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Genetics and Genomics of Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a rare disorder that may be hereditable (HPAH), idiopathic (IPAH), or associated with either drug-toxin exposures or other medical conditions. Familial cases have long been recognized and are usually due to mutations in the bone morphogenetic protein receptor type 2 gene (*BMPR2*), or, much less commonly, 2 other members of the transforming growth factor-β superfamily, activin-like kinase-type 1 (*ALK1*) and endoglin (*ENG*), which are associated with hereditary hemorrhagic telangiectasia. In addition, approximately 20% of patients with IPAH carry mutations in *BMPR2*. We provide a summary of *BMPR2* mutations associated with HPAH, most of which are unique to each family and are presumed to result in loss of function. We review the finding of missense variants and variants of unknown significance in *BMPR2* in IPAH/HPAH, fenfluramine exposure, and PAH associated with congenital heart disease. Clinical testing for *BMPR2* mutations is available and may be offered to HPAH and IPAH patients but should be preceded by genetic counseling, since lifetime penetrance is only 10% to 20%, and there are currently no known effective preventative measures. Identification of a familial mutation can be valuable in reproductive planning and identifying family members who are not mutation carriers and thus will not require lifelong surveillance. With advances in genomic technology and with international collaborative efforts, genome-wide association studies will be conducted to identify additional genes for HPAH, genetic modifiers for *BMPR2* penetrance and genetic susceptibility to IPAH. In addition, collaborative studies of *BMPR2* mutation carriers should enable identification of environmental modifiers, biomarkers for disease development and progression, and surrogate markers for efficacy end points in clinical drug development, thereby providing an invaluable resource for trials of PAH prevention. (J Am Coll Cardiol 2009;54:S32–42) © 2009 by the American College of Cardiology Foundation

Pulmonary arterial hypertension (PAH) is a rare disorder with an estimated incidence of approximately 2 cases per million per year (1,2). It is characterized by a sustained increase in mean pulmonary artery pressure (>25 mm Hg at rest or 30 mm Hg with exercise), normal pulmonary capillary wedge pressure, and increased pulmonary vascular resistance. For adults, mean age at presentation ranges from 36 to 50 years, although individuals of any age can be affected (2,3). Prior to the advent of modern therapies, life expectancy for adults with idiopathic pulmonary arterial hypertension (IPAH) was <3 years from diagnosis; for children, it was <10 months (4).

Pulmonary arterial hypertension may be heritable (HPAH), idiopathic, or associated with drug or toxin exposures (fenfluramine derivatives or toxic oil syndrome), or other medical conditions, including connective tissue diseases, human immunodeficiency virus infection, congenital heart disease, and portal hypertension. Familial cases have long been recognized (5), and in 2000, bone morphogenetic protein receptor type 2 (*BMPR2*) was identified following linkage analysis (6–8) as the gene responsible for more than 70% of HPAH and approximately 20% of IPAH cases (9–12). Crude indirect estimates of the population carrier frequency for *BMPR2*

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mutations lie in the frequency range of 0.001% to 0.01% (13). Two further receptor members of the transforming growth factor (TGF)-β cell signaling superfamily are also recognized as uncommon causes of HPAH. Heterozygous mutations in activin-like kinase-type 1 (*ALK1*) (14) and endoglin (*ENG*) (15) cause hereditary hemorrhagic telangiectasia (HHT) and may rarely lead directly to the development of PAH.

Heritable PAH is inherited as an autosomal dominant trait with incomplete penetrance and an estimated lifetime risk of 10% to 20% (16). The disease is more frequent in women, with a ratio of at least 1.7:1 women to men (2,17,18). Both incomplete penetrance and the significantly skewed gender ratio suggest interactions between *BMPR2* disease mutations and environmental exposures that may include hormones, together with a role for modifying genes. The latest classification scheme now replaces the term *familial PAH* with HPAH, at least in part to recognize the fact that up to 20% of cases previously thought to be IPAH harbor identifiable mutations in *BMPR2* and therefore pose a hereditary risk to other family members. Only 6% of PAH patients reported a family history of PAH in the prospective National Institutes of Health registry (18). A family history of PAH may go unrecognized in IPAH cases with *BMPR2* mutations, as a consequence of either incomplete penetrance or de novo (spontaneous) mutations. Heterozygous *BMPR2* sequence variants have been identified in a small subset of patients with PAH associated with relatively brief exposure to fenfluramine (13,19) or with congenital heart disease (20), raising the question as to whether such factors represent disease triggers in the face of inherited susceptibility in some patients. In contrast, *BMPR2* mutations have not been identified in PAH associated with the scleroderma-spectrum of disease or with human immunodeficiency virus (21,22).

HPAH and IPAH have a similar clinical course. HPAH is associated with a slightly younger age of onset and a slightly more severe hemodynamic impairment at diagnosis, but with similar survival (23). Patients with PAH and disease-causing *BMPR2* mutations are, however, less likely to respond to acute vasodilator testing during right heart catheterization and are unlikely to benefit from treatment with calcium channel blockade (23–25).

Genetic Anticipation

Families with *BMPR2* mutations have been reported to have genetic anticipation, or earlier age of diagnosis in subsequent generations (17). However, no systematic population-based study has been performed to avoid the ascertainment bias that could result in the recruitment and study of families associated with earlier-onset disease in more recent generations. Furthermore, the usual genetic mechanisms for anticipation, including trinucleotide repeat expansions, are not present in *BMPR2*. The question of genetic anticipation can be better addressed in future registries in which all patients with HPAH and IPAH can be genetically characterized and unbiased family studies can be performed.

Abbreviations and Acronyms

ALK1 = activin-like kinase type 1
BMPR2 = bone morphogenetic protein receptor type 2
ENG = endoglin
GRR = genotypic relative risk
GWA = genome-wide association
HHT = hereditary hemorrhagic telangiectasia
HPAH = heritable pulmonary arterial hypertension
IPAH = idiopathic pulmonary arterial hypertension
PAH = pulmonary arterial hypertension
TGF = transforming growth factor

The TGF-β Family and PAH

The TGF-β superfamily comprises a large series of cytokine growth factors that control a host of cellular functions, among them proliferation, migration, differentiation, apoptosis, and extracellular matrix secretion and deposition. Displaying high evolutionary conservation across species, TGF-β members are segregated into several subfamilies, notably the prototypic TGF-β ligands, receptors, and accessory molecules, activins, and the largest of these groups, the bone morphogenetic proteins (26). The implication of *BMPR2*, *ALK-1*, and *ENG* as causal factors in hereditary and associated forms of PAH has emphasized the critical importance of this pathway to the integrity of the pulmonary vasculature (27).

BMPR-II Structure and Signal Transduction

The 4 functional domains of TGF-β type II receptors are typically highly conserved across the family. They consist of an N-terminal ligand binding domain, a single transmembrane region, a serine/threonine kinase, and a cytoplasmic tail domain. Particular to BMPR-II, however, is the presence of an exceptionally long postkinase cytoplasmic domain, primarily encoded by exon 12 of the gene. A second isoform, generated by alternative splicing of exon 12, is expressed ubiquitously at the mRNA level, although the in vivo function of the mature polypeptide remains enigmatic (28).

Signal transduction routed through the TGF-β pathway has been extensively interrogated over the last 2 decades; in contrast, BMP signaling remains less well described. The paradigmatic BMP pathway is a phosphorylation relay of signaling intermediaries initiated at the cell surface and culminating in the nucleus (Fig. 1) (29). A heterotetrameric complex consisting of type I receptors, for example ALK1, BMPR1A or BMPR1B, and BMPR-II, amalgamate to bind extracellular dimeric ligand. These interactions promote close receptor species proximity and activation of the type I receptor by the constitutive kinase BMPR-II. The type I receptors, in turn, bind and phosphorylate members of the receptor R Smad family, namely SMAD1/5 or 8. When activated, the affinity of the R-Smads for a nuclear chaperone, Smad-4, common across the TGF-β system, is

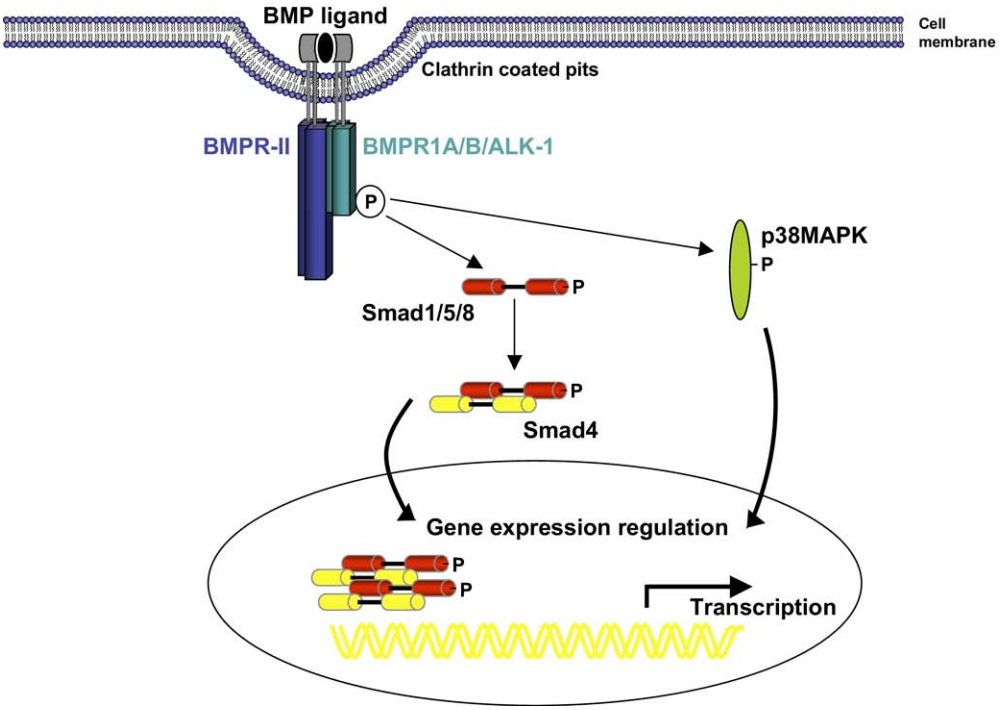

Figure 1 **BMPR2 Signal Transduction**

Upon binding ligand, the type II receptor phosphorylates a type I partner, which may be BMPR1A/B or upon activation by the ligands BMP9/10, ALK-1. This leads to a propagation of cytoplasmic signal transduction through the R-Smads 1/5/8, or, independently, via the p38MAPKs. In association with Smad4, the R-Smads translocate to the nucleus and, in complex with transcriptional cofactors, modulate the expression of a target set of genes.

augmented. These complex-bound signaling molecules shuttle to the nucleus, where, in concert with specific nuclear cofactors and repressors, they influence regulation of a limited set of target genes (26). Within the framework of an essentially uncomplicated canonical pathway, the specificity and flexibility of signaling outcomes are tightly maintained by the myriad, cell-specific levels of regulation extending from sequestration of extracellular ligand to inhibitive competition of type I receptors by nonfunctional decoy receptors and extensive cross-talk with other signaling pathways including p38MAPK, the cytoskeleton-associated proteins Tctex-1 and LIMK-1 (26,30–32), and c-src and RACK-1 (33,34).

The burgeoning understanding of TGF-β receptor structure and function has been an invaluable aid in determining the likely impact of *BMPR2* mutations in PAH. Conversely, and of equivalent importance, analysis of the spectrum and pattern of these defects, in conjunction with directed functional studies, has confirmed and better resolved those regions of the receptor necessary for normal physiologic activity.

Major Genetic Risk Factors in PAH

Through the compilation of previously reported mutation data, combined with an ongoing global collaboration targeted at conducting systematic and comprehensive screening of patient samples, a combined total of 298 *BMPR2* mutations have been identified (35). Of these, 88 are novel (Table 1) (36–39). These mutations were identified in independent probands, including those with a known PAH family history, sporadic onset of disease, and PAH associated with other disorders. All mutations studied to date appear to have risen on unrelated genetic backgrounds, suggesting that genetic founder events are uncommon (35). Indeed, approximately 41% (122 of 298) of all recorded *BMPR2* mutations are small deletions or insertions at sites of low-complexity sequence or as a consequence of C>T transitions, presumably resulting from the relatively common process of spontaneous cytosine deamination. Thus, despite the fact that PAH typically presents as a late-onset disease, ancestral mutations in this gene are at best rare, possibly because of a deleterious effect on reproductive fitness. In addition, we describe uncommon genetic susceptibilities, including *ALK-1* defects in PAH and PAH associated with HHT.

Mutation in *BMPR2* Constitutes the Primary Genetic Risk in PAH

A wide range of mutation detection methodologies have been employed to screen patients for point mutations and

large gene rearrangements, among them direct sequencing, melting curve analysis, denaturing high-performance liquid chromatography, Southern blotting, and multiplex ligation-dependent probe amplification. To date, mutations have been recorded in over 70% of subjects with 1 or more affected relatives, whereas in idiopathic cases, mutation detection rates between centers range from 10% to 40% (12,19,36,40). Of interest, mutations of TGF-β superfamily members have also been observed in associated forms of PAH, albeit at far lower frequencies (35). The spectrum of *BMPR2* mutations in hereditary and spontaneous disease is analogous, comprising all major classes of mutations. Mutations predicting premature truncation of the polypeptide chain are the most prevalent (203 of 298, 68%). Although there is another putative PAH locus at 2q32 that was mapped in part by using stress echocardiography to detect asymptomatic obligate carriers (41), no gene has yet been identified from this interval. Thus, to date, mutations in *BMPR2* remain the primary genetic susceptibility for PAH.

Truncating Mutations of *BMPR2* in Heritable and Idiopathic PAH

Mutations predicted to introduce premature truncation codons to the *BMPR2* open reading frame encompass nonsense (85 of 298, 29%), frame-shift (73 of 298, 24%), splice-site (26 of 298, 9%), and gene duplications/deletions (19 of 298, 6%). An exception of note is a double substitution of 2 consecutive bases (GC>AT) identified in a large PAH kindred 944 base pairs upstream of the translation start site (36). Bioinformatic assessment indicated that the mutation, within the context of flanking sequence, would likely generate the formation of an aberrant translational start signal, consequently leading to incorporation of a premature stop codon in the first exon of the gene. Allele-specific polymerase chain reaction assays utilizing variant and cell-based studies to examine activation of the nonsense-mediated decay (NMD) surveillance machinery have confirmed both degradation and loss of the mutant-harboring transcript. Indeed, both conventional studies on patient cell lines and novel in vitro technologies demonstrate that the majority of mutations in this class are rapidly removed from the cell via the NMD pathway (36,42). Along with the observation of large proximal and whole deletions of *BMPR2*, these findings now firmly substantiate haploinsufficiency as the predominant molecular mechanism underlying *BMPR2* predisposition to hereditary and idiopathic forms of disease (11,13,43).

Distribution and Impact of Missense Mutations Across Conserved Functional Domains of *BMPR2*

Missense mutations of *BMPR2* in HPAH/IPAH cluster mainly in regions of the gene-encoding receptor domains indispensable to signaling activity and are confined to exons 2, 3, 6 to 9, 11, and 12. The extracellular ligand-binding domain of BMPR-II adopts a precisely folded conformation, exquisitely dependent on the formation of 5 disulphide bridges by 10 cysteine residues dispersed across exons 2 and 3, invariant in the majority of type II receptors (44). Amino acid substitutions in this domain are common, particularly at the cysteine residues, with 17 of 22 independent cysteine mutations affecting 8 of the 10 conserved residues. Subcellular analysis of all tested cysteine mutant constructs demonstrates substantial cytosolic retention, likely to be due to a profound loss of conformational integrity (45,46).

The BMPR-II catalytic domain shares the fundamental structural and functional characteristics of members of the extensive eukaryotic protein kinase superfamily. The kinase region is compartmentalized into 12 subdomains of variable importance to the processes of adenosine triphosphate binding, substrate recognition, and phosphate group transfer (47). However, dispersed across the subdomains are 12 critical, highly conserved, amino acid residues. Missense mutations are typically restricted to regions crucial to kinase activity, as best exemplified by substitution of the native arginine residue at the invariant position 491, a site of frequent and recurrent mutation in PAH. Disruption of the key structural interaction between this residue and a glutamic acid at position 386 effectively renders the receptor kinase inactive. Whereas noncysteine substitutions traffic normally to the cell surface, all kinase mutations occurring in these catalytically important domains display a near complete abolition of signaling through the Smad pathway (45,46).

Variants of Unknown Significance in PAH

Amino acid substitutions are seldom observed in the cytoplasmic domain of BMPR-II (7 of 298, 2%), in contrast to truncating mutations, which account for approximately 15% of the overall mutation load in PAH. Furthermore, missense mutations in this region are functionally distinct from classical PAH-causing defects, as they are associated with normal levels of Smad activation (45,46). Instead, in transient transfection-based assays, disease-specific substitutions of the cytoplasmic tail appear to constitutively activate p38MAPK and impede phosphorylation of the dynein light chain Tctex-1 (32,46). Thus, this domain of the receptor is significant in relaying receptor signal through Smad-independent pathways. The relevance of this finding to disease pathogenesis is currently under investigation.

The mutation spectrum in PAH associated with other conditions or acknowledged risk factors, for example, fenfluramine use, is distinct from the mutation spectrum in hereditary/idiopathic disease (35). PAH patients with fenfluramine exposure exclusively harbor missense mutations, in comparison to only one-third of patients with HPAH. Moreover, the substitutions occur at positions not recognized as having a major impact on receptor function. For example, none of the 5 reported variants in the extracellular domain observed in patients with PAH and congenital heart

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Table 1 Pathogenic *BMPR2* Mutations in PAH

Location	Mutation Category	Domain	Nucleotide Change	Amino Acid Change	Frequency in This Study	Comment	Reference
5′ UTR	Missense		c.*-944/5GC>AT		1	H	Aldred et al. (36)
5′ UTR and Exon 1	Deletion		c.?_-540_76+?del		1	H	This analysis
Exon 1	Missense		c.28C>T	p.T10W	1	I	Baloira et al. (37)
	Nonsense		c.71C>A	p.A24E	1	I	This analysis
Intron 1	Splice-site		c.76+5G>A		1	I	This analysis
Exon 2	Nonsense	ECD	c.124C>T	p.Q42X	1	I	Fujiwara et al. (38)
	Frameshift	ECD	c.186insTACC	p.G63fsX1	1	H	This analysis
	Frameshift	ECD	c.189_207delins14	p.S64EfsX32	1	ASD/PAH	This analysis
	Deletion	ECD	c.189-209del21	p.del6470(STCYGLW)	1	H	This analysis
	Nonsense	ECD	c.201T>G	p.Y67X	1	H	This analysis
	Missense	ECD	c.247G>A	p.G83R	1	I	This analysis
Exons 1–4	Deletion	ECD	c.1-?_419+?del		1	I	This analysis
Exons 1–8	Deletion	ECD	c.1-?_1128+?del		1	I	This analysis
Intron 2	Splice-site	ECD	c.247+1delCAAGTG		1	H	This analysis
	Splice-site	ECD	c.247+1_+4 delGTAA	p.C84_S140del	1	I	This analysis
	Splice-site	ECD	c.248-2A>G		1	H	This analysis
	Splice-site	ECD	c. 248-5 delTATAGGinsAC		1	H	This analysis
Exon 3	Missense	ECD	c.248G>A	p.G83E	1	H	This analysis
	Missense	ECD	c.250T>C	p.C84R	1	H	This analysis
	Missense	ECD	c.250T>G	p.C84G	1	I	This analysis
	Frameshift	ECD	c.261insA	p.87fsX9	1	H	This analysis
	Missense	ECD	c.295T>C	p.C99R	1	H	This analysis
	Missense	ECD	c.296G>T	p.C99F	1	I	This analysis
	Missense	ECD	c.296G>A	p.C99Y	1	H	This analysis
	Nonsense	ECD	c.339C>A	p.Y113X	1	I	This analysis
	Nonsense	ECD	c.339C>G	p.Y113X	1	I	Fujiwara et al. (38)
	Missense	ECD	c.350G>A	p.C117Y	1	H	This analysis
	Nonsense	ECD	c.354-355TA>AG	p.C118X	1	H	This analysis
	Missense	ECD	c.367T>C	p.C123R	1	I	Fujiwara et al. (38)
	Missense	ECD	c.370A>G	p.N124D	1	I	This analysis
	Missense	ECD	c.377A>G	p.N126S	1	I	This analysis
	Deletion	ECD	c.248-?_418+?del		2	H,H	This analysis
Exon 2–3	Deletion	ECD	c.77-?_418+?del		5	H,H,H,H,I	This analysis
Intron 3	Splice-site	ECD	c.418+1G>C		1	H	This analysis
	Splice-site	ECD	c.418+5G>A		2	H	This analysis
Exons 4–5	Deletion	ECD	c.419-?_621+?del		2	H,I	This analysis
Exon 5	Nonsense	TM	c.583G>T	p.E195X	1	PVOD	This analysis
	Missense	TM	c.604A>T	p.N202Y	1	PVOD	This analysis
Exon 6	Frameshift	KD	c.612delA	p.L204fsX5	1	H	This analysis
	Nonsense	KD	c.631C>T	p.R211X	2	H	This analysis
	Frameshift	KD	c.660insG	p.G220fsX4	1	NK	This analysis
	Frameshift	KD	c.690-691delAGinsT	p.K239fsX21	1	H	This analysis
	Missense	KD	c.794A>G	p.E265G	1	I	This analysis
	Missense	KD	c.797G>C	p.R266T	1	NK	This analysis
	Missense	KD	c.806G>T	p.A268V	1	I	Baloira et al. (37)
Intron 6	Splice-site	KD	c.852+1G>C		1	I	This analysis
	Splice-site	KD	c.853-1G>C		1	H	This analysis
Exon 7	Nonsense	KD	c.928A>T	p.R310X	1	I	This analysis
	Missense	KD	c.932G>A	p.G311E	1	H	This analysis
Exon 8	Missense	KD	c.1019T>C	p.L340P	1	H	This analysis
	Nonsense	KD	c.1189-1190delTG	p.C347X	1	H	This analysis
	Missense	KD	c.1042G>A	p.V348E	1	H	This analysis
	Frameshift	KD	c.1095delC	p.R365fsX8	1	H	This analysis
	Frameshift	KD	c.1099-1103delGGGGA	p.E368fsX1	1	H	This analysis
	Deletion	KD	c.968-?_1129+?del		1	H	This analysis

Continued on next page

Table 1 Continued

Location	Mutation Category	Domain	Nucleotide Change	Amino Acid Change	Frequency in This Study	Comment	Reference
Intron 8	Splice-site	KD	c.968-5A>G		1	H	This analysis
Exon 9	Missense	KD	c.1157A>T	p.E386V	1	H	Fu et al. (39)
	Missense	KD	c.1171G>A	p.A391T	1	I	This analysis
	Nonsense	KD	c.1207C>T	p.Q403X	1	H	Fujiwara et al. (38)
	Frameshift	KD	c.1214delA	p.D405fsX6	1	H	This analysis
	Nonsense	KD	c.1248-1251delATTT	p.F417X	1	H	This analysis
	Frameshift	KD	c.1271delTCCCAGinsCGGAGA	p.F424fsX10	1	H	This analysis
Intron 9	Splice-site	KD	c.1276+1G>A		1	H	This analysis
	Splice-site	KD	c.1277-9A>G		1	H	This analysis
Exon 10	Nonsense	KD	c.1297C>T	p.Q433X	2	I,NK	This analysis
	Frameshift	KD	c.1392delA	p.A465fsX9	1	H	This analysis
Exon 11	Missense	KD	c.1460A>T	p.D487V	1	H	This analysis
	Missense	KD	c.1472G>A	p.R491Q	1	I	This analysis
Exon 12	Missense	CD	c.1598A>G	p.H533R	1	I	This analysis
	Frameshift	CD	c.2410-2413delGTCA	p.V804fsX1	1	H	This analysis
	Frameshift	CD	c.2441-2442delAC	p.H814fsX2	1	H	This analysis
	Frameshift	CD	c.2504insA	p.T835fsX6	1	PAH+collagen disease	This analysis
	Frameshift	CD	c.2609del T	p.L870fsX1	1	I	This analysis
	Nonsense	CD	c.2626C>T	p.Q876X	1	H	This analysis
	Nonsense	CD	c.2695C>T	p.R899X	2	I	This analysis
	Nonsense	CD	c.2789C>G	p.S930X	2	I	This analysis
Exon 13	Missense	CD	c.2945A>G	p.L982R	1	H	This analysis

Numbering is based on +1 of the initiation methionine.

ASD = atrial-septal defect; CD = cytoplasmic domain; ECD = extracellular domain; F = familial; I = idiopathic; KD = kinase domain; NK = not known; PAH = pulmonary arterial hypertension; PVOD = pulmonary veno-occlusive disease; TM = transmembrane; UTR = untranslated region.

disease or associated with appetite suppressant intake alter cysteine residues, and 2 residues altered in exon 5 encode a receptor region of no known functional significance (13,20,47). Significantly, a recent in vitro study of this mutation series has indicated that the signaling capacity of receptors harboring atypical variation approaches physiologic levels (42). Akin to the cytoplasmic tail substitutions, the relationship of these variants to the etiology of PAH is difficult to assess. A possible interpretation may be that these alleles produce PAH in the setting of additional genetic or environmental risk factors.

Rare Disease Alleles Underlying PAH

HHT is an autosomal dominant vascular disorder characterized by the appearance of cutaneous telangiectasias and arteriovenous malformations. The disease is caused by pathogenic mutations of either the TGF-β type I receptor ALK-I or accessory receptor endoglin (ENG) (Table 2) (15,27,48,49), and, rarely, Smad 4, which is also associated with juvenile polyposis. A small proportion of HHT patients have PAH that is clinically and histopathologically indistinguishable from other heritable forms of PAH, whereas others have PAH due to pulmonary arteriovenous fistulas (27). The underlying causative factor in these patients is, typically, mutations of *ALK-1*. Up to 20% (16 of 83) of all detected mutations in *ALK-1* are associated with the development of PAH, and of these, 81% (13 of 16) are consistently observed with PAH (50,51). The majority of these defects comprise missense mutations and cluster in functional domains of the receptor, namely the kinase domain and NANDOR box. In rare instances, mutations of *ALK-1* (n = 9) appear to cause IPAH or HPAH without HHT (Table 3) (27,38,48). Of interest, 4 mutations are confined to 2 discrete positions (amino acids 479 and 484)

Table 2 *ENG* Mutations Underlying PAH

Location	Domain	PAH Classification	Nucleotide Change	Amino Acid Change	Reference
Exon 5	ECD	PAH+HHT	c.682-686del	p.S228fsX102	Harrison et al. (27)
Exon 10	ECD	PAH+HHT	c.1334delT	p.M445fsX44	Harrison et al. (27)
Exon 11	ECD	PAH+HHT+dexfenfluramine	c.del410G	p.Q470fsX20	Chaouat et al. (15)
Intron 12	ECD	PAH+HHT	c.1429-22T>C		Harrison et al. (48) Mache et al. (49)

Numbering is based on +1 of the initiation methionine of ALK1.

HHT = hereditary hemorrhagic telangiectasia; other abbreviations as in Table 1.

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Table 3 *ALK-1* Mutations Underlying PAH

Location	Domain	PAH Classification	Nucleotide Change	Amino Acid Change	Age at Onset	Reference
Exon 2	ECD	IPAH	c.430C>T	p.R144X	NK	This analysis
Exon 5	ECD	IPAH	c.536A>C	p.D179A	51 yrs	Harrison et al. (27)
Exon 7	KD	HPAH	c.936C>G	p.H312Q	14 yrs	Fujiwara et al. (38)
Exon 8	KD	IPAH	c.1142T>C	p.L381P	9 yrs	Fujiwara et al. (38)
Exon 9	KD	HPAH	c.1270C>A	p.P424T	7 yrs	Fujiwara et al. (38)
Exon 10	KD	IPAH	c.1436G>A	p.R479Q	7 yrs	Fujiwara et al. (38)
	KD	IPAH	c.1436G>C	p.R479P	NK	This analysis
	KD	IPAH	c.1451G>A	p.R484Q	18 months	Harrison et al. (27)
	KD	HPAH	c.1451G>A	p.R484Q	2 yrs	Fujiwara et al. (38)

Numbering is based on +1 of the initiation methionine of ALK1.
IPAH = idiopathic pulmonary arterial hypertension; other abbreviations as in Tables 1 and 2.

of the kinase domain, suggesting that the native residues, both arginines, may be important for maintenance of the pulmonary architecture (45). However, an important caveat is that these patients usually present with early-onset disease and may go on to develop HHT at a later stage (38,48).

Genetic Testing for PAH

Clinical genetic testing is available for PAH for *BMPR2*, *ALK1*, and *ENG*. In most cases, genetic analysis will commence with analysis of *BMPR2* unless there are specific clinical symptoms or family history to suggest HHT, such as mucocutaneous telangiectasias, recurrent epistaxis, gastrointestinal bleeding, or arteriovenous malformations in the pulmonary, hepatic, gastrointestinal, or cerebral circulations. Genetic testing may be offered to any individual with a family history of PAH or IPAH (without other known affected family members), and physicians may have a duty to inform these patients of the possibility that PAH could develop in other family members. It may be necessary to go back to stored blood or DNA if the only affected family member is deceased and if those materials are available for testing.

Evaluation of *BMPR2* should begin with full sequencing of all 13 exons, including splice junctions. If no mutation is identified by sequence analysis, further characterization for genomic deletions and rearrangements should be evaluated by an appropriate methodology, such as multiplex ligation-dependent probe amplification. Using these combined approaches, approximately 70% of HPAH patients will have identified mutations in *BMPR2* (36). Clinical genetic testing is available in North America and Europe, with the current cost of testing ranging from approximately U.S. $1,000 to $3,000 to analyze the first member of a family. Testing other family members for a family-specific mutation is U.S. $300 to $500. Genetic testing should involve pre-test and post-test genetic counseling, ideally with a genetic counselor experienced in pulmonary hypertension. As a consequence of the incomplete penetrance and variable age of onset, identification of a *BMPR2* mutation may have a complex and serious psychosocial impact on the family and is often associated with feelings of guilt in the parent who has passed on mutation to the children. Genetic testing is most helpful when it is able to identify members of the family who are not genetically at risk for PAH, and who can then forgo serial evaluation for detection of PAH.

The most common reasons that persons pursue genetic testing are to inform their children of their hereditary predisposition or to make informed decisions about family planning (52). In the past, many patients opted not to pursue genetic testing because of anxiety regarding genetic discrimination. Recognition of these concerns has led a number of countries to introduce either voluntary or legal codes to protect individuals requesting genetic counseling and formal testing. For example, in the U.S., the Genetic Information Nondiscrimination Act, passed in May 2008, protects members of both individual and group health insurance plans from discrimination in coverage or cost of health insurance coverage and also protects against discrimination in employment based upon a genetic predisposition (53). Genetic testing of children should be performed with caution, because of the potentially significant psychological impact on a child, particularly overt anxiety for the future development of a potentially fatal disease in the absence of currently known effective disease-prevention strategies.

Clinical Monitoring of Individuals at Risk

Clinical monitoring of patients with a family history of PAH or carriers of the *BMPR2* mutation has not been evaluated rigorously. Consideration has been given to annual clinical examination, echocardiogram, stress echocardiography, Doppler echocardiography during supine bicycle exercise, and right heart catheterization at rest and with exercise. The 1998 World Pulmonary Hypertension Conference suggested that first-degree relatives of known HPAH patients should be screened annually using clinical examination and echocardiography. It is hoped that with regular surveillance, individuals can be diagnosed earlier in their disease and benefit from early treatment. Although there are currently no data to suggest that early diagnosis

will improve the outcome, such studies are in progress (54,55).

Reproductive Planning

Prenatal testing is available for *BMPR2* mutations if a familial mutation has been identified. For a prospective parent carrying a known *BMPR2* mutation, there is a 50% risk of transmitting this mutation to any offspring (56). Prenatal testing can be performed by chorionic villus sampling as early as the 10th week of pregnancy. However, partly in consequence of the reduced penetrance of PAH disease mutations, in our experience, very few families have pursued this option. Additionally, pre-implantation genetic diagnosis is available, whereby families use in vitro fertilization, and genetic testing is performed on the embryo prior to embryo transfer. Only embryos without the familial *BMPR2* mutation are transferred to the uterus. This option may prove more appealing, particularly to women who are carriers of the *BMPR2* mutation and have been advised not to pursue a pregnancy because of the increased risk of developing PAH symptoms themselves. In such cases, in vitro fertilization is already required for surrogacy. However, pre-implantation genetic diagnosis with in vitro fertilization is costly, may not be covered by insurance in the U.S., and is not available in all European countries. Pre-implantation genetic diagnosis has some associated diagnostic errors (57).

Identifying Novel, Highly Penetrant Genes for HPAH

A small percentage of HPAH families have multiple affected individuals but do not have identified mutations in *BMPR2*, despite full sequencing of exons and splice junctions and testing for genomic alterations (9–12). Although many of these families have limited numbers of living affected family members available for research, HPAH in these families may be due to locus or allelic heterogeneity that could be identified by sequencing of candidate genes, or in rare cases, analyzed by linkage if sufficient numbers of affected family members are available. As our ability to cost-effectively sequence and interpret greater amounts of DNA grows, we should be able to analyze the *BMPR2* mutation-negative HPAH cases for mutations in the whole *BMPR2* genomic locus and for other physiologic candidate genes.

Genetic Modifiers of Risk for PAH

The complex clinical features of PAH, including variable age of disease onset both within and between families and sex-dependent penetrance, imply the existence of additional factors capable of modulating disease susceptibility (58). Moreover, the likely existence of environmental modifiers is highlighted by our observation of at least 7 pairs of monozygotic twins discordant for disease (R. C. Trembath, personal communication, September 2008). To date, a number of studies examining the contribution of variations in candidate genes considered to play a biological role in the etiology of PAH have been conducted (40,59–68). However, all the case/control analyses suffer from critically small sample cohorts that would be unlikely to identify genetic modifiers of moderate effect size. Further, validation through independent replication studies, a crucial second stage of association analyses, has been lacking in most of the studies performed to date (Table 4) (35,59–64,66–68).

Genome-Wide Association (GWA) Analysis in IPAH

Driven by the HapMap project (69) and the development of genotyping platforms facilitating analysis of several hundred thousand independent loci, an understanding of the human haplotypic architecture of sequence variation and the identification of loci conferring even modest risks for disease are now feasible. To achieve the power required to conduct a GWA study and to detect signals of modest genotypic relative risk (GRR), large numbers of cases and controls and

Table 4 Candidate Gene Association Studies Conducted in PAH

Gene	PAH Classification	Patient Sample (n)	Control Sample (n)	Association (Patient/Control Frequency)	Significance (p Value)	Reference
ACE	Not specified	60	158	DD genotype (0.45/0.28)	0.01	Abraham et al. (59)
	Not specified	51	200	No association	N/A	Hoeper et al. (63)
	Hypoxic	48	30	I allele (0.67/0.38)	0.003	Aldashev et al. (60)
5-HTT	PAH+appetite suppressants	89	84	II genotype (0.65/0.27)	<0.001	Eddahibi et al. (62)
	IPAH/CTEPH	74/35	Unknown	No association	N/A	Koehler et al (64)
	HPAH/IPAH/APAH	133/259/136	253	No association	N/A	Machado et al. (35)
	HPAH/IPAH	166/83	125	II genotype with early onset in FPAH	<0.02	Willers et al. (67)
Endoglin	PAH+systemic sclerosis	23	140	6bINS (0.11/0.24)	<0.01	Wipff et al. (68)
PGIS	CTEPH	90	144	No association	N/A	Amano et al. (61)
Kv1.5	IPAH	NO responders 42	NO nonresponders 37	SNP4 a allele; SNP17 a allele (0.07/0.23; 0.016/0.06)	0.01/0.05	Remillard et al. (66)

ACE = angiotensin converting enzyme; APAH = associated pulmonary arterial hypertension; CTEPH = chronic thromboembolic pulmonary hypertension; D = deletion; HPAH = heritable pulmonary arterial hypertension; HTT = hydroxy-tryptamine (serotonin) transporter; I = insertion; Kv1.5 = potassium channel subunit 1.5; NO = nitric oxide; *PGIS* = prostacyclin synthase gene; PH = pulmonary hypertension; other abbreviations as in Tables 1 and 3.

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international multicenter collaboration will be required (70,71).

GWA Study Design in IPAH

A staged approach will be taken, using a common genotyping platform and concurrent screens. This will provide a dataset of >1,000 cases and ≤3,000 controls. All patients will be screened for mutations in *BMPR2* and *ALK-1,* and mutation carriers will be removed from the analysis. Data from the 2 screens may be combined in a meta-analysis (72). Based on power calculation simulations, these numbers are considered sufficient to detect variants of moderate effect size (GRR 1.3). The second stage of the study will be independent replication by a GWA performed on a combined cohort of North American IPAH cases and controls. Again, the datasets will be combined in a meta-analysis, and loci displaying significant association across the 3 studies will be chosen for further interrogation. In this way, the false-positive report rate is anticipated to be limited and the likelihood of true associations with IPAH increased (73).

Publicly available data from the HapMap consortium on the extent of linkage disequilibrium in regions displaying positive signals will be used to fine-map associated loci and establish a panel of tagging single nucleotide polymorphisms. Where strong association is identified with low-frequency haplotypes, candidate genes will be resequenced to capture rare, potentially causal variants. All potentially functional single nucleotide polymorphisms will subsequently be analyzed in all patient and control cohorts. A range of well-established statistical tools, including conditional logistic regression, will be used to identify those variants most likely to be true susceptibility alleles.

Modifiers of *BMPR2* Mutation in Heritable Disease

The major PAH centers worldwide have access to at least 250 kindreds with identified *BMPR2* mutations. Risk alleles from the IPAH GWA study will be assessed in affected probands from these families by comparison to age- and sex-matched unaffected mutation carriers to determine whether, in the framework of *BMPR2* mutation, they act as modifiers in familial disease as well as IPAH.

Future Research

Identification of a substantial number of genetically at-risk individuals offers the potential to develop an extremely valuable resource for future studies, including assessment of the natural history of PAH, development of biomarkers for disease onset and progression, the identification of environmental and genetic modifiers, and the opportunity to test methods for primary prevention. Biomarker development will benefit from advances in expression profiling, proteomics, and metabolomics, by longitudinally testing individuals at increased genetic risk. Interestingly, the largest number of genetically at-risk individuals is within families of patients currently diagnosed with IPAH, given the relatively higher prevalence of this form of PAH. Through clinical genetic testing programs or by enrollment in research programs, such kindreds will provide an invaluable resource for studying PAH and potentially identifying preventive measures. Clearly these types of ambitious studies will require large numbers of patients as well as collaboration among multiple centers around the world. However, such a cohort of asymptomatic *BMPR2* mutation carriers is likely to provide one of the most powerful resources for an in-depth understanding of PAH pathogenesis and prospects for prevention.

Author Disclosures

Dr. Elliott is employed by Intermountain Healthcare. Intermountain Healthcare has filed a patent based on Dr. Elliott's invention for the use of BMPR2 mutation analysis to assess vasoreactivity in pulmonary arterial hypertension. Intermountain Healthcare, with Dr. Elliott as Principal Investigator, has received grant support from Actelion, Pfizer, Encysive, CoTherix, and United Therapeutics. Dr. Elliott serves as a member of the Registry to Evaluate Early and Long Term PAH Disease Management sponsored by Actelion. Drs. Machado, Eickelberg, Geraci, Hanaoka, Loyd, Newman, Phillips, Soubrier, Trembath, and Chung report no conflicts of interest.

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