on the presence and severity of these underlying cardiopulmonary processes, which may include WSPH group 1, 2 (secondary to left heart disease), and/or 3.

There are limited data on the response to PH-targeted therapy in TS21. A few small studies assessing the efficacy of bosentan in adults with CHD, PH (including a high number with Eisenmenger syndrome), and TS21 demonstrated that it has both short- and long-term benefits on hemodynamics and 6-minute walk distance.[24-26] Pediatric data are lacking and it is likely that patients are treated according to existing pediatric PH treatment algorithms, given experience at the authors' PH centers and anecdotally. More granular data from large pediatric cohorts treated at experienced PH centers would be of great benefit.

TS21 health supervision guidelines currently recommend screening echocardiograms after birth and later in childhood if clinical concerns exist as well as a polysomnogram before age 4.[27] Housten and colleagues[28] more recently published additional screening recommendations to address the cardiopulmonary comorbidities that influence PH development and advocate for more stringent screening with yearly echocardiograms in patients with underlying respiratory conditions. It is clear that even as the most studied genetic syndrome associated with PH, additional work is needed to better understand disease phenotype in TS21.

Trisomy 13 and Trisomy 18

Trisomy 13 (TS13) and trisomy 18 (TS18) are the next most commonly encountered chromosomal trisomies after TS21. These disorders have high early mortality and prevalence of PH. While a majority of infants with both TS13 and TS18 die within the first month of life, there is a subset that exhibits longer-term survival. In a multistate population study, 5-year survival was 9.7% in TS13 and 12.3% in TS18.[29] Multiple congenital anomalies are common in both TS13 and TS18. CHD is present in 60% to 80% of patients, with VSD, ASD, and tetralogy of Fallot being most common.[30,31] Extracardiac anomalies commonly encountered in TS13 include orofacial anomalies, abdominal wall defects, limb defects, and central nervous system abnormalities.[31] Extracardiac anomalies commonly encountered in TS18 include tracheoesophageal fistula, cleft lip, diaphragmatic hernia, and spinal dysraphism.[31]

PH is frequently cited as a potential complication of both TS13 and TS18, though data on the prevalence and hemodynamic severity are limited. From clinical experience, we can state the among those with these syndromes, the burden of PH, and its severity, is high and seemingly on the rise. This may be because intensive treatment has improved survival beyond early infancy for more patients; nonetheless, CHD and PH are common contributors to a high burden of morbidity and mortality.[32]

A particular challenge with evaluating PH in these disorders is the high prevalence of CHD, which likely plays some role in the development of pulmonary vascular disease. The role of lung parenchymal and vascular development has not been rigorously evaluated. Tahara and colleagues[33,34] analyzed lung tissue of a small set of patients with TS18 with CHD and found hypoplasia of the small pulmonary arteries, alveolar wall thickening, alveolar hypoplasia, and histologic

findings of mild pulmonary vascular disease. Notably, the role of unrepaired CHD is impossible to ignore when considering these findings. Cardiac surgical intervention in patients with TS13 and TS18 remains controversial due to the short average lifespan of these patients, though care paradigms may be shifting toward more intervention as a subset of patients has longer-term survival. Patients are often considered for surgery on a center- and patient-specific basis for palliative or comprehensive cardiac surgeries. The Pediatric Cardiac Care Consortium demonstrated that in-hospital mortality after cardiac surgery is high in TS13 (28%) and TS18 (13%). However, those who survive to discharge had a median survival of 15 to 16 years.[35] Over half of the patients in this cohort with a preoperative cardiac catheterization had PH, though mean pulmonary vascular resistance was higher (5.1 versus 3.5 WU) in those who died during hospitalization.[35] Similarly, Kaneko et al[36] reported that in a Japanese cohort, PH was present in 78% of hospitalized TS13/TS18 patients.

22q11 Deletion Syndrome

22q11 deletion syndrome (often referred to as DiGeorge syndrome) was reported in 21 (1.4%) of 1475 reported cases in the PPHNet Registry.[12] *22q11* deletion syndrome has a wide spectrum of clinical phenotypes. The most common associated features are facial dysmorphisms, congenital heart disease, thymic hypoplasia, parathyroid hypoplasia, developmental delay, laryngeal abnormalities, renal/urinary tract anomalies, and ophthalmologic abnormalities.[37] CHD is present in approximately 80% of patients, most commonly with conotruncal defects, including tetralogy of Fallot, pulmonary atresia with VSD, truncus arteriosus, interrupted aortic arch, and VSD.[38] Pulmonary blood flow can range from critically limited to excessive depending on the underlying CHD, which may impact the development of PH. Data on the prevalence of PH in 22q11 deletion syndrome are limited, with 1 case report describing suprasystemic perioperative PH in an infant with truncus arteriosus undergoing repair at 34 days of life.[39] There is also possible increased risk for postoperative PH after CHD repair, with a single-center retrospective cohort study identifying postoperative PH in 13% (8/62) of patients with 22q11 deletion syndrome who underwent cardiac surgery.[40]

SINGLE-GENE–RELATED SYNDROMES

There are several genetic syndromes characterized by pathologic variants in single genes which have associations with PH. We selected those with a more robust literature base to highlight, with less well-described syndromes compiled in Table 1.

Table 1. Additional Genetic Syndromes Associated With Pulmonary Hypertension

Syndrome	Gene	Syndrome features	Evidence for PH association
Alveolar capillary dysplasia with misalignment of the pulmonary veins	*FOXF1*	Diffuse disorder of the neonatal lung with respiratory distress and pulmonary hypertension; CHD (especially misalignment of the pulmonary veins but also may see left ventricular hypoplasia and/or other defects) with varied possible additional anomalies of cardiac, gastrointestinal, and genitourinary systems	41, 42
Adams-Oliver	*ARHGAP31*, *DOCK6*, *EOGT*, *RBPJ*, *NOTCH1*, *DLL4*	Aplasia cutis congenita; limb abnormalities; CHD; vascular anomalies; central nervous system anomalies	43, 44
Alagille	*JAG1*, *NOTCH2*	CHD (especially pulmonary arterial anomalies); cholestasis; butterfly vertebrae; ophthalmologic abnormalities[45]	46, 47
Cantú	*ABCC9*, *KCNJ8*	Facial dysmorphism; macrocephaly; CHD; skeletal abnormalities	48
Dursun	*G6PC3*	Triad of HPAH, leucopenia, and atrial septal defect; also, congenital neutropenia	49, 50
FLNA syndrome	*FLNA*	CHD; PAH; developmental impairments	51
Gaucher disease	*GBA1*	Hepatomegaly; splenomegaly; bone abnormalities; cytopenia; potential for neurologic impairment	52
Holt Oram	*TBX5*	CHD (especially ASD); cardiac conduction abnormalities; hand malformations	53-55
Kleefstra	*9q34.3* microdeletion	CHD; developmental delay; seizures; sleep abnormalities	56-58
Klippel-Trenaunay		Capillary malformations; varicose veins; disturbed growth of bone and/or soft tissue	59
POEMS	None	Plasma cell dyscrasia with polyneuropathy (P); organomegaly (O); endocrinopathy (E); M protein (P); skin changes (S)	60-62
Pierre Robin sequence	None	Retrognathia; glossoptosis; airway obstruction; may be associated with other syndromes	63-66
Sickle cell disease	*HbS*	Red blood cell disorder with ramifications including primary pulmonary vascular disease and diastolic heart failure	67, 68

Abbreviations: PH indicates pulmonary hypertension; CHD, congenital heart disease; HPAH, heritable pulmonary arterial hypertension; PAH, pulmonary arterial hypertension; ASD, atrial septal defect.

TBX4 Syndrome

While *BMPR2* remains the most commonly identified mutated gene in PAH cases (typically but not exclusively in familial PAH or IPAH cases), there is a growing recognition of other pathologic genes, including *TBX4*. *TBX4* is the second most commonly mutated gene in PAH populations.[3,4] In pediatric patients, up to 8% of cases are associated with *TBX4* mutations, while the proportion is slightly less among adult PAH patients. As with *BMPR2* gene mutations, patients with *TBX4* may present at any age; however, there is an almost bimodal distribution, with a large proportion presenting as young children and another proportion presenting in adulthood after approximately age 40 years.[3] Intriguingly, the phenotypic presentations are quite varied, with many young children presenting with some combination of persistent PH of the newborn which may or may not resolve, developmental lung disease, congenital heart defects, neurodevelopmental variation, and skeletal abnormalities.[69-71] In contrast, while some older children may present with more of a primary pulmonary vascular disease phenotype, this is the more common presentation among adults, who have a much more "pure" PAH phenotype at first evaluation. However, the adults also have syndromic spectrum disease, with skeletal abnormalities (eg, small patella syndrome), parenchymal lung defects, and airway anomalies.[72,73] As with *BMPR2* and other single-gene mutations associated with PAH, *TBX4* spectrum disease has reduced penetrance and phenotypic variability, although further work is necessary to explore the impact and pathogenesis of mutations in this gene upon the human condition.

Hereditary Hemorrhagic Telangiectasia

Hereditary hemorrhagic telangiectasia (HHT) is characterized by mucocutaneous telangiectasias, recurrent epistaxis, arteriovenous malformations (including pulmonary, gastrointestinal, hepatic, and cerebral locations), and PH. It is inherited in an autosomal-dominant manner due to pathogenic variants in *ENG*, *ACVRL1*, or *SMAD4*.[74-76] As with *BMPR2*, these genes are involved in the TGF-β signaling pathway. There are 2 primary mechanisms for the development of PH in HHT: secondary to the high output state associated with hemodynamically significant arteriovenous malformations or due to the development of intrinsic pulmonary vascular disease as a form of HPAH. HHT-associated genes account for approximately 1% of identified genetic causes of IPAH in both pediatric and adult cohorts.[3,8,77] While the sequelae of telangiectasias and arteriovenous malformations have a variable time course, PH most commonly develops in adulthood in HHT patients with median age of onset in the fifth to seventh decade of life.[77] PH is present in approximately 10% to 20% of patients with HHT and imparts significant increase in mortality.[78,79] As recently reviewed, data on response to therapy and survival are somewhat mixed in the literature to date, with opportunity for enhanced understanding of genotype-phenotype associations.[80]

Pulmonary Veno-Occlusive Disease and Pulmonary Capillary Hemangiomatosis

Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are rare pulmonary and pulmonary vascular disorders characterized clinically by hypoxemia, PH, reduced diffusing capacity as measured by pulmonary function testing, and abnormal chest imaging by computed tomography. While classically considered distinct entities, PVOD and PCH have long been suspected to exist as 2 ends of the same spectrum due to their shared and distinct features.[81] Consistent with this, in 2014, investigators separately demonstrated pathogenic biallelic mutations (both copies of the gene have mutations) in the gene *EIF2AK4* among familial cases of PVOD and PCH, helping to cement the pathophysiologic connection between PVOD and PCH.[82,83] The discovery of *EIF2AK4* was significant not only due to the connection between PVOD and PCH; because of the challenges with diagnosis with both entities, lung biopsy in the setting of relevant clinical findings is often of serious consideration prior to formal diagnosis of PVOD or PCH. However, with the discovery of *EIF2AK4*, in some circumstances biopsy may be avoided if biallelic pathogenic *EIF2AK4* mutations are detected.

But it is important to recognize that not all cases of PVOD or PCH associate with *EIF2AK4* mutations, and much room for enhanced understanding remains. In addition, the precise mechanisms of disease onset and progression remain an area of opportunity for discovery, with increasing evidence that both PVOD and PCH may develop in response to some sort of insult, such as enhanced pulmonary venous and capillary pressure, toxic insult, or a combination of factors (recently reviewed by Weatherald et al[84]). While complete phenotype-genotype information is lacking, unfortunately, survival among *EIF2AK4* mutation carriers is poor, often lower than among individuals with *BMPR2* mutation-associated PAH.[85]

Noonan Syndrome

Noonan syndrome is a multisystem disorder with variable phenotype, which includes characteristic facial features, CHD, short stature, hearing loss, and skeletal malformations. This syndrome is caused by mutations in genes within the RAS/mitogen-activated kinase signaling pathway, most commonly *PTPN11*, *SOS1*, and *RAF1*. Between 50% and 80% of patients with Noonan syndrome have cardiac involvement, most commonly with pulmonary stenosis, hypertrophic cardiomyopathy, or ASD.[86,87] PAH is not a typical feature of this syndrome, though was first described in a 1982 case report of a 2-month-old with Noonan syndrome and PAH without concomitant CHD.[88] A few other case reports and small case series have since been published describing severe PAH in patients with Noonan syndrome, ranging in age from the neonatal period to early adulthood.[86,89,90] While specific gene mutations are not described in all reports, it is notable that PH in the setting of *RAF1* mutations were found in the 2 patients described by Hopper and colleagues[89] and an additional patient in the description of a cohort of *RAF1*-positive patients.[89,91] This suggests that further investigation in the specific genetic alterations in those with Noonan syndrome and PAH will be

important to best identify patients most at risk.

HETEROGENEOUS DISEASES

Mitochondrial Disease

Mitochondrial disease is a diverse group of diseases caused by dysfunction within the mitochondrial respiratory chain. These rare disorders can affect virtually all organ systems, with variable phenotypes, including liver disease, neurologic impairment, skeletal muscle weakness, acid-base disturbance, and cardiac involvement. The most common cardiac phenotype in mitochondrial disease is hypertrophic cardiomyopathy, though PH was first described in 2005.[92] PH has been reported in several case reports and small series with a variety of specific mitochondrial disorders.[93-99] The majority of described patients seemingly had PAH, though 1 study describes a patient with an underlying cardiomyopathy with significant biventricular dysfunction and a second describes a patient with severe restrictive lung disease, which suggests the potential for group 2 and group 3 PH in this population.[96] Few of these studies describe PH-specific therapy, though response to therapy is variable in the few who do describe treatment. Hung and colleagues[98] describe echocardiographic improvement with treatment with furosemide and mitochondrial supplements, whereas Sproule and colleagues[95] describe no improvement in PAH despite therapy with inhaled nitric oxide, epoprostenol, and mitochondrial supplements. The potential for improvement with mitochondrial supplements is particularly interesting and warrants further investigation as a potential means to reverse PH in this population.

CONCLUSION

It is evident that PH is encountered in multiple pediatric- and adult-onset genetic syndromes. There is a high incidence of associated lung disease and CHD, making it challenging to discern whether the primary risk for PH is secondary to the underlying genetic syndrome, cardiac and/or pulmonary disease, or a combination of both. Comprehensive human cohort studies are helpful to identify potential genetic associations with PH and relative frequency of these coinciding relationships. With the exception of the chromosomal trisomies, much of the literature describing these associations is comprised of a limited number of case reports. Additional research is needed to better delineate the incidence of PH and its impact on morbidity and mortality in these genetic syndromes.

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REGULAR ARTICLE

Genetic Counseling and Testing for Pulmonary Arterial Hypertension in the United States

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INTRODUCTION

Group 1 pulmonary arterial hypertension (PAH) refers to a set of rare proliferative vascular diseases that result in a marked increase in pulmonary arterial pressure and resistance due to narrowing of the pulmonary arterioles.[1] Vasoconstriction, fibrosis, cell proliferation, and thrombosis contribute to these pathological processes.[2] In some cases, PAH may be associated with risk factors such as drug or toxin exposure (eg anorexigens) or other diseases (such as connective tissue disease, HIV infection, portal hypertension, congenital heart disease, or schistosomiasis). When no underlying cause is found, PAH is labeled "idiopathic PAH" (IPAH). There has been increasing recognition that genetic factors play a role in the development of familial and sporadic IPAH.

Heritable PAH (HPAH) is a subgroup of PAH that includes cases with more than one affected family member (familial PAH [FPAH]) and/or cases with an established causative genetic variant, regardless of family history (ie, apparently sporadic IPAH found to have predisposing germline variant on genetic testing). The definition of HPAH also applies to genetically mediated pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis (PVOD-PCH), which are pathologically distinct forms of PAH with hemodynamic similarities. In addition, some individuals with PAH presumed associated with anorexigen use[3] or congenital heart disease[4] have also been shown to have an underlying genetic cause.

While multiple single-gene causes and candidate genes for HPAH have been discovered (Tables 1 and 2), the *BMPR2* gene remains the most common and well-studied cause of HPAH. At least 10% to 40% of IPAH and 70% to 80% of FPAH have been shown to have an underlying pathogenic variant[10] and a proportion of PVOD-PCH has been shown to be caused by biallelic pathogenic variants in the *EIF2AK4* gene.[11,12] Inclusion of newly discovered PAH genes increases genetic test yield in FPAH to 92% to 95% (B.G., unpublished data, October 2021).

HPAH is predominately inherited in an autosomal-dominant pattern conferring a 50% risk for first-degree relatives to inherit the familial susceptibility variant. Most HPAH genes exhibit incomplete penetrance and variable expressivity, with the exception of *EIF2AK4*-mediated PVOD-PCH, which is completely penetrant and inherited in an autosomal-recessive pattern.

The purpose of this article is to offer a perspective on the current utility of genetic counseling and testing for PAH; to provide a framework for appropriate anticipatory guidance, supportive counseling, and incorporation of genetic test results into patient care; and to identify areas for future genetics research.

GENETIC COUNSELING

Genetic counseling refers to professional guidance and support provided

Key Words—pulmonary arterial hypertension, pulmonary hypertension, genetic counseling, genetic testing, PAH susceptibility variant
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Table 1. Genes With Well-Established Links to PAH

Gene	% of PAH cases[5]	Features of associated syndromic disease	Mode of inheritance	Penetrance of PAH
ACVRL1[a]	< 1%	HHT2 (OMIM: 600376): telangiectasias, recurrent epistaxis, arteriovenous malformations, gastrointestinal bleeding and anemia	AD	Incomplete
BMPR2[a]	75%[b]	N/A	AD	Incomplete. 14% in males; 42% in females
CAV1[a]	< 1%	N/A	AD	Incomplete
EIF2AK4[a]	10%-25% of sporadic PVOD-PCH[8]	N/A	AR	Complete
ENG[a]	< 1%	HHT1 (OMIM: 187300) telangiectasias, recurrent epistaxis, arteriovenous malformations, gastrointestinal bleeding and anemia	AD	Incomplete
GDF2[a]	< 1%	HHT5 (OMIM: 615506): telangiectasias, recurrent epistaxis, arteriovenous malformations, gastrointestinal bleeding and anemia	AD	Incomplete
KCNK3[a]	1%-3%	N/A	AD	Incomplete
SMAD9[a]	< 1%	N/A	AD	Incomplete
TBX4[a]	< 1%	Ischiocoxopodopatellar syndrome with or without PAH (OMIM: 147891): skeletal abnormalities, short stature, airway diverticulosis, congenital heart defects[9]	AD	Incomplete

Abbreviations: AD, autosomal-dominant mode of inheritance; AR, autosomal-recessive mode of inheritance; HHT, hereditary hemorrhagic telangiectasia; OMIM, online Mendelian inheritance in man; PAH, pulmonary arterial hypertension; PCH, pulmonary capillary hemangiomatosis; PVOD, pulmonary veno-occlusive disease.

[a]Childhood and adult onset of PAH reported.[4,6,7]

[b]Heritable PAH: accounts for ~25% of idiopathic cases; rare cause of pulmonary veno-occlusive disease (OMIM: 265450).

Table 2. Genes With a Potential Link to PAH

ATP13A3	*SMAD1*
BMPR1B	*SMAD4*
KCNA5	*SOX17*
KDR	*TET2*

to patients and their relatives who seek to learn about the inherited nature of a disease, the recurrence risk in family members, and the availability of proactive measures such as clinical surveillance and/or genetic testing for an identified familial variant, when applicable. A key aspect of genetic counseling is also the interpretation and incorporation of genetic test results into clinical care. Genetic counseling is provided by trained specialists such as a geneticist or a genetic counselor and is regarded as a valuable and essential medical service by clients and providers.[13-15]

Several professional societies recommend genetic counseling with genetic testing (Table 3) for individuals with HPAH or IPAH, familial or sporadic PVOD-PCH, and for first-degree relatives once a familial susceptibility variant has been identified,[16-21] as well as for individuals with congenital heart disease–associated PAH,[20] anorexiant PAH,[18,21] and hereditary hemorrhagic telangiectasia–related PAH.[21]

Genetic counseling occurs in two steps, pretest and posttest (Table 4; Figure 1). Genetic counseling prior to undergoing genetic testing is essential to guide the patient or at-risk relative in making an informed decision about testing, and provides valuable disease-specific education and resources that are beneficial to the patient and their family members, even if genetic testing is not pursued.

Pretest genetic counseling begins with collection and assessment of the affected individual's medical and family history which can allow for the diagnosis of unrecognized familial disease, assessment of inheritance pattern, and identification of at-risk relatives in the family, as well as influence the choice of tests if a particular gene is suspected to be involved. Education surrounding PAH symptoms, inheritance, and the genetic testing process, including cost and insurance coverage, as well as benefits, limitations, and potential psychosocial risks of genetic testing, are addressed to allow thoughtful decision-making around testing. Ideally, genetic testing starts with an affected individual as explained in Table 4.

Posttest genetic counseling involves communication with the patient regarding the test result, its interpretation, recommendations for the patient if applicable, and recommendations for relatives based on the result and family history. Posttest genetic counseling also involves provision of resources including a family letter to assist in informing at-risk relatives regarding their potential risk for PAH and recommendations for additional evaluation.

Table 3. Professional Society Recommendations for Genetic Counseling, Genetic Testing and Clinical Surveillance

Professional society	Genetic counseling[a]	Genetic testing[a]	Cascade clinical screening in at-risk individuals with susceptibility variant	Cascade clinical screening in FDRs when HPAH family is genotype negative	Cascade clinical screening in FDRs of individuals with IPAH
American College of Chest Physicians, 2004[16]	Recommended for at-risk relatives in case of FPAH; IPAH should be advised of the option	Recommended for at-risk relatives in case of FPAH; IPAH should be advised of the option	Doppler echocardiogram	Doppler echocardiogram	Not addressed
American College of Cardiology Foundation/ American Heart Association, 2009[17]	Recommended for HPAH and FDRs at-risk for *BMPR2* mutation	Recommended for HPAH and cascade testing for BMPR2 mutation	Annual echocardiogram[b]	Not addressed	Not addressed
European Society of Cardiology/ European Respiratory Society, 2015[18]	Recommended for HPAH, IPAH, anorexiant PAH, and PVOD-PCH	Recommended for HPAH, IPAH, anorexiant PAH, HHT-related PAH, and familial or sporadic PVOD-PCH	Resting echocardiogram recommended. Consider repeating annually	Resting echocardiogram recommended. Consider repeating annually	Not addressed
American Heart Association–American Thoracic Society, 2015[19]	Recommended for IPAH and HPAH	Recommended for IPAH, HPAH, and FDR of genotype positive patients	Serial echocardiograms or other non-invasive studies	Screening with symptoms	Not addressed
European Pediatric Pulmonary Vascular Disease Network, 2019[20]	Recommended for HPAH and IPAH	Recommended for IPAH, HPAH, and FDR of genotype positive patients; consider for drug-induced PAH and CHD-related PAH	Echocardiogram every 1-3 years, initiate in childhood	Serial echocardiograms	Evaluation with symptoms
6th World Symposium on Pulmonary Hypertension, 2019[21]	Recommended for HPAH, IPAH, anorexiant PAH and FDR of genotype positive patients	Recommended for HPAH, IPAH, anorexiant PAH, HHT-related PAH and FDR of genotype positive patients	CPET and annual echocardiogram	Not addressed	Not addressed

Abbreviations: CHD, congenital heart disease; CPET, cardiopulmonary exercise test; FDR, first-degree relative; FPAH, familial pulmonary arterial hypertension; HHT, hereditary hemorrhagic telangiectasia; HPAH, hereditary pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension; PCH, pulmonary capillary hemangiomatosis; PVOD, pulmonary veno-occlusive disease.

[a]FPAH, HPAH, IPAH, PAH, PVOD-PCH: refers to the affected individual.

[b]Refers to *BMPR2* variant carriers only.

GENETIC TESTING FOR PAH AND INTERPRETATION OF GENETIC TEST RESULTS

Due to genetic heterogeneity and an inability to distinguish the underlying genetic cause based on personal and family history in most instances, a comprehensive PAH gene panel including all genes with clear association to PAH is generally recommended and is similar in cost to performing single-gene analysis. If involvement of one gene is strongly suspected, single-gene testing with reflex to the panel in case of a negative result may be considered. Whole exome or whole genome sequencing are generally not needed for sporadic adult-onset IPAH as they have similar yield as a panel test but are more expensive. Whole exome or whole genome sequencing may be considered in large HPAH families with a negative result on panel testing, or in pediatric PAH due to the higher likelihood of novel gene discovery in these populations, and the involvement of genes that are not typically associated with PAH.[4]

Unlike many medical tests, the results of genetic testing for most disorders, including PAH, provide a probabilistic result rather than a binary genetic/not genetic outcome. Panel-based testing identifies multiple genetic variants, and these must be carefully interpreted by the testing laboratory to categorize each variant as pathogenic, likely pathogenic, variant of uncertain significance, likely benign, or benign based on the strength of evidence for or against pathogenicity.[22] While there may be ample evidence available to categorize some variants, other variants may lack evidence to classify as disease-causing or benign,

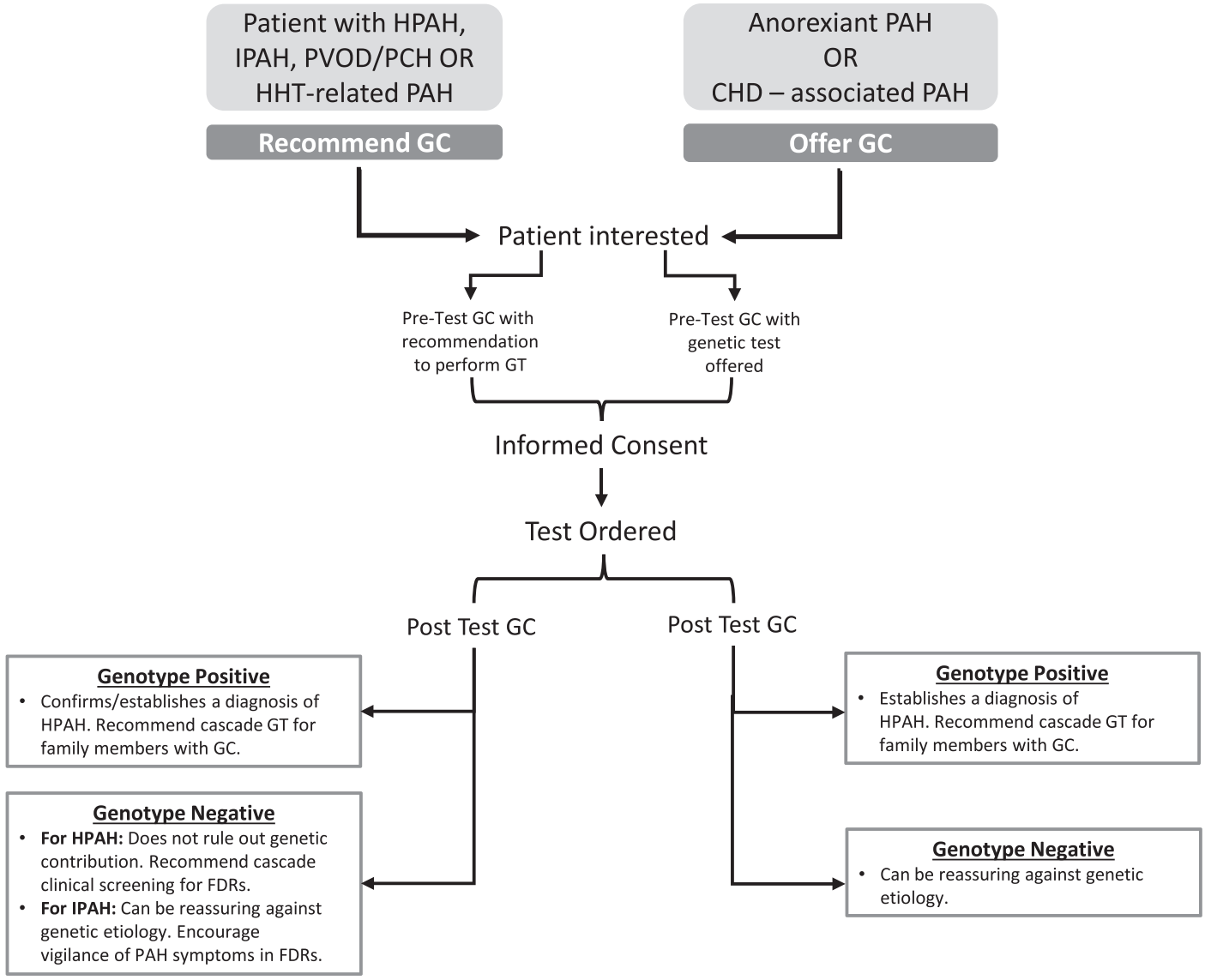

Figure 1: Framework for genetic counseling and testing in pulmonary arterial hypertension. CHD indicates congenital heart disease; GC, genetic counseling; GT, genetic testing; FDR, first-degree relative.

and are placed in the uncertain category. As genetic testing of PAH patients becomes more routine, new evidence is likely to result in reclassification of variants. The results are therefore complex, and best handled by professionals with in-depth genetics knowledge.

Genotype-Positive Result and its Integration Into Medical Care

Identification of a pathogenic or likely pathogenic variant in a PAH-associated gene is considered a positive result. Pinpointing a genetic variant causative of PAH establishes the diagnosis of HPAH in individuals previously diagnosed with IPAH or associated PAH, and confirms genetic etiology in those with FPAH. On occasion, the involvement of a specific gene could have medical management implications for the affected individual. For example, identification of biallelic *EIF2AK4* mutations establishes a diagnosis of PVOD-PCH without a lung biopsy, as well as guides treatment, as vasodilators are contraindicated[8] while early referral for lung transplantation is indicated.[8,19] Identification of *TBX4*-mediated disease in a case of resolved persistent pulmonary hypertension of the newborn (PPHN) would indicate a need for annual surveillance with echocardiogram due to the risk of recurrence.[6] Importantly, knowing the familial susceptibility variant allows for cascade genetic testing (ie, targeted testing for the familial variant in at-risk relatives). Cascade genetic testing even in young children is considered necessary due to the availability of effective medical interventions.

Family members harboring an FPAH-susceptibility variant have an increased risk of developing PAH and other features associated with the identified gene. They can also pass the variant to their offspring. Genetically mediated PAH and PVOD-PCH can manifest as early as infancy,[8,23] and therefore, clinical screening with echocardiograms is initiated in childhood,[20] and repeated every 1 to 3 years or sooner if symptoms develop to facilitate early detection and treatment of PAH.[17,20] In the recently published outcomes from the

Table 4. Framework for Genetic Counseling and Testing in PAH

Pretest Genetic Counseling
Review patient's medical history and collect standard 3-generation pedigree • Attention to disease characteristics within the family such as age at symptom development, age at diagnosis, age at death, consanguinity, and the presence of syndromic features such as those seen in hereditary hemorrhagic telangiectasia and small patella syndrome. • Family history should be updated over time due to its changing nature.
Provide education about disease symptoms, prognosis and genetics
Discuss mode of inheritance • Identification of at-risk relatives.
Recommend genetic testing based on guidelines
Review utility and limitations of testing • Test utility: Identification of known or likely pathogenic variant explains disease and establishes a diagnosis of HPAH in individuals with IPAH, PVOD-PCH, or associated PAH. Additionally, it allows for cascade testing to accurately identify at-risk relatives and for using genetic information in reproductive decision-making. • Limitations: Incomplete detection rate (i.e. not all genes for PAH have been discovered); reduced and sex-influenced penetrance; and variability in clinical presentation, severity, and age at diagnosis (known as variable expressivity).
Explore psychosocial considerations • Impact and feelings surrounding the possibility of a positive or negative result • While cascade testing can assist in identification of at-risk relatives, a cure for PAH does not currently exist. However, clinical screening may lead to identification of disease at an early stage when treatment may slow progression of PAH. • Discuss protection afforded by the Genetic Information Non-discrimination Act for asymptomatic at-risk relatives against discrimination by health insurance and employers, with some caveats. Current lack of protections against discrimination by life insurance, long-term care and disability insurance.
Explore readiness for testing, and support patient's decision
Order appropriate genetic test in the ideal test candidate (ie affected individual) if patient consents: • Testing the affected individual first provides information whether an identifiable susceptibility variant is present or not. In contrast, a negative result in an unaffected relative without knowing the specific familial PAH susceptibility variant would not clarify whether the unaffected relative has not inherited the familial variant, or still harbors a familial variant that is not identifiable due to limitations in current knowledge around PAH genetics.
Provide resources • Include information regarding useful websites (eg Pulmonary Hypertension Association), and relevant clinical trials. • If genetic testing is not pursued, provide a family letter to coordinate cascade clinical screening in HPAH families.
Posttest Genetic Counseling
Review of results and discussion of cascade genetic testing and/or cascade clinical surveillance recommendations based on genetic test results and family history a. Genotype-positive result: • If a PAH susceptibility variant is identified in the affected individual, provide family letter to coordinate cascade genetic testing and/or cascade clinical surveillance. ○ Autosomal-dominant HPAH: cascade testing starts with first-degree relatives and expands to other relatives where applicable. ○ Autosomal-recessive PVOD-PCH: cascade genetic testing is recommended for the affected individual's siblings. • For individuals seeking to learn about prenatal diagnosis or preimplantation genetic testing when the familial susceptibility variant is known, refer to a prenatal genetic counselor. ○ Decisions surrounding prenatal diagnosis or preimplantation genetic testing need consideration of several factors including comfort level with chance (incomplete and gender-influenced penetrance), personal and religious preferences, and financial and emotional burden surrounding the process and procedure.
b. Familial PAH with genotype negative result: • Provide family letter to coordinate cascade clinical surveillance if a PAH susceptibility variant *is not* identified in the affected individual.
c. If a VUS is identified, coordinate segregation studies when indicated. Recontact the patient when a VUS is reclassified. d. Consider DNA banking in case of uninformative genetic test result. DNA banking may be done pretest in some circumstances.

Abbreviations: HPAH, hereditary pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension; PCH, pulmonary capillary hemangiomatosis; PVOD, pulmonary veno-occlusive disease; VUS, variant of uncertain significance.

DELPHI-2 study,[24] in which 55 adult asymptomatic *BMPR2* mutation carriers received annual multimodal screening for a minimum of 2 years, the overall incidence of PAH was 2.3% per year. Individuals were diagnosed with PAH at an earlier stage than is typical in the absence of proactive clinical screening. Additionally, the 5 patients who were diagnosed with PAH via screening were started on oral combination therapy and at the end of 6 years, they remained in a low mortality-risk category.[24]

If the FPAH-susceptibility variant is known, prenatal diagnosis and preimplan-

tation genetic testing become available family planning options for affected and at-risk asymptomatic males who wish to ensure biological offspring have not inherited predisposition to PAH.[25,26] Pregnancy in affected or unaffected at-risk females is discouraged as it can be life-threatening. It is also unclear if preimplantation genetic testing with gestational surrogacy is safe, as the effect of ovarian stimulation in females with a PAH-susceptibility variant remains unknown.

Guideline authors recommend that adult at-risk individuals in a family receive genetic counseling so they can make an informed decision about undergoing genetic testing and/or serial clinical screening for PAH.[17,19,21] It is also common practice for at-risk relatives choosing not to undergo family-variant testing to have serial clinical screening.

Family members who test negative for the FPAH-susceptibility variant have the general population risk of about 1 in a million to develop PAH, and their children are not at risk for inheriting the familial variant.

Genotype-Negative Result and its Integration Into Medical Care

The absence of a PAH susceptibility variant on a comprehensive PAH panel test in the affected individual is considered a genotype-negative result. In the case of FPAH, a negative genetic test result in the proband is clearly uninformative, does not rule out monogenic etiology, and indicates there are a proportion of genes or genetic mechanisms responsible for FPAH that are as-yet undiscovered. Professional societies recommend clinical surveillance for asymptomatic first-degree relatives with serial echocardiograms even when the affected relative is genotype-negative,[16,17,20] and screening is ideally repeated annually.[18] In the case of sporadic IPAH, a negative genetic test is reassuring, but does not rule out a genetic etiology. Clinical screening for first-degree relatives in this context is not indicated,[25] but the proband is encouraged to inform close relatives regarding the diagnosis and symptoms so they can seek an evaluation if cardiorespiratory symptoms arise.[20]

In most cases, identification of one or more variants of uncertain significance is treated like an uninformative negative result with respect to cascade clinical surveillance, that is, clinical surveillance is recommended for at-risk relatives in FPAH families while surveillance is not recommended for family members of patients with IPAH. In the case of a novel or suspicious variant of uncertain significance identified in a family with HPAH, segregation studies may be performed to understand its role in PAH susceptibility.

Retesting affected individuals with a negative result when new PAH genes are identified should also be considered.

DISCUSSION

Due to the utility of genetic counseling and testing in identifying family members at-risk for PAH, guidelines recommending these services for PAH have been available for over 15 years. Yet, it appears that these recommendations are not often followed by medical providers in the United States, largely owing to a lack of knowledge surrounding PAH genetics, and perceived lack of relevance of genetic counseling and testing in clinical care.[27] Other reasons for not utilizing genetic services highlighted in the same study[27] included high cost or insurance noncoverage for genetic services and a lack of access to genetic counselors.

Additionally, a 2020 survey of patients with IPAH conducted by the United Kingdom Pulmonary Hypertension Association highlighted preference of a majority (74%) of patients with IPAH to undergo genetic testing, and their desire to have cascade genetic testing offered to relatives if the specific genetic cause was pinpointed (80%).[28] Further, PAH is typically advanced by the time it is diagnosed, but with studies starting to document improved outcomes with available therapy even in severe PAH,[24,29,30] it is imperative to implement proactive measures (ie genetic testing with serial evaluation) in individuals at risk for HPAH, to detect disease at an early stage when the impact of therapy can be maximized. Genetic testing has become extremely affordable in the last 5 years. Postnatal targeted testing for a familial variant is in the range of US$200 with many insurance companies covering genetic counseling as well as genetic testing. Therefore, the time has arrived for better utilization of genetics services for patients with PAH. It may be easier to obtain insurance coverage for recommended serial screening in the presence of a PAH susceptibility variant. Genetic counselors specialized in cardiovascular disease or general genetics are ideally suited to coordinate genetics-related care. Providers without local access to a genetic counselor can request services through telegenetics companies.

Future Directions for Precision Genetics in PAH

Despite recent advances in gene discovery for HPAH, there remains a proportion of gene-elusive FPAH cases. Further, in families with a known predisposition, utility of predictive testing is still complicated by incomplete penetrance of disease. Studies to discover the missing genetic contributors to HPAH, as well as to understand contributors to reduced penetrance and variable expressivity are needed. In addition, the most effective clinical screening protocol for individuals at high risk of HPAH addressing at which age evaluations should be initiated, which screening modalities should be utilized, and what the frequency of screening should be in genotype positive versus negative families is yet to be established.

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Genetic Counseling and Pulmonary Arterial Hypertension

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INTRODUCTION

As the role of genetics within pulmonary arterial hypertension (PAH) continues to expand, it has become evident over time that there is value and utility in incorporating genetic counseling into this field. Genetic counselors provide a comprehensive service that is tailored to addressing many of the complexities and nuances of genetics and PAH for patients and their families.

WHAT IS GENETIC COUNSELING?

As defined by the National Society of Genetic Counselors, genetic counseling is the process of helping people understand and adapt to the medical, psychological, and familial implications of genetic contributions to disease (Table 1). This process integrates the following: (1) interpretation of family and medical histories to assess the chance of disease occurrence or recurrence; (2) education about inheritance, testing, management, prevention, resources, and research; and (3) counseling to promote informed choices and adaptation to the risk or condition.[1] Genetic counselors have advanced training in human genetics and psychosocial counseling. In the United States, most genetic counselors have a master's degree in genetic counseling from an accredited genetic counseling program. Most employers require their genetic counselors to be certified by the American Board of Genetic Counseling (ABGC), and to maintain their certification by completing ongoing continuing education. In states where genetic counselors are licensed, all genetic counselors with licensure must be ABGC-certified.

ROLE OF GENETIC COUNSELING IN PAH

Genetic counselors play an integral role in multidisciplinary clinics, including pulmonary hypertension clinics. Expert consensus guidelines recommend genetic counseling and genetic testing to patients with familial PAH and idiopathic PAH.[2-5] Current practice guidelines recommend genetic counseling prior to genetic testing, specifically to address the complex genetic basis of PAH, issues of incomplete or variable penetrance, surveillance for at-risk family members, concerns about genetic discrimination, and psychosocial concerns associated with the diagnosis. After genetic testing, genetic counselors can facilitate cascade testing of at-risk family members if a pathogenic mutation is identified and can discuss reproductive testing options if desired.[5]

GENETICS OF HERITABLE PAH

Heritable PAH is inherited in an autosomal dominant manner with reduced penetrance. This means that each child of a parent with PAH due to a gene mutation has a 50% chance of inheriting the genetic mutation that caused the condition. However, not everyone who

Table 1. PAH and Genetics—Important Definitions

Term	Definition
Idiopathic PAH	PAH without an identifiable cause; typically, a diagnosis of exclusion
Familial PAH	PAH that occurs in ≥ 2 family members
Heritable PAH	Includes familial PAH and simplex PAH (ie, a single occurrence in a family) when a pathogenic mutation in one of the known genes has been identified
Penetrance	The proportion of people with a genetic mutation who exhibit symptoms of the disorder
Reduced penetrance	When the penetrance of a mutation is below 100%; that is, when not all individuals carrying a pathogenic mutation develop signs or symptoms of the condition
Variable expressivity	Differing clinical features among individuals carrying the same pathogenic mutation
De novo	Spontaneous genetic mutation not inherited from a parent

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Table 2. PAH Genes

Genes associated with heritable PAH (adapted from Elliott 2021)[10]	
Gene	**% of patients with identifiable mutations**
BMPR2	15.3
TBX4	1.3 (more common in children)
KCNK3	< 1.0
GDF2	< 1.0
KLF2	< 1.0
SMAD4	< 1.0
ATP13A3	< 1.0
BMPR1B	< 1.0
KCNA5	< 1.0
SMAD1	< 1.0
SMAD9	< 1.0
SOX17	< 1.0
AQP	< 1.0
CAV1	< 1.0
ACVRL1	< 1.0
ENG	< 1.0

carries the familial mutation will develop PAH, also known as reduced penetrance. For example, penetrance for *BMPR2* mutations has been estimated at ~20% overall with sex-dependent penetrance due to higher penetrance observed in female (42%) versus male (14%) carriers.[6,7] Penetrance for individuals with hereditary PAH due to mutations in *ACVRL1*, *KCNK3*, *CAV1*, *SMAD9*, or *BMPR1B* is not yet known.

Pathogenic mutations are identified in about 20% to 30% of patients with idiopathic PAH, and in about 75% of patients with familial PAH.[4,8,9] Genetic testing can be useful for familial risk assessment if a pathogenic mutation is identified. However, given the incomplete clinical sensitivity of current genetic testing for familial PAH, a negative result does not rule out a genetic cause of, or contribution to, a patient's PAH (Table 2).

In adult populations, most heritable PAH (~75%) is caused by mutations in the *BMPR2* gene.[11] Other genes associated with hereditary PAH have been identified, but in aggregate account for a very small percentage in adult populations (Table 2).

Table 3. American College of Medical Genetics Variant Classification Categories

Variant	Definition
Pathogenic	Variants or gene alterations that are known to disrupt gene function and cause disease
Benign	Variants in a gene that are found in many unaffected people and do not increase risk for disease
Variant of uncertain significance (VUS)	Gene alterations not commonly found in the general population for which it is unclear whether the variant causes disease or not. Rare genomic variation is extremely common and very difficult to interpret especially in relatively common adult-onset conditions with high clinical variability and incomplete penetrance. In the future, as more is learned about these genes, new information may clarify the risk associated with these specific alterations. The category of VUS has been further divided into subcategories ("*likely pathogenic*" and "*likely benign*") to reflect the substantial difference in variant data affecting interpretation.

Emerging data indicate that there are fundamental differences in the genetics of pediatric-onset versus adult-onset PAH. A greater proportion of patients with pediatric-onset PAH have identifiable genetic mutations as compared to those with adult-onset PAH (35% versus 11%, respectively).[12] While *BMPR2* mutations are the most common cause of heritable PAH in both pediatric and adult populations, the genetics of pediatric-onset PAH are much more heterogenous. In one larger cohort of 412 pediatric- and adult-onset familial PAH and ideopathic PAH cases, rare deleterious *TBX4* variants were reported in 13 cases (3%) with a significant enrichment of variants among pediatric (12/155; 7%) compared to adult-onset (1/257; 0.4%) patients. Variants in the *SOX17* transcription factor have been shown to be similarly enriched in pediatric PAH cases (19/273; 7%), compared with adult-onset PAH (13/3,455; 0.4%).[12] Additionally, de novo genetic variants (spontaneous mutations not inherited from a parent) contribute to a significant proportion, approximately 15%, of pediatric PAH cases.

GENETIC VARIANT CLASSIFICATION

Certain guidelines have been established for the interpretation of genetic variants; specifically, the American College of Medical Genetics has published guidelines that have helped to standardize the classification of genetic sequence variants for the medical community.[13] For clinicians that order genetic testing or those on the clinical team reviewing these results, it is important to know the different classifications of variants. The standard terminology that is used to describe variants identified in genes that cause Mendelian conditions are as follows: "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign" (Table 3).

It is important to take into consideration the rapidly changing landscape of genetic testing when ordering PAH genetic panels. For any patient undergoing testing that receives a "negative" result (meaning no pathogenic findings) or a "VUS" (a variant of uncertain significance), we typically recommend that patients check with their genetic counselor or medical care team every 2 to 3 years to inquire about changes, updates, or reclassification regarding their genetic testing results. Some genetic testing laboratories will contact the ordering provider if there is a change to the interpretation of genetic testing results, but not all do, therefore the onus ultimately falls on the patient to recontact their genetic provider to ensure testing is up to date and as comprehensive as possible. Moreover, patients are encouraged to recontact their genetic provider with any subsequent changes to their personal or family's cardiovascular history so that their genetic counselor and care team can assess whether additional testing is recommended.

COORDINATION OF GENETIC TESTING

A critical role of a genetic counselor is to help families coordinate genetic testing. There are various laboratories that

Table 4. PAH Gene Panels Based on Laboratory[a]

Laboratory	GeneDx	Prevention Genetics	Invitae	Blueprint Genetics
Genes	*ACVRL1*	*ACVRL1*	*ACVRL1*	*ACVRL1*
	BMPR2	*BMPR1B*	*AQP1*	*ABCC8*
	CAV1	*BMPR2*	*ATP13A3*	*AQP1*
	EIF2AK4	*CAV1*	*BMPR2*	*ATP13A3*
	ENG	*EIF2AK4*	*CAV1*	*BMPR1B*
	GDF2	*ENG*	*EIF2AK4*	*BMPR2*
	KCNK3	*GDF2*	*ENG*	*CAV1*
	SMAD9	*KCNA5*	*GDF2*	*EIF2AK4*
		KCNK3	*KCNK3*	*ENG*
		SMAD9	*SMAD9*	*FOXF1*
		TBX4	*SOX17*	*GDF2*
			TBX4	*KCNA5*
			KCNA5	*KCNK3*
			BMPR1B	*KLF2*
				NFU1
				NOTCH3
				RASA1
				SARS2
				SMAD4
				SMAD9
				SOX17
				STRA6
				TBX4

[a]Gene panels are subject to change, please refer to each laboratory for updates.

offer clinical genetic testing for PAH; however, not all panels include the same genes, and thus it is paramount for the genetic provider to critically assess and determine which laboratory and/or gene panel will provide the highest yield. For example, an important consideration is whether the panel encompasses genes that target pediatric or adult-onset PAH; specifically, not all panels include the *TBX4* or *SOX17* genes, both of which are enriched in pediatric cases (Table 4).[12] Turnaround time can also vary between laboratories, generally from 14 days to 1 month or greater. Most laboratories perform genetic analysis on blood, saliva, or buccal specimens, but for it is important to check with specific labs should testing be done on any other specimen types (ie, postmortem, blood spot, skin punch) as not all labs have the same capabilities. Cost can also vary among laboratories. Not all accept insurance and patient self-pay pricing can vary from $250 to ~$900. Lastly, there are certain laboratories that offer family-variant testing options (typically for pathogenic or likely pathogenic variants), so it is recommended to inquire with the specific laboratory about options for follow-up testing of at-risk relatives.

RESOURCES FOR GENETIC COUNSELING

Even though the field of genetic counseling is growing, there still may be difficulty in finding a genetics provider to coordinate counseling or testing in your geographical area. If you do not have access to a cardiovascular genetic counselor within your hospital or clinic, here are some resources that can be used to either find a genetic counselor in your area or solicit commercial telehealth genetic counseling services which can be accessed from anywhere in the country:

https://findageneticcounselor.nsgc.org/
https://informeddna.com/
https://www.genomemedical.com/
https://www.gene-matters.com/

CONCLUSION

A genetic counselor is a master's-level trained clinical provider that can help facilitate genetic testing for patients and their families with heritable PAH. As genetic testing for PAH continues to rapidly expand, genetic counselors' skill-set and knowledge of the complexities, nuances, and new technologies available are well suited to assist PAH clinical teams and their patients. This collaboration helps to ensure informed decision making surrounding genetic testing for patients and their family members who may also be at risk and can subsequently receive surveillance and interventions that could be life-saving.

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Genetics and Pulmonary Hypertension

This winter, Greg Elliott, MD, Professor of Medicine at the University of Utah School of Medicine, and Emeritus Professor at Intermountain Healthcare, and Usha Krishnan, MD, Pediatric Cardiologist and Professor of Pediatrics at Columbia University Medical Center, gathered with Wendy Chung, MD, PhD, Professor of Pediatrics and Chief, Division of Clinical Genetics, Columbia University; Paul Yu, MD, Associate Professor of Medicine at Harvard Medical School and Brigham Women's Hospital in Boston; and Eric Austin, MD, Pediatric Pulmonologist at Vanderbilt University Medical Center, and Associate Professor at Vanderbilt, to discuss the genetics of pulmonary hypertension.

Dr Elliott: Let's begin by introducing the participants in today's Pulmonary Hypertension Roundtable focused on the genetics of pulmonary hypertension. I'm Greg Elliott. I'd like to introduce my comoderator, Dr Usha Krishnan.

Dr Krishnan: Hi, it's a great honor to be here. I'm a pediatric cardiologist, with my main interest being Pulmonary Hypertension and a healthy curiosity in the genetics of PH. I'm excited to learn from the experts on this panel. We're joined by Drs Wendy Chung, Paul Yu, and Eric Austin. I'm going to request each of them to introduce themselves, and we'll start with Wendy, and then Paul, and then Eric.

Dr Chung: Hi, I'm Wendy Chung. I'm very pleased to be here. I'm a pediatric geneticist, also based out of Columbia University with a long-standing interest in pulmonary hypertension, and I'm always learning because we don't know everything about genetics or causes of pulmonary hypertension.

Dr Yu: Thanks. I'm Paul Yu. I'm an adult cardiologist with an interest in pulmonary hypertension and a research effort focusing on signaling in the bone morphogenetic protein (BMP) and TGF-beta signaling pathway. I've been interested in the genetics of pulmonary hypertension as a way to get new insights into the mechanisms of disease, and, hopefully, to identify new treatment targets. I have a small academic practice and follow some patients with pulmonary hypertension, and I'm part of a pulmonary vascular disease program at our institution. Some of my research is informed from that clinical experience, but just as importantly, from clinical PAH experts in our communities such as the ones we have on the call here, with whom I have the privilege of consulting and working.

Dr Austin: Hello, I'm Eric Austin. I direct the pediatric PH program at Vanderbilt. I've had the privilege of working with each of the people on this call, and many other people in the field for many years, with an interest in pulmonary hypertension. I have a laboratory working on translational investigations of individuals with pulmonary hypertension, and also human cohort studies including clinical trials trying to translate what we can learn.

I know less about genetic science than everyone on here, but I do my best, and really feel it's important. I'm grateful that we have this topic because our pediatric PH patients and their families really embrace this, so I'm grateful for this opportunity with you as well.

Dr Elliott: Eric, I'll take my prerogative as one of the moderators to introduce the audience to some of the history of our current understanding of the genetics of pulmonary hypertension. I'd like you to start by just giving the audience some idea of the work and the discoveries that shape the current understanding of heritable pulmonary hypertension.

Dr Austin: Absolutely. Pulmonary hypertension, as we know it as a heritable disease, was originally described in the 1950s by Dr Dresdale up in New York, who described early families who had pulmonary hypertension that ran through the family. That work continued through the years, and investigators began to think about "how can we capture these families and use them to understand the biology and the genetics of the disease better over time".

In the '90s, there was a great interest as the genetic revolution was happening, and really well on its way in discovering what are the underpinnings genetically that cause PH, particularly, pulmonary arterial hypertension, what we then called primary pulmonary hypertension, in families. Familial pulmonary arterial hypertension (PAH) (a subtype of heritable PAH) is 2 or more individuals who have pulmonary hypertension in the family. Many familial PAH patients and families consented to participate in studies in which investigators in the '90s really tried to determine the shared genetic cause of PAH within and across families.

Multiple groups were instrumental in making this discovery. There were international collaborations in North America and to the UK and beyond. There was work at Columbia University that was led as well. Large investigations culminated in 2 distinct papers that both found that the primary gene at the time, that we understood associates with PAH, was the gene known as bone morphogenetic protein receptor type II, or BMPR2. Those investigators, collectively, really put in a tremendous amount of work in the discovery of BMPR2 as the predominant cause of familial, and now, what we call heritable PAH. And, those family participants were instrumental.

There are certainly other causes, and people immediately, of course, began to try and understand not only why and how does that cause disease, also, they tried to figure out—is BMPR2 also applicable to other forms of PAH? Sub-

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sequently, it was determined that somewhere in the order of 15% to 20% of idiopathic PAH may harbor mutations in BMPR2. People such as Dr Yu began to think about signaling and why does BMPR2 contribute to the pathogenesis of PAH. People such as Dr Chung and colleagues began to think, "Well, wait a minute. Not only do we need to expand on our understanding of BMPR2, but can we go beyond BMPR2 and understand other causes of genetic-associated PAH?"

An early discovery was in the disease HHT, which we already knew caused a form of primary pulmonary hypertension in a small number of HHT patients (HHT is short for hereditary hemorrhagic telangiectasia). This led to the discovery that not only BMPR2, which is a TGF-beta superfamily member, can cause PAH, but so can HHT-associated genes that are mutated, endoglin and ALK1. Those reports came from here in North America as well as in the UK. Dr Elliott was part of many of those studies. Discovering that now we had BMPR2, ALK1, and endoglin, in the early 2000s subsequent work studying more and more families led to more and more discoveries of genetic-associated underpinnings of PAH.

Dr Chung leads a lot of those right now, with many of us in both adults and children, but other people across the pond, in Europe, particularly, in the United Kingdom and in France, have investigated these, as well as individuals in Asia. It is now known that a large percentage of pediatric PAH and a decent percentage of adult PAH is actually related to genetic causes. Not only BMPR2, not only ALK1 and endoglin, but other irregularities in other genes as well.

So, we've gone beyond TGF-beta to other genes that may or may not be related to TGF-beta signaling of a great interest, including TBX4, SOX17, and EIF2AK4. EIF2AK4 is associated with a less common form of pulmonary hypertension, but still incredibly important, known as PVOD-PCH-spectrum disease. While there is much more to share, I hope that gives a decent quick overview, Dr Elliott, about where we've been and where we are now as a field. So much exciting stuff going on and a lot more to learn, but we've learned that genetics truly is majorly important to the pathogenesis of many PAH forms.

Dr Krishnan: Thank you so much, Eric.

Dr Elliott: Eric, thank you. You just covered an incredible amount of work in a short time. All the people who contributed, and there were, as you mentioned, so many people around the world who contributed to these discoveries, they would be really impressed to hear how fast you ran through it in comparison with how long it took them to assemble all the pieces of this big puzzle and begin to make sense out of it all. Just the discovery of BMPR2 took years of hard work.

Paul and Wendy, do you have anything to add to that history so well stated before we move on?

Dr Chung: No. I'll just say that, as Eric said, he rattled off a bunch of different genetic causes. They're not all created equal in terms of the proportion of PAH patients with variants in these genes or with the same associated clinical features. We're also still learning about genetics. We still don't understand the cause of PAH for most people. Even in some cases where we have clear family histories of multiple people in the family with pulmonary hypertension and we think it should be genetic, we haven't figured it out yet. We have figured out a lot but not everything.

Dr Elliott: Wendy, that's a terrific point. We're not out of a job, there's more work to be done. Paul?

Dr Yu: I agree. I think, as Eric so nicely summarized, some of these mutations are complex in that they can present as different phenotypes besides pulmonary hypertension, including the HHT syndromes. I think that's one really important unanswered question, of how the same mutations in 1 gene can manifest differently as HHT or as PAH, or in some cases, as PVOD, as your group, Greg, recently reported. These different presentations can sometimes be found among different members of a family carrying the identical mutation. What are the additional genetic or nongenetic factors that cause these various mutations to manifest as any disease, and when they do, what causes them to manifest as a particular disease on this spectrum of vascular disorders?

Dr Krishnan: Right now, what we know is really the tip of the iceberg. As far as the genetics of pulmonary hypertension, there's so much more to learn. With that background, let's pivot the discussion to the current knowledge of genetics and how can we use it to help our patients. Wendy, very often, cardiologists and pulmonologists and other practitioners are not comfortable discussing the genetics with their PAH patients. What should doctors tell their patients about heritable PAH? When should genetic counselors be involved and what kind of panels and what is available everywhere? Could you please start the discussion and then we can have Paul and Eric to comment after you?

Dr Chung: Sure. I would say there are different scenarios that I see clinically; I'm going to start with the adults because that's where we see more individuals with pulmonary hypertension. There are some individuals with a family history of pulmonary hypertension. Historically, we haven't recognized everyone with a family history, because a generation ago, people may not have been diagnosed with pulmonary hypertension. In some cases, people didn't necessarily communicate the information within their family. My point being that you don't always get a positive family history, even when you go back afterwards and look carefully at the family.

Then in those cases, hopefully, the physician caring for the patient has at least gotten a cursory family history to know there might be something. Oftentimes, that discussion naturally comes up because people say, "Oh, my uncle Joe had this and is this the same thing in me? Should I be worried about my kids?" That conversation naturally comes up, and depending on how people feel about it, it's driven by the patient who is worried about their children or their nieces and nephews or

other relatives. The genetics of pulmonary hypertension are tricky because even if you have the genetic variant, it does not mean 100% that you'll develop the disease.

In fact, there are differences between men and women. Females are at about 40% lifetime risk. Males are at about 20% lifetime risk. We can't necessarily say exactly if or when any one person will develop pulmonary hypertension. At this point, I don't have a cure that I can give someone to prevent the pulmonary hypertension. That level of uncertainty for many people doesn't sit well. They'd rather not know unless there's news you can use, something that you could clearly do to prevent this condition. Otherwise, it causes some people angst or concern. They worry about discrimination, they worry about a lot of things, but that causes a lot of people to just be ostriches, bury their head in the sand and not have to deal with it.

There's also an interesting chicken and egg problem. We don't know about a large number of people who are at risk from whom we could actually learn quite a bit in terms of primary prevention and getting to the point where we could potentially prevent this disease.

There's the bigger group of folks which Eric was mentioning. The people who don't have a family history of pulmonary hypertension. Of those IPAH adults, 20% have one of these BMPR2 mutations, and a few more have another gene. For whatever reason, they don't recognize this because they really truly don't have a family history of pulmonary hypertension, and other people who might have the genetic susceptibility just simply aren't demonstrating any evidence of disease.

Again, when they think about their kids for instance, even though they don't realize they have the gene, they still potentially have passed on that genetic susceptibility to the next generation. Even though they're ignorant to it, the gene's still there. A lot of the doctors don't feel comfortable bringing it up in those cases because it's just pointing a finger at something that people don't want to even think about. They don't want to think their kids might be at risk, they don't want to think they might themselves have passed on a genetic predisposition. They don't want to feel the guilt associated with that because, again, they don't feel like there's news you can use. They're just like an ostrich—bury their head in the sand and go on from there. That's, I would say, the culture, sociologically, that we often see in adults.

I think it's going to be a completely different story if we get to the point where we can actually do something for disease prevention. If we had that magic bullet in terms of early diagnosis, early treatment, and early intervention to slow down or halt the disease, it would be a completely different discussion. But this is the chicken and the egg. If we don't do those studies, if we don't identify people early, if we don't start treatment early, we'll never know, we'll never know if those interventions work, and so we're stuck.

Eric and I are pediatricians. Parents of young children, they have a very different perspective on this. They're watching their little children, oftentimes, they're really little, and their children are sometimes dying of this disease. For them, they are oftentimes not finished having their children. They think of having other children, they think about their other children who are still at risk, and they're panicked. It's a terrible thing to lose a child. To go through that and the heartache that goes with it is just unbearable, and they can't imagine that, so they will do anything and everything to make sure that they can either save a child who's already with them and do anything to help a child who's already sick. If they're thinking about having more children, they want to avoid being in this situation again in the future.

I find parents of children are very aggressive, information-seeking, and want to have answers. They'll do whatever they can to keep their families safe and healthy.

Dr Krishnan: What about tests though? When do you order whole exome sequencing vs whole genome sequencing, and when do you just order a PH panel? When are genetic counselors involved in this process? Is it better to involve a genetic counselor to talk about the genetic background of PAH to families?

Dr Chung: There's no one way to skin a cat, so to speak. I think your strategy is based on your institution, based on the expertise that's there. I'll also be radical and say we're doing much of this now by video conferencing. There are ways of being able to get expertise that may not be in your clinic, so to speak, but you can teleport someone to be able to come into your clinic or to be able to deliver these services. Genetic counselors have a masters in genetic counseling and human genetics. They're very nice people, very knowledgeable, and patient.

They can sit for sometimes hours, talking to families to educate them about what this is, be able to understand their family, understand social dynamics, and do all the paperwork to get the job done. They explain things in a way that the family understands, and help with preauthorization to ensure the family doesn't get stuck with big bills, and communicate information to other family members. The problem is we don't have enough genetic counselors.

We also don't have enough genetic counselors who understand the genetics of pulmonary hypertension. Even though you might have a fantastic genetic counselor upstairs or down the hall from you, if you don't train them about pulmonary hypertension, and if they don't listen to this podcast and learn about it, then they're not as strong an asset for your program. We can educate them in terms of integrating them, and I would go for a model of integration, pushing in and bringing the genetic counselors into your program to be able to help you. If that's not possible, you can reach out to your genetics team and refer them over. My advice is, get 1 go-to person at your institution who really knows what they're doing and works well with you, and works with the laboratories since there are certain laboratories that are good in terms of good, reputable, and patient-friendly. They have billing policies that don't leave people stuck with big bills.

Your other question about what tests to order, I'll just make it very simple to say that you can do targeted testing with just pulmonary hypertension genes. The number of those genes, as Eric describes, keeps growing over time as we

find more genes. Sometimes, a test that you ordered 5 years ago is out of date because we've identified more genes over time and you miss something. That's a problem in terms of genetic testing. You may have to go back and order that test again if you miss something, and a family clearly has a strong family history, and you don't give up.

The other thing that we've developed as geneticists, is THE genetic test, T-H-E genetic test. For some people that's overwhelming, either in terms of cost or information, but for those people who get a negative targeted test and are really information-seeking want to go for the gusto, and go for what we call an exome. In particular, if it's a child, I include both the parents or include multiple family members who have pulmonary hypertension so we can do a comparison within the family genetically and compare what is genetically the same to narrow down that search space and find that pulmonary hypertension gene.

When I do that in kids, I very often find a gene I have never, ever seen before in pulmonary hypertension. We are still at that cutting edge of still searching and finding new information. The good news is that many times that mutation is de novo, or new in the child with pulmonary hypertension, and won't happen again in other kids. So parents can breathe a sigh of relief that they're not going to have to worry about that for their other kids or for future kids.

Dr Krishnan: Is that why mutations found in children are different from mutations in adults?

Dr Chung: Yes. For mutations in kids, if you think about it from a population genetics point of view, this is true of isolated pulmonary hypertension, but it's also true for pulmonary hypertension associated with other conditions we see in children. For instance, we see more diaphragmatic hernias and congenital heart disease in children associated with pulmonary hypertension. If you think about it, 2 generations ago, or even 1 generation ago, before we had fantastic surgeons, before we had fantastic pulmonologists or cardiologists, those children were born, and they didn't make it very long. They died of their disease, and they didn't live to have children of their own.

They couldn't pass those genes on because they didn't make it into their 20s to be able to have kids. From a population point of view, those mutations were born and died and born and died every generation. It's really only now that some of my patients are actually living long enough that they can be able to have children of their own, if they can survive. We are certainly now getting some kids up until the age where they might be able to have their own kids.

Dr Krishnan: Wow! Paul and Eric, any comments before we go on to the next question?

Dr Yu: I think that what Wendy said was absolutely helpful for understanding the role of genetic counseling and family planning for people who are currently unaffected. I wanted to ask our panel members how each of you feels that the knowledge of having a particular mutation affects your treatment plan and your approach to the affected individuals, also known as the probands? Given that we know from Dr Elliot's work and Dr Marc Humbert's work, that people with BMPR2 mutations are more severe clinically at the time that they present, and may have a more challenging disease, do positive genetic testing results affect how aggressively we should approach those patients, above and beyond their clinical status and hemodynamic parameters that you're already factoring into those decisions?

Dr Austin: I'll take that to start. I think that's a great question. What you're alluding to Paul, is true, that the metrics that we use to determine severity of PAH in the current era, including features that we learned from work by Drs Elliott, Humbert, and others, is that mutation carriers with PAH have metrics suggestive of a more severe disease condition than people who have nonheritable PAH. For me, as a practicing pediatric PH physician, this knowledge does influence the way I think about things.

The truth is, as you know, there's a lot of data now that upfront, early aggressive therapy, in many PAH patients, may be the right choice. But, I'm particularly aggressive in our practice and I think others are (but I can't speak for others of course) with PAH patients with a known PAH-associated gene mutation. I suspect there is a common scenario in which we think, "Well, we've got a person, they found a BMPR2 mutation in this person, so I'm going to be even more aggressive to move to a three-drug therapy option including some form of prostacyclin or prostacyclin-derivative fairly quickly." I am biased in that direction, and I do talk, frankly, to families about this potential treatment approach.

But, I try not to be overly influential about it, because we just don't have a robust amount of data. Wendy, I hope I didn't interrupt your response.

Dr Chung: No, no. Full disclosure, I'm not the one who's actually prescribing the medications here. All of my other colleagues are the ones who are actually doing the management, but I think about the comparisons between other genetic conditions and pulmonary hypertension I would argue more aggressive treatment and even earlier before vascular remodeling, could cut this off at the beginning early on in this process.

Realizing that not everyone at genetic risk is going to get sick, we have to have real time markers to see who's progressing to develop pulmonary hypertension. But let's not wait for symptoms, let's think about how we can intervene earlier. Mutations in BMPR2 are dominantly inherited, and if you have 1 of these genes, there's 50/50 chance that your brother, your sister, your kids have this as well, so identifying 1 person in the family, you can potentially save lives other members of your family by giving them a heads up with this information.

Dr Elliott: Yes. As a moderator, I want to jump in for a couple of points. One, Wendy, to your point, we in Utah, and, Eric, some of your senior colleagues at Vanderbilt have seen an occasional family member who lives in a rural community where they were thought to have asthma until they presented with very advanced PAH. Had the BMPR2 mutation been uncovered early and

treatment started early, we would expect that they would have had a better outcome. Discovery of pathogenic BMPR2 mutations creates an opportunity for earlier recognition and treatment of this progressive, often fatal, disorder.

One other gene marker that's important on the treatment side, is EIF2AK4, which causes pulmonary veno-occlusive disease (PVOD) / pulmonary capillary hemangiomatosis (PCH). PVOD/PCH doesn't respond well to our current PAH therapies, probably because it's a very different pathway, isn't it? It's not a TGF-β pathway like BMPR2 or ALK1. In fact, some of our PAH therapies can precipitate pulmonary edema in patients with PVOD/PCH. There's a great example of the value of genetic testing. Recognizing EIF2AK4 mutations when it looks like idiopathic or familial PAH can very definitely influence our sense of prognosis, disease progression, and response to therapy, so I would always put that in there.

Fortunately, the data that we've seen so far tells us that PVOD/PCH caused by EIF2AK4 mutations is rarely found in patients diagnosed with Group 1 PAH, at least in adults. Two studies, 1 study in the United States and a larger study in Great Britain and Europe, reported that only about 1% of patients diagnosed with IPAH had occult PVOD caused by biallelic EIF2AK4 mutations. The percent may be higher in families thought to have familial PAH.

Dr Krishnan: The recognition of a gene coding for PVOD really triggers a lung transplant referral as there is no treatment, and the condition is one of rapid progression to death.

Dr Elliott: Yes, I think that's a good point. Here in Utah, our approach is to give the patient with PVOD/PCH an early referral to the lung transplant program. We know that these patients are unlikely to benefit from PAH-specific therapies, and their prognosis for survival is generally poor by the time they seek medical attention and are diagnosed.

Dr Elliott: Maybe we should move ahead, Usha, to your question about penetrance.

Dr Krishnan: Okay. I was going to address this question to Paul initially, and then to Eric. Paul, most of us who are not geneticists sometimes find it difficult to understand what penetrance means. What can you tell us about the penetrance of PAH with mutations in genes like BMPR2? Can you help our audience understand incomplete penetrance? Are there epigenetic or environmental factors that can influence penetrance?

Dr Yu: Thanks Usha. Wendy nicely addressed part of this question with her earlier comments. As she noted, there was a great study from Dr Austin and his colleagues at Vanderbilt several years ago that looked at 53 different families with heritable pulmonary hypertension. From that large set of families, there was a unique opportunity to observe multiple generations for long periods of time to get us better estimates than we had been previously able to obtain from cross-sectional studies, meaning at one static point in time, to answer this question of penetrance.

In that study, the estimates of penetrance were a little bit higher overall than previous estimates, maybe 25% over the lifetime of patients with BMPR2 mutations, for example, where previous estimates were somewhat lower, in the range of 15% to 20%. This study shows that the lifetime penetrance of mutations in BMPR2 and other genes is a concept that is evolving as we have more numbers of unaffected and affected mutation carriers identified, and more patient-years of observation. As Wendy pointed out, there might be a difference between men and women, as in this Vanderbilt study men were found to have 14% while women were found to have 42% penetrance. This factor, gender itself, is obviously a modifier of penetrance. We don't know exactly what the mechanisms are, if they are related to the influence of sex hormones, or the influence of other factors.

In addition to sex hormones, there are genetic differences between men and women, as well as epigenetic and environmental differences. There could be as-of-yet unidentified factors, and there's a ton of research activity focused on addressing this question, where the factors that modify penetrance are an important part of the puzzle. Several large studies that came from Wendy's group, and Bill Nichols' group, and Nick Morrell's group sequencing the genomes of thousands of individuals with Group 1 PAH found that the mutations that cause heritable PAH, now totaling over a dozen and a half mutations identified over the course of the past 20 years, are also found at substantial rates in patients who do not necessarily present phenotypically as heritable PAH but rather as other etiologies of PAH. While we have generally been more suspicious of these mutations and thus more likely to pursue genetic testing in our patients who have a strong family history, or who appear to fit within a syndromic pattern, especially when they're pediatric patients, that strategy may be too limiting, it turns out. One of the first such large-scale sequencing studies from Bill Nichols and Wendy looked at the exomes sequenced from almost 2600 patients and found that some of these mutations are enriched in patients thought to have PAH from exposure to stimulants, or from congenital heart disease, liver disease, or even HIV. In answering to your question about penetrance, these sequencing studies suggest that some of the clinical factors that we already know predispose to PAH can probably interact with genetic changes to promote penetrance.

A possible corollary to this idea is that we might start looking for mutations more broadly in our patients, to encompass more Group 1 PAH patients, some of whom have less obvious syndromic findings, or who may have de novo mutations or unclear or unavailable family histories, but I'd welcome your thoughts on that.

Dr Austin: That's an interesting question. Some people are very aggressive about Group 1 PAH mutation screening because of what you said, that we have probably a number of individuals with other PAH forms with PAH-associated gene mutations. For example, early work showed that about 6% of congenital heart defects, actually, with PAH were associated with BMPR2 mutations. Wendy could comment on what the updated percent-

ages are in that scenario, however. So, I think you're absolutely right, Paul.

It's fascinating to think that the genetic-associated PAH was once thought of as a niche group. I think it was 6% of the 1980s NIH Registry had a family history. It turns out that 6% really, is probably much lower than in actuality; but, even if it was just that 6%, genetics has dramatically informed what we know about other forms of PAH that had no known family history. Anyway, we are now screening in our clinic, congenital heart, idiopathic, and familial forms routinely.

But, we do not go beyond that routinely, unless we have some other syndromic-associated target. Wendy, you could probably comment better about the distribution in congenital heart disease and other forms. I'd be fascinated to actually be reminded of that data.

Dr Chung: We've looked at things that go from both directions. With Erika Berman Rosenzweig, and Usha Krishnan, we've taken individuals who came to them because they had pulmonary hypertension, and we see who has a history of congenital heart disease, or diaphragmatic hernia, or family history of PH, or none of the above. The numbers are still modest. We're talking about 200-ish families that we've studied that way, but we've seen some of the genes. Some of the genes, for instance, a gene associated with Noonan syndrome, was in a person we didn't recognize as having Noonan syndrome.

We did afterwards, looking back and saying, "Oh, well, yes, maybe it could be," but didn't recognize this gentleman had Noonan syndrome and went through his whole childhood without being diagnosed. We've also done it in the other direction, which is interesting because in many cases, I'll see the newborns with a diaphragmatic hernia or congenital heart disease. I'll ask myself, is this a baby who's going to have trouble with pulmonary hypertension in the future? Can I make any prediction based on what I'm seeing with the genetics that'll help our pulmonary hypertension team in terms of monitoring or early intervention, early recognition, and early treatment?

Kids are different. Kids are not just little people in little packages. There really are developmental differences. There's a difference in terms of the way the pulmonary vasculature develops. It's in part a plumbing issue, with the heart and plumbing into the pulmonary vasculature as well.

Sometimes it's a problem in the anatomical neighborhood or field effect, so with diaphragmatic hernia, sometimes we see, developmental lung problems, and it probably isn't just the vasculature but could be alveologenesis in general. Then in some of these cases it's broader. I have to admit, if you'd asked 10 years ago, how many pulmonologists were looking down at kids' toes or feet or knees, or asking about hip problems, they would say, "No, we never take off the shoes." The thing that I'm alluding to is we know about a mutation gene called TBX4, that we used to call small patella syndrome. We were focused on their small knees or kneecaps.

Other people were looking at their lungs, but people weren't putting everything together. When you do, we don't understand why within the same family with the same TBX4 mutation, someone has a hip issue when they're 30, but they don't have any pulmonary problems. I've got families where we find pulmonary hypertension, but not until the 70s, and then I'll have in that same family, a little one who's got pulmonary hypertension at the age of 10, and we don't know why. There may be a second or third contributing factor, be it genetic, be it infectious, be it something else.

There are probably other things that go into this equation and help us determine risks, but it's a numbers game. In terms of being able to get to the to truly see what is influencing risk, we need to study many people.

Dr Austin: There is an interesting study that was recently published out of France from David Montani and Marc Humbert and colleagues, in which I believe they followed 55 individuals who had BMPR2 mutations who were otherwise well, without PAH. They showed over a 2 to 3 year period about 1% of males and 3.5% of females actually were diagnosed with PAH. I think this gets at the notion that Wendy alluded to that if we could capture people who are genetically at risk, but not with overt disease early, could we somehow alter that trajectory. This reservoir of people who actually have a genetic risk in these families but don't have disease is incredibly high. I hope that some day we can provide them some disease-modifying therapeutic approach.

Dr Elliott: In the short time that we have left, we have maybe 3 questions that remain for discussion. First, does anyone have experiences to share over insurance coverage in the United States and limitations of genetic counseling or testing, or do you feel like that's not a barrier so much?

Dr Chung: I'll just say there are a couple of different insurance questions. Number 1, testing is largely covered by insurance. I don't want people to be reluctant because they're afraid they're going to get bills for thousands of dollars; that doesn't happen anymore. That's number 1. Number 2 is some people worry about what I'll call discrimination. Whether it's insurance discrimination or something else, for those individuals who are not showing any signs or symptoms of pulmonary hypertension, they're worried. "If I've got the genetic predisposition, am I going to have trouble getting health insurance, life insurance, long-term care, disability insurance? Is someone going to hire me? Am I going to get into the right college?" Whatever it is, but people are worried.

With this, what I will say is, there's a federal law in place to protect individuals from having your health insurance rates raised or being denied health insurance. It's a very good law. I'm not worried about health insurance, but here's the rub. It doesn't protect in terms of life insurance, long-term disability, insurance long-term care, so people worry about that. Again, I haven't personally seen it happen, but people worry about that. What I have seen is that some people will get their life insurance, and then they'll get their genetic testing. As long as you've got your insurance policy in place and you pay your premiums, you

should be okay in terms of having a policy. For people who are really concerned, that's one way of approaching it.

Dr Elliott: Wendy, that's very helpful. Eric, any comment about using genetic tests to facilitate a diagnosis in a PH patient?

Dr Austin: Yes. You did specifically discuss wisely earlier, EIF2AK4 with regard to PVOD and PCH, and whether that could spare us a lung biopsy and really inform. That is really a very heavy hitter and a major player for us. If we're concerned at all about a spectrum of disease that may not respond well to vasodilator therapy such as PVOD/PCH-spectrum disease, that would be a large contributor to the way genetics really impacts a person's diagnosis.

If we found a BMPR2 mutation in a person with PAH for whom we thought it was idiopathic but we weren't sure, that would make us probably feel better that we were dealing with PPH in the traditional form, primary pulmonary hypertension, and not as worried about connective tissue disease or other forms. Although, as Paul said earlier, there are some concerns that there are individuals out there who have maybe two hits in that. I think for the sake of brevity, I'll say that in my experience the most is that the PVOD/PCH is where genetics with diagnosis is most key, but it's also true that genetics is incredibly important for informing familial understanding as we just have been talking about for a while.

Dr Krishnan: Paul, a question just leading from that would be, if you have a genetic diagnosis, is there any approach, any advances, or anything in the pipeline regarding treating patients with certain mutations?

Dr Yu: There may be 2 ways to answer that. I think that genetics have been really helpful in highlighting potential therapeutic strategies. I'll circle back to your question about whether we have specific treatments for those genetic causes, but as I mentioned earlier, our lab is interested in regulation of the bone morphogenetic protein or BMP and TGF-beta signaling pathway. We know that there are a whole variety of vascular syndromes that can be caused from genetic mutations in the pathway, of which PAH is one, as are HHT, and occasionally PVOD. What we've been excited about are a couple of different approaches that try to reorder or redistribute the signaling in this pathway.

The pathway is complicated by the fact that there are 33 different signaling proteins in this pathway that impart signals to different cells in the body at specific times to coordinate the growth and remodeling of all of our tissues, and these include BMP proteins, as well as activin and TGF-beta proteins. There are 12 different receptors for these proteins, and a number of co-receptors. It has become clear to researchers in this field that this is a very modular system that's designed to help organisms establish important patterns required for embryonic development—in other words the blueprint of life—how do we form our limbs, how we make our digits, how we establish left and right sides of our bodies? The same signals that occur at precisely timed intervals and in precise locations during our development are also part of the carefully orchestrated sequence events that give us our pulmonary vessels, or our pulmonary vascular tree. It is likely these same signals maintain the stability of these structures in adulthood or govern the way they are repaired from injury.

In adults, we found that we can use several types of novel drug agents to try to bias the signaling in this pathway to treat diseases, based on the concept that some of the proteins are responsible for bad or disease-promoting remodeling of tissues, and others may be more protective. The genetics of PAH have suggested that the BMP signaling proteins, including BMPR2 itself, are protective factors that when lost can predispose to PAH, and that the signaling of other factors in this pathway, such as TGF-beta and activin proteins, might contribute to disease. Given the complexity of the system, most of these new types of drugs bias BMP, activin, and TGF-beta signaling only by broad strokes.

Recently we were involved in a translational effort for a molecule called sotatercept, which is basically an activin receptor that was reengineered to become an activin-blocking protein. The thinking was that activin signals generally seem to oppose BMP signaling, and that activin signals might become maladaptive in PAH. Blocking activin signals might restore the balance toward BMP signals that are insufficient in PAH disease. The genetics of PAH were the direct inspiration for this approach. The activin and TGF-beta signaling molecules are not found to be deficient or mutated in PAH, but rather increased—which led us to think that their imbalanced or unopposed signaling could be a driver of PAH. Both activin and TGF-beta turn out to be pretty good drivers of fibrosis, and thickening of vascular smooth muscle, which are 2 processes that describe changes in the lungs of people with PAH. Sotatercept is a potent blocking agent for activin signals, while appearing to leave most BMP signals more or less intact, and was special in that it had already been tested for safety in about 400 other volunteers and patients in previous clinical studies. Namely, it had been used in clinical trials for anemia, especially anemia due to beta thalassemia, and so we had many years of patient experience to learn about the effective and safe doses of this drug. When we used a rodent version of sotatercept, ACTRIIA-Fc, in animal models of pulmonary hypertension, it appeared to be very effective in not only in lessening the impact of disease as it was developing, but also appeared to be effective in reversing established disease.

The findings in rodent models of pulmonary hypertension were so conclusive that they led to a clinical effort called the PULSAR trial that was recently published in New England Journal of Medicine. In this study, sotatercept was seen to improve pulmonary vascular resistance in patients with moderate to severe pulmonary hypertension following 6 months of treatment. There are now follow-up studies, including STELLAR, a large Phase 3 study looking at this concept in more patients with PAH. We're hopeful about the results of STELLAR, and optimistic that it will pave the way for other follow-up strat-

egies that will augment BMP signaling or modify activin or TGF-beta signals in different ways to either enhance the therapeutic effect, safety, or both.

The human genetics of PAH really paved the path toward understanding which of the proteins are potentially helpful versus those that might be promoting the disease. I don't think that we have enough information yet to know if these treatments will end up being more or less effective for specific types of mutations, or without any mutations at all. For example, in the PULSAR Trial, the majority of patients were not known mutation carriers, and there were not enough patients enrolled with any of the known mutations to make conclusions about whether or not any of these mutations influenced the success. Hopefully, in the follow-up studies, we'll get more information on these subtle but important details.

Dr Krishnan: That's an exciting note to end this conversation with some hope in the air. That, I think is really, really important. Thoughts from everyone?

Dr Elliott: Actually, Usha, I just want to thank the participants and organizers of this roundtable discussion. This was a terrific conversation. It really is exciting to end our conversation with the hope that future generations of PAH patients will benefit from novel therapies aimed at the molecular causes of their disease which were discovered through genetic studies.

This whole concept of precision medicine, using molecular pathways and knowledge of pathogenesis, is incredibly exciting to someone like me who has lived through 40 plus years of advances in the treatment of PAH. I'm old enough to have given hydralazine when we were first trying to vasodilate the pulmonary vascular bed. I'm here to tell you, that was primitive therapy. We have come a long way. We'll close it there.

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