Published in final edited form as:

Semin Respir Crit Care Med. 2013 October ; 34(5): 568–580. doi:10.1055/s-0033-1355443.

HERITABLE FORMS OF PULMONARY ARTERIAL HYPERTENSION

Eric D. Austin, MD, MSCI and

Department of Pediatrics, Division of Pulmonary, Allergy, and Immunology Medicine, Vanderbilt University School of Medicine, MCN DD-2205, Nashville, TN 37232-2578, Office phone: 615.343.7617, Fax: 615.343.9951

James E. Loyd, MD

Department of Medicine, Division of Pulmonary, Allergy, and Critical Care Medicine, 1161 21st Avenue South, T-1218 MCN, Vanderbilt University School of Medicine, Nashville, TN 37232-2578, Office phone: 615.322.3412, Fax: 615.343.7448

Eric D. Austin: Eric.austin@vanderbilt.edu; James E. Loyd: Jim.loyd@vanderbilt.edu

Abstract

Tremendous progress has been made in understanding the genetics of heritable pulmonary arterial hypertension (HPAH) since its description in the 1950s. Germline mutations in the gene coding bone morphogenetic receptor type 2 (*BMPR2*) are detectable in the majority of cases of HPAH, and in a small proportion of cases of idiopathic pulmonary arterial hypertension (IPAH). Recent advancements in gene sequencing methods have facilitated the discovery of additional genes with mutations among those with and without familial PAH (*CAVI*, *KCNK3*). HPAH is an autosomal dominant disease characterized by reduced penetrance, variable expressivity, and female predominance. These characteristics suggest that genetic and non-genetic factors modify disease expression, highlighting areas of active investigation. The reduced penetrance makes genetic counseling complex, as the majority of carriers of PAH-related mutations will never be diagnosed with the disease. This issue is increasingly important, as clinical testing for *BMPR2* and other mutations is now available for the evaluation of patients and their at risk kin. The possibilities to avoid mutation transmission, such as the rapidly advancing field of pre-implantation genetic testing, highlights the need for all clinicians to understand the genetic features of PAH risk.

Keywords

bone morphogenetic protein receptor type 2; caveolin-1; *KCNK3*; *CBLN2*; pulmonary arterial hypertension; genetics; modifiers; hereditary hemorrhagic telangiectasia

BACKGROUND

Pulmonary arterial hypertension (PAH) was first described by Dresdale et al in 1951 as a sporadic disease entity known as primary pulmonary hypertension (PPH).¹ In 1954, Dresdale et al described the familial transmission of PPH in a kindred, thus providing the

first known reports of familial PAH (FPAH), which is a heritable condition.² In the wake of years of genetic research, it is now recognized that PAH comes in many forms, including idiopathic (PAH), heritable (HPAH), and associated with drug exposures (such as anorectic fenfluramine compounds) or other medical conditions.³ As described below, HPAH may occur as a known familial disease, or in a patient determined to have IPAH with a known genetic variant associated with PAH.

In the 30 years following the initial description of PAH in families, reports of additional families with PAH appeared periodically in the literature, resulting in a total of 13 U.S. families reported by 1984. A follow up analysis of these 13 families in 1984 revealed an additional 8 new cases, as well as the description of a new family located in Tennessee. This 14th family contained the largest number of affected family members described to date, with six deaths due to pulmonary hypertension during two generations.⁴ Analysis of those 14 families showed vertical transmission with male to male transmission that proved an autosomal dominant mode of inheritance. Over time, it also became clear that a proportion of cases of pulmonary hypertension which appeared to occur as 'sporadic' disease had a familial basis that was not obvious due to the reduced penetrance that allowed some carriers to remain unaffected yet able to silently pass their genetic susceptibility (gene mutation) to their offspring. In fact, the current international classification scheme, which incorporates the label HPAH, does so at least in part to recognize the fact that approximately 20% of cases previously thought to be idiopathic (IPAH) harbour identifiable mutations and therefore pose a hereditary risk to other family members.^{5,6} It is notable that while genetic mutations are appropriately linked to FPAH, due to the higher prevalence of IPAH compared to FPAH the majority of PAH patients with heritable disease are patients labeled IPAH at diagnosis.

DETERMINING THE MAJOR GENE ASSOCIATED WITH HPAH

In the mid to late 1980s, the National Institutes of Health (NIH) prospective registry of PPH provided the foundation for the clinical definition of PAH, and facilitated interaction of participating investigators to collect and organize sufficient numbers of families to provide robust statistical power for a genome wide search for HPAH loci.⁷ Independently, two teams of investigators mapped the locus for the gene, named *PPH1*, for HPAH to chromosome 2q31-32 in 1997.^{8,9} Subsequently, both teams also demonstrated that germline mutations in the gene encoding bone morphogenetic protein receptor type-2 (*BMPR2*), a TGF- β superfamily of receptors member, was the gene responsible for the majority of cases of the autosomal dominant familial disease now known as HPAH.^{10,11}

It is currently recognized that not all families (~20%) with PAH have mutations identified in *BMPR2* despite comprehensive testing. Not surprisingly, the identification of *BMPR2* mutations highlighted the potential relevance of the TGF- β Superfamily signalling pathway overall to PAH pathogenesis. Furthermore, in rare instances HPAH may present in association with hereditary hemorrhagic telangiectasia (HHT), a disease caused by mutations in Activin-like Kinase Type 1 (*ALK1*) and Endoglin (*ENG*)—the products of both of these genes are also members of the TGF- β superfamily of receptors.^{12,13} However, as discussed below, the inability of *BMPR2* to account for all cases of HPAH has prompted

intense investigation for alternative loci which may represent sites of rare variants (mutations) that associate strongly with PAH.

TRANSMISSION PATTERNS AND CLINICAL EXPRESSION OF HPAH

Penetrance refers to the frequency with which a specific genotype (e.g., a *BMPR2* gene mutation) results in the expression of a specific trait (phenotype; e.g., HPAH) by the individual with that genotype. As with some other genetic diseases, the penetrance of a *BMPR2* mutation is reduced. Reduced penetrance of *BMPR2* germline mutations can make establishing familial transmission difficult since generations of mutation-carrying individuals may not express disease.^{4,14} Thus, only about 27% of individuals with a known genetic mutation in *BMPR2* will develop detectable PAH. However, reduced penetrance appears to be a feature of all forms of HPAH, regardless of *BMPR2* gene mutation or not. In addition, expressivity of the disease (variations in the phenotype among those with a given genotype and disease penetrance), is variable. For example, the age of onset can occur at any age, and while most die within 1–5 years of diagnosis without therapy some HPAH patients do survive for many years. While not a feature specific to the inheritable form of PAH, female predominance (~ 2:1 female: male ratio) is another major feature of HPAH.¹⁵ A typical family with PAH is represented in Figure 1.

A notable development in the study of HPAH is the recent reversal of a prior concept among PAH investigators, including our group at Vanderbilt. Based upon several studies showing progressively earlier age of onset of HPAH in subsequent generations, a phenomenon known as genetic anticipation was suspected to occur among families despite a lack of biologic explanation.^{14,16–18} While not definitively disproven, Larkin and colleagues recently performed linear mixed effects models and limited time-truncation bias by restricting the date of birth to analyze our HPAH families at Vanderbilt for genetic anticipation. This more rigid analytic approach demonstrated no evidence for genetic anticipation.¹⁹ While not absolutely dismissed, there is thus no current evidence to support genetic anticipation as a global feature of HPAH.

Reduced Penetrance

Mutant *BMPR2* alleles with reduced penetrance segregate in HPAH kindreds afflicted by this autosomal dominant disease, indicating that heterozygosity for a mutation is required but is not sufficient to cause clinical expression of HPAH in most cases.²⁰ To date, the mechanisms which reduce penetrance are unknown, but are likely to include additional genetic and/or environmental modifiers of disease expression.²¹ Discovery of the factors which modify disease penetrance are an area of active investigation, given the potential to impact not only HPAH, but perhaps other forms of PAH as well.

At Vanderbilt University, we follow a research cohort of nearly 200 families with HPAH, each with reduced *BMPR2* penetrance. Larkin et al recently analyzed our largest 53 families with PAH. To estimate penetrance, 1,683 total siblings at risk, of whom 842 were likely to carry a mutation, were studied. Of 232 total PAH patients, there were 177 females and 59 males. The overall penetrance in this study cohort was 27%, although it was different

according to gender. Specifically, the female penetrance was 42%, while the male penetrance was 14%.¹⁹

Skewed gender ratio

Since the 1950s, it has been known that 2 or more females are diagnosed for every male. In fact, aside from a *BMPR2* mutation, gender is the most reliable determinant of HPAH, as suggested by the differential penetrance according to gender noted above. Whether the female predominance reflects increased risk for females or decreased risk for males remains unclear, although recent work has provided some clues in this regard, particularly in terms of sex hormone variations among *BMPR2* mutation carriers with HPAH compared to healthy *BMPR2* mutation carriers. Understanding the mechanism(s) by which gender modulates disease is an area of active investigation, as it may one day improve diagnosis and therapy of all forms of PAH.

The connection of sex hormones to HPAH, and other forms of PAH, is certainly not a new idea. Pregnancy, a time of female sex hormone surge, and iatrogenic female sex hormone exposures are acknowledged risks for all types of PAH patients and supported by several reports.^{22–24} However, recent data from humans with HPAH has suggested more advanced genotype-phenotype considerations with regard to sex hormones and *BMPR2* mutation carriers.

Initially, using an unbiased gene expression array to study immortalized lymphocytes from female *BMPR2* mutation carriers, West et al detected reduced expression (10-fold lower) of the *CYP1B1* gene among female *BMPR2* mutation carriers with HPAH compared to those who did not have HPAH.²⁵ The critical role of CYP1B1 in the metabolism of estrogens prompted a follow up study, which demonstrated that among females with a *BMPR2* mutation, common variants in *CYP1B1* that cause reduced CYP1B1 activity associated strongly with HPAH; furthermore, study of a nested cohort of those women with HPAH demonstrated a urinary estrogen metabolite breakdown consistent with the hypothesis that female *BMPR2* mutants with HPAH metabolize estrogen into the more estrogenically active 16 α -hydroxyestrone compound more so than unaffected *BMPR2* mutation carriers.²⁶

Variable expressivity

The variable age of onset of HPAH, which can affect individuals at any age from early childhood to late adulthood, reflects the variable expressivity of disease.²⁷ While there is a paucity of data convincingly outlining the impact of *BMPR2* genotype upon clinical outcomes and therapeutic response in PAH, this is beginning to change. For example, Sztrymf et al reported more severe disease among patients with a *BMPR2* mutation. While ascertainment bias of family members could account for the 10 year difference in age at diagnosis reported, the majority of subjects with a *BMPR2* mutation (59%) had no known family history to prompt earlier clinical evaluation. In addition, the age at death was approximately 15 years younger among *BMPR2* mutation carriers (Figure 2). Finally, compared to patients without a mutation, *BMPR2* mutation carriers had more severe hemodynamic compromise at diagnosis, including higher mean pulmonary artery pressure,

lower cardiac index, and higher pulmonary vascular resistance. Interestingly, *BMPR2* mutation status did not appear to impact survival.²⁸

While the study by Sztrymf et al did not demonstrate a survival difference based upon mutation status, there are attempts to use *BMPR2* mutation type and status to select therapy in HPAH. Because a small proportion of PAH patients have good long term response to calcium channel blockers, Elliott et al sought to compare the results of vasoreactivity testing according to *BMPR2* mutation type.^{29,30} This retrospective study demonstrated that compared to patients without a *BMPR2* mutation, patients with nonsynonymous *BMPR2* mutations were nearly 9 times less likely to demonstrate vasoreactivity by standard testing (vasoreactivity in 3.7% of 27 patients with nonsynonymous *BMPR2* variations versus 35% of 40 patients without mutation (P=0.003)).³¹ Rosenzweig et al found similar results, as well as worse hemodynamic parameters consistent with those seen in the French report, in a cohort of adult and pediatric patients with PAH and a *BMPR2* mutation.³²

MUTATIONS (RARE VARIANTS) IN THE TGF- β SIGNALING SUPERFAMILY AND HPAH

While the discovery that *BMPR2* mutations constitute the genetic basis of the majority of cases of HPAH was a great step forward in the understanding of this disease, a complete genetic and molecular understanding remains elusive, although great progress has been made in the past decade.³³ For example, while it is the most frequently found mutation in HPAH, the precise manner in which *BMPR2* dysfunction or deficiency causes PAH remains unclear. In addition, the *BMPR2* gene is not the only locus at which a detectable mutation results in phenotypic expression of PAH—several new loci have been recently identified. While much work remains to be done on *BMPR2* and HPAH, the discovery of novel loci has brought tremendous opportunity to expand our knowledge about the pathogenesis of HPAH.

The initial discovery of *BMPR2* gene mutations was made using microsatellite markers and linkage analyses, which focused the search for the gene ultimately identified as *BMPR2* to chromosome 2, in the region of q31-33.^{8,34,35} Subsequently, a positional candidate gene approach identified *BMPR2*, which is a member of the transforming growth factor beta (TGF- β) superfamily of receptors, as the responsible gene.³⁶ Germline mutations in this large gene (~192 kb), which resides on chromosome 2q33 and spans 13 exons cause HPAH in most affected kindreds.^{10,11} The translation of a *BMPR2* transcript generates a polypeptide, composed of four distinct functional domains, of 1,038 amino acids in humans.³⁷ The *BMPR2* protein is a constitutively active serine-threonine receptor kinase whose downstream signaling has profound effects on developmental processes, including vasculogenesis.

Because the TGF- β superfamily of receptors contains many proteins encoded by many genes which signal in a variety of ways including via SMAD protein signaling, additional candidate genes exist which may associate with PAH. However, to date, germline genetic mutations in those genes have not been widely described among families with HPAH, or among those with IPAH. One exception to this is the *SMAD9* gene, which is located on chromosome 13. A *SMAD9* gene, which encodes a Smad8 protein, was recently found to be

mutated in one patient with sporadic PAH.³⁸ This discovery makes sense given the importance of TGF- β signaling to PAH, although additional cases have yet to be reported.

Mutations at two additional loci directly related to the TGF- β superfamily can cause a PAH phenotype as well, although this form of PAH results in conjunction with a larger heritable disease known as Hereditary Hemorrhagic Telangiectasia (HHT). HHT is a vascular dysplasia characterized by mucocutaneous telangiectasias, recurrent epistaxis and gastrointestinal bleeding, as well as arteriovenous malformations of the pulmonary, hepatic, and cerebral circulations. Mutations in additional components of the TGF- β signaling superfamily receptor complex, activin receptor-like kinase 1 (*ALK1*) located on Chromosome 12 and endoglin (*ENG*) on Chromosome 9, contribute to the pathogenesis of HHT.^{39,40} Pulmonary hypertension can occur in HHT, but typically this is a secondary form related to a high cardiac output state (not 'classic' PAH). However, PAH that is clinically and histologically identical to HPAH and IPAH has recently been described in multiple unique kindreds with HHT associated with detectable mutations in *ALK1*, implicating *ALK1* as an additional locus for HPAH.^{41,42} Interestingly, an *ENG* germline mutation was recently described in a patient with HHT and PAH that was associated with dexfenfluramine exposure, a genetic-environmental interaction which has also been described in *BMPR2* mutation carriers.⁴³ As has also been described for *BMPR2* mutation carriers, investigators recently reported a father and his child with an *ENG* mutation resulting in abnormal transcript splicing causing markedly reduced levels, suggesting haploinsufficiency leading to disease expression; while he himself was unaffected, the man's child was affected with PAH as a youth.⁴⁴

The locus heterogeneity identified in genes that all encode members of the TGF- β signaling pathway in patients with HHT and PAH suggests that defects in this pathway can provoke pulmonary vascular disease. While no direct interaction between the gene products of the *BMPR2*, *ALK1* or *ENG* genes is known, and these receptors do not appear to share activating ligands, the receptors all signal intracellularly via the Smad family of co-activators.^{45,46} In this manner, the report of a *SMAD9* mutation is not surprising. Although the exact mechanisms have yet to be elucidated, it is evident that variations at different genetic loci that signal via the TGF- β superfamily of receptors can result in a similar phenotypic expression, and that a better understanding of this signaling will improve understanding of HPAH.

MUTATIONS (RARE VARIANTS) IN ADDITIONAL GENES NOT IN THE TGF- β SIGNALING SUPERFAMILY AND HPAH

While TGF- β gene mutations (rare variants), especially in the *BMPR2* gene, are present in the majority of families with HPAH, some families have clear autosomal dominant transmission of disease but no detectable TGF- β germline mutations. With the advent of next generation sequencing platforms, it is now possible to perform a broad, unbiased, evaluation of the exome (and genome) to search for additional rare variants (genetic mutations) of strong effect which strongly associate with human disease.⁴⁷ This approach is now being actively applied to study both HPAH and IPAH cases for the discovery of novel loci of high impact among those without detectable PAH-associated mutations.

We recently studied four HPAH patients from a large family without detectable genetic mutations in the TGF- β pathway by whole-exome sequencing as a discovery approach to uncover novel gene mutations associated with HPAH. Analysis was performed to identify the single nucleotide and insertion and deletion variants shared among the four individuals using BWA (Burrows-Wheeler Aligner)⁴⁸ and SAMtools⁴⁹ and screened using dbSNP build (129) on the human assembly hg18, SIFT, and the 1000 Genomes Project to remove known variants in the human population. Ultimately, we identified one specific shared gene mutation also present in a 5th HPAH patient in that family, which was felt to represent a rare variant of significance to HPAH development in that family. The mutation was in the Caveolin-1 (*CAVI*) gene, at a highly conserved region with high likelihood of detrimental functional consequences. Subsequent evaluation of an additional 62 unrelated HPAH and 198 IPAH patients (all without detectable *BMPR2* mutations) uncovered the independent finding of a *de novo* *CAVI* mutation in an unrelated child with IPAH—that child's mutation was nearly identical to that found in the initial family (both frameshift mutations in exon 3: c.474delA (P158P fsX22) and c.473delC (P158H fsX22)). Those variants were genotyped in 1000 ethnically-matched Caucasian, European controls and were not identified in any healthy individuals, supporting the association with PAH.⁵⁰

While *CAVI* mutations appear to associate with PAH with reduced penetrance, the prevalence of mutation among those without a *BMPR2* mutation appears low: 1 out of 63 (1.6%) families with PAH tested and 1 out of 198 (0.5%) IPAH patients tested. However, the association of *CAVI* mutations with PAH has strong biologic plausibility. *CAVI* encodes caveolin-1, a membrane protein required to form flask-shaped invaginations of the plasma membrane known as caveolae that function in membrane trafficking, cell signaling, cholesterol homeostasis, and other crucial cellular processes.^{51–57} Although found in many cell types, caveolae are abundant in endothelial cells.^{58,59} Mice lacking caveolin-1 or the related protein caveolin-2 have airway and pulmonary vascular abnormalities, while expression of caveolin-1 in endothelial cells of caveolin-1 knock out mice rescues a number of these defects.^{60–65} In a possible link to *BMPR2*, caveolar function is important to multiple signaling pathways and cellular processes, including *BMPR2* and nitric oxide signaling.^{66–78} Finally, changes in the expression or localization of caveolin-1 have previously been reported to occur in PAH patients.^{79–82} Thus, the finding that *CAVI* is mutated in human PAH does not come as a surprise. But, how the *CAVI* mutation might directly contribute to HPAH pathogenesis in humans remains an area of intense investigation.

Not long after the discovery of *CAVI*, another novel gene was discovered using whole-exome sequencing. Again, a gene with strong biologic plausibility that is not part of the TGF β signaling pathway superfamily was identified in families with PAH and in IPAH patients. Using a similar approach to that taken with the *CAVI* discovery, Ma and Roman-Campos et al independently demonstrated mutations in the gene *KCNK3* (Potassium Channel, Subfamily K, Member 3), which encodes the human TASK-1 protein. Three unrelated PAH families and three IPAH patients contained different missense mutations in highly conserved regions of the gene. Each mutation was predicted to have damaging consequences, and all of the mutations identified did result in loss of function according to

electrophysiological studies. To date, the frequency of *KCNK3* mutations among those without detectable mutations in *BMPR2*, *ALK1*, *ENG*, and *CAVI* is higher among PAH families than data presented for *CAVI* (3.26% (3 out of 92) of PAH families and 1.32% (3 out of 228) of IPAH cases had detectable *KCNK3* mutations).⁸³

As with *CAVI* mutations, the biologic plausibility for *KCNK3* mutations is strong. TASK-1 is a pH sensitive potassium channel in the two pore domain superfamily.⁸⁴ Ion channels play a critical role in both vasoconstriction and vascular remodeling and have been an area of scientific interest among pulmonary hypertension researchers for many years. A complicated interplay between potassium and calcium ion channels helps to regulate membrane depolarization via calcium. Specifically, reduction in potassium channel expression promotes calcium influx to pulmonary vascular smooth muscle cells, promoting vasoconstriction. In contrast, enhanced potassium channel activity, as may occur with increased gene expression, causes pulmonary vascular vasodilation.⁸⁵ Thus, the discovery of *KCNK3* mutations in PAH is not surprising, and with time additional mutations in other ion channel related genes may be uncovered. An additional result of the *KCNK3* discovery is the prospect for novel therapeutics since pharmacological manipulation of currents through TASK-1 channels is possible.⁸⁶

Table 1 lists the currently known rare variants associated with familial PAH and IPAH patients.

THE BMP PATHWAY

BMPR2 is ubiquitously expressed and highly conserved throughout nature as a receptor for a family of cytokines known as bone morphogenetic proteins (BMPs). As members of the TGF- β superfamily of receptors, BMPs play a crucial role in the regulation of mammalian development. They are regulators of embryonic lung morphogenesis as well as bone and cartilage development.⁸⁷ Although genetic studies strongly implicate the TGF- β superfamily in the regulation of pulmonary vascular cell growth and differentiation, the precise molecular mechanisms involved are unclear.

It is clear that heterodimerization of the serine/threonine transmembrane kinases, *BMPR1* and *BMPR2*, is critical to *BMPR2* signaling. Four functional domains comprise *BMPR2*: ligand binding, kinase, transmembrane, and cytoplasmic domains. Activation of the heterodimeric *BMPR2/BMPR1* receptor complex leads to phosphorylation of a series of cytoplasmic mediators, which include the Smad family. Following phosphorylation, Smad proteins 1, 5, and 8 complex with Smad 4 for translocation into the nucleus to regulate target gene transcription in concert with specific nuclear repressors and cofactors.⁴⁵ In this manner, the Smad signaling pathway appears to participate in the inhibition of cell growth and the induction of apoptosis.⁸⁸ It is postulated that *BMPR2* mutations eliminate a critical growth regulatory function in pulmonary vascular cells by disrupting Smad activation, although other substrates related to BMP signaling have also been implicated.⁸⁹

PREVALENCE OF *BMPR2* MUTATIONS IN HERITABLE PAH

Despite locus heterogeneity, *BMPR2* mutations are responsible for PAH in the vast majority of HPAH, as well as some cases of PAH without family history. Germline *BMPR2* mutations appear to cause PAH regardless of ethnic group.^{90–92} A variety of testing methodologies have been used to detect *BMPR2* mutations, including sequencing of genomic DNA, Southern blot analysis, high-performance liquid chromatography, and melting curve analysis. The majority of mutations encode frameshift, nonsense, or splice site donor/acceptance site mutations.⁹³ Multiplex Ligation-dependent Probe Amplification (MLPA) analysis of genomic DNA with confirmation by real-time PCR has expanded investigators ability to detect *BMPR2* mutations.⁹⁴ It is now generally accepted that ~ 70% of families with documented PAH in one or more members have a detectable mutation in *BMPR2* in affected individuals.³ This agreement further supports the primary role of *BMPR2* in the central pathogenesis of disease in HPAH.³⁷

BMPR2 MUTATIONS IN IDIOPATHIC PAH

Given the clinical and pathologic similarity to HPAH, it is not surprising that mutations in *BMPR2* cause some cases of IPAH. These mutations may be either inherited low-penetrance alleles or *de novo* events not present in the parents or siblings.³⁷ While the detection rates vary in part due to the availability of detailed family histories, mutations are typically detected in about 15% of IPAH patients (6–40%).^{6,37,95,96} Of note, while the precise rate of *BMPR2* mutations in the general population is unknown, it is extremely low.³⁶

GENETIC MODIFIERS OF HPAH DUE TO *BMPR2* MUTATIONS

Not all *BMPR2* mutations result in identical clinical presentations, not surprisingly. And, the functional impact of *BMPR2* mutations has been incompletely investigated to date, with variable consequences for signaling activity reported.^{97,98}

Complete disruption of BMP signaling may lead to absence of critical mechanisms in the antiproliferative and differentiation mechanisms in the pulmonary vasculature.⁹³ Haploinsufficiency (HI) has been generally accepted as the predominant molecular mechanism by which a *BMPR2* mutation predisposes to PAH.^{90,99} Consistent with this, ~50–60% of the reported pathogenic mutations should result in a premature truncation codon (PTC), which if it resides in certain exons will trigger decay of the mRNA with no protein produced (HI condition).⁹⁰ In addition, various deletions within exons 2–13 have been reported, which lead to nonfunctional peptides.^{94,95} While it is clear that alleles resulting in HI effects cause PAH, it is unclear what percentage of *BMPR2* mutations that result in disease have dominant negative (DN) effects on BMP signaling.^{97,100} *In vitro* functional studies have shown DN effects on BMP signaling due to mutations or truncations in the kinase domain of *BMPR2*.¹⁰¹ While some data suggest that DN mutations are more detrimental than HI mutations, whether reproducible phenotypic differences will emerge is unknown.¹⁰²

Recent work by Hamid et al suggests that while characterization of the mutation type is important, it is not the only component of *BMPR2* allelic variation that correlates genotype

with phenotype. In fact, the level of production of *BMPR2* transcript by the remaining wild-type allele, particularly in the setting of HI mutations, may be a critical modifier of disease expression. Studying lymphoblastoid cell lines derived from *BMPR2* mutation carriers with four different HI mutations, real-time PCR assays were used to determine wild-type and mutated *BMPR2* transcript levels. As expected, HI mutant alleles contribute very little to the total *BMPR2* transcript levels (0–2.5%), so that the wild-type *BMPR2* allele is the major determinant of total transcript amount. Interestingly, HPAH patients had significantly lower wild-type *BMPR2* transcript levels compared to unaffected mutation carriers with the same HI mutation. Because the *BMPR2* receptor forms a heteromeric complex with *BMPR1a* and *BMPR1b*, deficiency of the normal *BMPR2* receptor could affect the stoichiometric balance on the cell surface, leading to decreased signaling through the receptor and a greater predisposition to disease penetrance. This finding that variations in the amount of normal *BMPR2* transcripts can affect penetrance supports the concept that at least among HI mutations, the extent of transcript production by the wild-type *BMPR2* allele may be a critical modifier of disease penetrance (Figure 3).¹⁰³

Reduced wild-type transcripts may explain the discovery by Atkinson et al that upon immunohistochemical staining of lung tissue from HPAH patients there is a near complete absence of *BMPR2* protein in those heterozygous for the mutation.¹⁰⁴ This finding was interesting because given the presence of one mutated germline allele and one normal (wild-type) germline allele, one would expect to see about 50% the amount of protein produced by the wild-type allele—unless affected HI mutation carriers are subjects with particularly reduced wild-type *BMPR2* transcript levels (alternatively, it is possible that all patient samples studied had a DN effect). Thus, in the setting of one *BMPR2* mutation, naturally low expression by the ‘healthy’ wild-type allele may be a risk factor for PAH penetrance.

An alternative model of disease modification is the prospect of a lung-specific alteration of protein function due to somatic mutation. In a *BMPR2* mutation carrier, this concept is similar to the loss of a second tumor suppressor gene in the ‘two-hit’ model of cancer development. The occurrence of somatic mutations of the *BMPR2* gene within pulmonary vasculature cells could cause or aggravate *BMPR2* haploinsufficiency, although previous work had failed to find this to be true among those with a germline *BMPR2* mutation.²¹ In contrast, Aldred and colleagues recently detected a somatic *SMAD9* gene mutation in the pulmonary vascular cells of one of two *BMPR2* mutation carriers with PAH, providing partial support for the ‘two hit model’ in *BMPR2*-PAH.¹⁰⁵

Reduced penetrance implies that a mutation of the *BMPR2* gene is required but insufficient alone for phenotypic expression. While Hamid et al have shown that variation in levels generated by the wild-type *BMPR2* allele can modify disease, it is likely that additional endogenous and exogenous modifiers also mediate the clinical expression of HPAH.²⁰ The most obvious endogenous factors are genetic in origin, especially those involving biologically relevant candidate genes. A number of such genes have been investigated and implicated, but findings have not been convincingly replicated.^{102,106–111} It is unknown whether these might be genes whose action promotes clinical expression of a *BMPR2* mutation in those who develop PAH, or whether these are protective modifiers that prevent disease in the mutation carriers who never develop disease.

The balance of signaling among the various members of the TGF β pathway of receptors, including BMPR2 and TGF β receptor signaling, may modify disease expression.¹² It has been suggested that a reciprocal relationship exists between pathways comprised of members of this superfamily of receptors, with the perturbation of BMPR2 signaling by a genetic mutation detrimental to this balance (Figure 4).¹¹² Interestingly, TGF β signaling appears enhanced in various forms of pulmonary hypertension, although *BMPR2* mutations may alter the cellular response to this activity.¹¹³ Zaiman et al recently found reduced markers of pulmonary vascular remodeling and prevention of disease onset following inhibition of the TGF β type 1 receptor using the rat monocrotaline model of pulmonary hypertension.¹¹⁴

Consistent with Zaiman et al's work and with genetic modifier studies of lung disease in cystic fibrosis, in a cohort of individuals with varying types of *BMPR2* mutations, we found that those subjects with genetic polymorphisms in the gene *TGF β 1* predicted to increase TGF β pathway activity were more likely to be penetrant.¹⁰² While biologically plausible, this finding requires replication and validation.

COMMON VARIATIONS IN GENETIC LOCI AMONG THOSE WITH HPAH AND IPAH, BUT WITHOUT DETECTABLE *BMPR2* OR OTHER PAH MUTATIONS

While rare variants (mutations) have been an area of intense focus in PAH, presumably common genetic variations influence disease expression as well. Until recently, the discovery of validated common variants has been challenging for *BMPR2*-associated PAH, and absent in non-*BMPR2* PAH. Recently Germain et al addressed this issue directly, and identified a new locus of importance in PAH pathogenesis. They conducted the first genome-wide association study of patients (*BMPR2* mutation not detected) with familial and idiopathic PAH. This enormous effort succeeded via the use of a two-stage approach including two independent case-control studies, which in total provided 625 PAH cases and 1,525 healthy subjects. Both the discovery and replication stages demonstrated a two-fold higher odds of disease among those patients with the common variant rs2217560[G] allele at the 18q22.3 locus. In a subsequent combined population analysis, the rs2217560[G] allele was associated with a higher odds of PAH: 1.97 (1.59–2.45; *P* value 7.47×10^{-10}).¹¹⁵ After careful examination, it was determined that the gene of highest likelihood to be of impact in this GWAS was the cerebellin-2 precursor (*CBLN2*) gene, which lies 52 kb upstream of the rs2217560 SNP.¹¹⁵

CBLN2 belongs to the cerebellin gene family, which encodes a group of secreted neuronal glycoproteins not previously linked to PAH.¹¹⁶ However, Germain et al measured higher *CBLN2* mRNA levels in the lungs of humans with PAH compared to controls, a finding particularly striking in endothelial cells from PAH patients (while pulmonary artery smooth muscle cell production of *CBLN2* mRNA was reduced). Meanwhile, exposure to increasing concentrations of *CBLN2* *in vitro* inhibited pulmonary artery smooth muscle cell proliferation, suggesting cross-talk between endothelial and smooth muscle cells mediated via *CBLN2* levels although this remains to be determined. While the precise role of *CBLN2*

variants to PAH remains to be determined, as does its applicability (or not) to other forms of PAH, this discovery identified a novel association that would not have otherwise been uncovered using a candidate gene approach.

BMPR2 AND ALTERNATIVE CAUSES OF PULMONARY HYPERTENSION

Mutations in *BMPR2* have not been consistently found in other causes of pulmonary hypertension. However, pulmonary veno-occlusive disease (PVOD), a rare form of pulmonary hypertension in which the vascular changes also affect small pulmonary veins and venules, has been linked to mutations in *BMPR2*. This has been confirmed in at least three independent studies.^{95,117,118}

The detection of *BMPR2* mutations in PVOD emphasizes that clinical heterogeneity can result from a *BMPR2* mutation. This clinical heterogeneity arises from the ability of different allelic mutations at a single locus to produce different disease phenotypes. Further, PVOD and PAH may well represent different ends of the same spectra of disease, with phenotype influenced by genetic and/or environmental modifiers.¹¹⁷

PAH related to certain drug exposures is well known. Specifically, PAH has been reported in conjunction with exposure to appetite suppressants, such as fenfluramine and dexfenfluramine.^{119,120} While the exact mechanism of PAH promotion has not been elucidated, these drugs must serve as environmental triggers to facilitate disease expression, possibly in genetically-susceptible individuals. Individual factors of susceptibility are particularly plausible given the low numbers of PAH in those exposed to fenfluramine (approximately 1 case per 10,000 people exposed).¹²¹ In 9% of unrelated patients with PAH associated with fenfluramine exposure, Humbert et al found distinct *BMPR2* mutations. Interestingly, when compared to other patients with PAH associated with fenfluramine, patients heterozygous for a *BMPR2* mutation expressed disease following a significantly shorter interval of exposure to fenfluramine.⁹⁹ While rare, the association of drug exposure and *BMPR2* mutations in PAH further emphasizes the importance of endogenous and exogenous factors in the expression of disease.

Roberts et al found *BMPR2* variations in 6% of 106 children and adults with congenital heart disease, regardless of pulmonary vascular pressures, but no studies have been undertaken using the expanded genetic analyses currently in use.¹²² Since the BMP pathway activity is known to be important in the embryologic development of the cardiovascular system, further studies are warranted.

GENETIC IMPLICATIONS FOR CLINICAL EVALUATION AND THERAPY

Information on the impact of *BMPR2* genotype upon clinical outcomes and therapeutic response in PAH has steadily increased over the past several years. For instance, a small proportion of PAH patients have good long term response to calcium channel blockers as predicted by vasoreactivity testing, so Elliott et al retrospectively compared the results of vasoreactivity testing with known *BMPR2* mutations (specifically, nonsynonymous *BMPR2* sequence variations).^{29,30} They found that patients with HPAH or IPAH with nonsynonymous *BMPR2* sequence variations were less likely to demonstrate vasoreactivity

by standard testing.³¹ In a cohort of adult and pediatric patients with PAH and a *BMPR2* mutation, Rosenzweig and colleagues found similar results, as well as worse hemodynamic parameters.³²

Interestingly, Sztrymf et al reported that having certain *BMPR2* mutations is associated with a more aggressive form of PAH. By examining their large registry of individuals with PAH in France, they found that 20% of patients without a family history of PAH had a detectable mutation in *BMPR2*, suggesting that heritable disease may be more common than currently appreciated. They also found that subjects with a *BMPR2* mutation had a more severe form of the disease, based on an earlier age at diagnosis and more severe hemodynamic abnormalities than those lacking a mutation. While survival was comparable in both groups, patients with *BMPR2* mutation were more likely to be treated with parenteral prostacyclin therapy or undergo lung transplantation.^{28,123}

Additional studies are needed to enable correlation of genotype and phenotype because of the heterogeneous molecular basis of reduced penetrance seen in PAH.¹²³ In addition, they provide clinical evidence that not all *BMPR2* mutations result in equal effects. Therefore, as noted above, HI mutations may result in less severe disease than those resulting in DN effects. Also, kindred individuals with the same *BMPR2* mutation may have different polymorphic alleles or environmental exposures that modify the penetrance and severity of their shared *BMPR2* mutations. One day, patients and their physicians may be able to implement a management strategy that is based in part upon one's genotype and environmental exposures.¹²³

GENETIC TESTING, SCREENING THOSE AT RISK, AND RELATED ISSUES

The issue of genetic testing for PAH on a clinical basis is challenging. As an autosomal dominant disease, the siblings or children of patients with HPAH, or of known heterozygotes for a *BMPR2* mutation, have an overall risk of 50% to inherit the disease-causing allele. With reduced penetrance such that ~27% of carriers will develop disease, this yields an estimated risk of 13.5% ($50\% \times 27\%$) to express disease for each child of a *BMPR2* mutation carrier.^{19,124} Thus, the majority of 1st degree (parent, sib, or child) relatives will be asymptomatic even though 50% will be carriers of a *BMPR2* mutation.

A negative test result for the *BMPR2* mutation for a family member of an affected *BMPR2* carrier is reassuring. If negative, an individual PAH family member's risk of disease falls from about 1 in 10 (13.5%, noted above) to that of the population, which is approximately 1 in 1,000,000 (100,000 fold risk reduction). However, if an asymptomatic individual at risk is found to carry the known familial *BMPR2* mutation, because of reduced penetrance the risk increases only 2 fold (from 13.5% to 27%) to express clinical disease throughout their lifetime.

Genetic testing on a clinical basis for HPAH is currently available. It is most efficiently used for unaffected individuals with a known family history of PAH, or PAH patients. Laboratory evaluation for a *BMPR2* mutation should start with full sequencing of all 13 exons, including splice junctions. Advanced methods, such as MLPA, may be needed to identify some mutations which involve deletions or duplications missed by genomic sequencing.³

Also, cDNA sequencing may be required to detect some mutations found deep in introns that perturb splicing. Genetic testing should only be provided in concert with professional genetic counseling by experienced counselors available before and after test results are available.^{36,124} It is important to consider that a positive test for a *BMPR2* mutation in a subject with IPAH converts the concept of disease from one of a 'sporadic' finding to that of a heritable family disease; this situation can be terrifying for the family, as kin are at risk to harbor the same mutation. Not surprisingly, the major reason subjects give for testing is to provide information about their children.¹²⁵

BMPR2 mutations are usually family-specific, without a common recurring mutation type. Given the vast number of potential mutations in the large *BMPR2* gene (> 425 are currently known), screening for a mutation is most efficient in an affected patient with IPAH or FPAH, so that the specific mutation in their family can be identified, if present. There is no rationale for clinical genetic testing for *BMPR2* mutations of relatives of a PAH patient unless a mutation is identified in the patient sample. With the discovery of several additional mutations that associate with PAH, there is a need to better understand the prevalence of those mutations among PAH patients without *BMPR2* gene mutations. The incorporation of targeted screening for mutations in those genes (e.g., *KCNK3*), or a panel of relevant genes, is an area of active platform design for a number of clinical labs and will likely be available in the near future at a cost structure that hopefully will remain relatively low.

Current clinical care screening recommendations for asymptomatic family members of patients with HPAH include a surveillance echocardiogram at 3 to 5 year intervals.¹²⁴ Based upon studies of families with HPAH, investigators in Germany proposed a screening process utilizing estimation of systolic pulmonary artery pressure at rest and during exercise by echocardiography. Specifically, Grunig et al reported an abnormal pulmonary artery pressure response to hypoxia during echocardiographic evaluation in individuals deemed at risk to develop PAH based upon a known *BMPR2* mutation or a possible 'risk haplotype'.¹²⁶ The development of echocardiogram and other advanced imaging modalities is critical to improve the earlier detection of PAH in at risk individuals. However, the use of stress echocardiography as a screening tool for PAH has not been widely accepted, and requires more extensive evaluation and independent confirmation. As such, it is not currently recommended for general use.

Finally, with the advent of modern fertility medicine approaches, families with *BMPR2* mutation carriers now have enhanced options in considering approaches to the conception of a child. In addition to pregnancy prevention and the avoidance of genetic testing, families now have the option to pursue pre-implantation genetic testing. Frydman et al recently demonstrated this approach, using pre-implantation genetic testing to support the clinical decision making and action of a couple at high risk of conceiving a child with a *BMPR2* gene mutation. In this circumstance, the male was a *BMPR2* mutation carrier from a family with a high PAH penetrance, highlighting both the importance of *BMPR2* avoidance as well as the safety of the conceiving mother to undergo pregnancy (pregnancy presumably carries enhanced risk for *BMPR2* mutation carriers, such that at present encouraging a female *BMPR2* mutation carrier to pursue pregnancy is controversial). While cost is currently one limiting feature of this approach in the US, pre-implantation genetic diagnosis may be

considered in selected families impacted by PAH in multiple kin.¹²⁷ However, the decision to proceed in this manner is certainly complicated for a variety of reasons, including the reduced penetrance of *BMPR2* gene mutations (no guarantee a mutation carrier will ever develop PAH) and the inherent risk of genetic variation due to the complexity of the process. As such, experienced genetic consultation will be a critical contribution to the clinical care of subjects who consider this potentially valuable care approach.¹²⁸

Conclusion and Future Directions

Tremendous progress in the evaluation of PAH has been made in relation to the genetic underpinnings of this disease. The discovery that mutations in the *BMPR2* gene underlie the majority of cases of HPAH, and of an important subset of cases of IPAH, has energized the field and prompted significant progress in understanding the molecular basis of PAH. So, too, has the association of HPAH in HHT with genes that encode additional members of the TGF β superfamily of receptors. However, much work is still needed to elucidate the cause(s) of the reduced penetrance and variable expressivity of *BMPR2* mutations, including the identity and role of various genetic and environmental modifiers of disease expression. In addition, studies should strive to elucidate the role of gender, the strongest disease modifier known.

The identification of novel genes and proteins involved in disease pathogenesis and progression, such as those involved in the serotonin and TGF- β signaling pathways, is an area of intense study. The endogenous and exogenous influences that converge to promote pulmonary vasculature-specific cellular proliferation and vascular remodeling require further investigation. Hopefully, a greater understanding of the genetic and molecular mechanisms of PAH will lead to earlier diagnosis, advanced pharmacogenetics, and perhaps one day in the future, disease prevention.

Acknowledgments

The authors thank the many patients and families who graciously contributed to this work, and Ms. Lisa Wheeler, whose service is invaluable as coordinator of the Vanderbilt Familial Pulmonary Arterial Hypertension study.

Support

This work was supported by NIH PO1 HL072058, NIH P01 HL108800, NIH K23 HL098743, and GCRC RR000095

References

1. Dresdale DT, Schultz M, Michtom RJ. Primary pulmonary hypertension. I. Clinical and hemodynamic study. *Am J Med.* 1951; 11:686–705. [PubMed: 14902839]
2. Dresdale DT, Michtom RJ, Schultz M. Recent studies in primary pulmonary hypertension, including pharmacodynamic observations on pulmonary vascular resistance. *Bull N Y Acad Med.* 1954; 30:195–207. [PubMed: 13141055]
3. Machado RD, Eickelberg O, Elliott CG, et al. Genetics and genomics of pulmonary arterial hypertension. *J Am Coll Cardiol.* 2009; 54:S32–42. [PubMed: 19555857]
4. Loyd JE, Primm RK, Newman JH. Familial primary pulmonary hypertension: clinical patterns. The American review of respiratory disease. 1984; 129:194–7. [PubMed: 6703480]

5. Thomas AQ, Gaddipati R, Newman JH, Loyd JE. Genetics of primary pulmonary hypertension. *Clinics in chest medicine*. 2001; 22:477–91. ix. [PubMed: 11590842]
6. Thomson JR, Machado RD, Pauciulo MW, et al. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. *J Med Genet*. 2000; 37:741–5. [PubMed: 11015450]
7. Rich S, Dantzker DR, Ayres SM, et al. Primary pulmonary hypertension. A national prospective study. *Ann Intern Med*. 1987; 107:216–23. [PubMed: 3605900]
8. Nichols WC, Koller DL, Slovis B, et al. Localization of the gene for familial primary pulmonary hypertension to chromosome 2q31-32. *Nat Genet*. 1997; 15:277–80. [PubMed: 9054941]
9. Morse JH, Barst RJ. Detection of familial primary pulmonary hypertension by genetic testing. *N Engl J Med*. 1997; 337:202–3. [PubMed: 9221353]
10. Lane KB, Machado RD, Pauciulo MW, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. The International PPH Consortium. *Nat Genet*. 2000; 26:81–4. [PubMed: 10973254]
11. Deng Z, Morse JH, Slager SL, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet*. 2000; 67:737–44. [PubMed: 10903931]
12. Newman JH, Phillips JA 3rd, Loyd JE. Narrative review: the enigma of pulmonary arterial hypertension: new insights from genetic studies. *Ann Intern Med*. 2008; 148:278–83. [PubMed: 18283205]
13. Sztrymf B, Yaici A, Girerd B, Humbert M. Genes and pulmonary arterial hypertension. *Respiration; international review of thoracic diseases*. 2007; 74:123–32.
14. Loyd JE, Butler MG, Foroud TM, Conneally PM, Phillips JA 3rd, Newman JH. Genetic anticipation and abnormal gender ratio at birth in familial primary pulmonary hypertension. *Am J Respir Crit Care Med*. 1995; 152:93–7. [PubMed: 7599869]
15. Simonneau G, Robbins IM, Beghetti M, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol*. 2009; 54:S43–54. [PubMed: 19555858]
16. Sztrymf B, Yaici A, Jais X, Simonneau G, Sitbon O, Humbert M. Genetics of pulmonary arterial hypertension: recent data and practical applications. *Rev Mal Respir*. 2005; 22:796–805. [PubMed: 16272982]
17. Pearson CE, Nichol Edamura K, Cleary JD. Repeat instability: mechanisms of dynamic mutations. *Nat Rev Genet*. 2005; 6:729–42. [PubMed: 16205713]
18. Armanios M, Chen JL, Chang YP, et al. Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. *Proc Natl Acad Sci U S A*. 2005; 102:15960–4. [PubMed: 16247010]
19. Larkin EK, Newman JH, Austin ED, et al. Longitudinal analysis casts doubt on the presence of genetic anticipation in heritable pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2012; 186:892–6. [PubMed: 22923661]
20. Gaine SP, Rubin LJ. Primary pulmonary hypertension. *Lancet*. 1998; 352:719–25. [PubMed: 9729004]
21. Machado RD, James V, Southwood M, et al. Investigation of Second Genetic Hits at the BMPR2 Locus as a Modulator of Disease Progression in Familial Pulmonary Arterial Hypertension. *Circulation*. 2005; 111:607–13. [PubMed: 15699281]
22. Irey NS, Manion WC, Taylor HB. Vascular lesions in women taking oral contraceptives. *Arch Pathol*. 1970; 89:1–8. [PubMed: 4188605]
23. Morse JH, Horn EM, Barst RJ. Hormone replacement therapy: a possible risk factor in carriers of familial primary pulmonary hypertension. *Chest*. 1999; 116:847. [PubMed: 10492306]
24. Sweeney L, Voelkel NF. Estrogen exposure, obesity and thyroid disease in women with severe pulmonary hypertension. *European journal of medical research*. 2009; 14:433–42. [PubMed: 19748850]
25. West J, Cogan J, Geraci M, et al. Gene expression in BMPR2 mutation carriers with and without evidence of Pulmonary Arterial Hypertension suggests pathways relevant to disease penetrance. *BMC Med Genomics*. 2008; 1:45. [PubMed: 18823550]

26. Austin ED, Cogan JD, West JD, et al. Alterations in oestrogen metabolism: implications for higher penetrance of familial pulmonary arterial hypertension in females. *Eur Respir J*. 2009; 34:1093–9. [PubMed: 19357154]
27. Runo JR, Loyd JE. Primary pulmonary hypertension. *The Lancet*. 2003; 361:1533–44.
28. Sztrymf BCF, Girerd B, Yaici A, Jais X, Sitbon O, Montani D, Souza R, Simonneau G, Soubrier F, Humbert M. Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. *Am J Respir Crit Care Med*. 2008; 177:1377–83. [PubMed: 18356561]
29. Montani D, Marcelin AG, Sitbon O, Calvez V, Simonneau G, Humbert M. Human herpes virus 8 in HIV and non-HIV infected patients with pulmonary arterial hypertension in France. *Aids*. 2005; 19:1239–40. [PubMed: 15990583]
30. Archer SL, Michelakis ED. An evidence-based approach to the management of pulmonary arterial hypertension. *Curr Opin Cardiol*. 2006; 21:385–92. [PubMed: 16755209]
31. Elliott CG, Glissmeyer EW, Havlena GT, et al. Relationship of BMPR2 Mutations to Vasoreactivity in Pulmonary Arterial Hypertension. *Circulation*. 2006; 113:2509–15. [PubMed: 16717148]
32. Rosenzweig EB, Morse JH, Knowles JA, et al. Clinical implications of determining BMPR2 mutation status in a large cohort of children and adults with pulmonary arterial hypertension. *J Heart Lung Transplant*. 2008; 27:668–74. [PubMed: 18503968]
33. Newman JH. Pulmonary hypertension. *Am J Respir Crit Care Med*. 2005; 172:1072–7. [PubMed: 15994464]
34. Morse JH, Jones AC, Barst RJ, Hodge SE, Wilhelmsen KC, Nygaard TG. Familial primary pulmonary hypertension locus mapped to chromosome 2q31-q32. *Chest*. 1998; 114:57S–8S. [PubMed: 9676630]
35. Morse JH, Jones AC, Barst RJ, Hodge SE, Wilhelmsen KC, Nygaard TG. Mapping of familial primary pulmonary hypertension locus (PPH1) to chromosome 2q31-q32. *Circulation*. 1997; 95:2603–6. [PubMed: 9193425]
36. Newman JH, Trembath RC, Morse JA, et al. Genetic basis of pulmonary arterial hypertension: current understanding and future directions. *J Am Coll Cardiol*. 2004; 43:33S–9S. [PubMed: 15194176]
37. Machado RD, Aldred MA, James V, et al. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat*. 2006; 27:121–32. [PubMed: 16429395]
38. Shintani M, Yagi H, Nakayama T, Saji T, Matsuoka R. A new nonsense mutation of SMAD8 associated with pulmonary arterial hypertension. *J Med Genet*. 2009; 46:331–7. [PubMed: 19211612]
39. Johnson DW, Berg JN, Baldwin MA, et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet*. 1996; 13:189–95. [PubMed: 8640225]
40. McAllister KA, Grogg KM, Johnson DW, et al. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet*. 1994; 8:345–51. [PubMed: 7894484]
41. Harrison RE, Flanagan JA, Sankelo M, et al. Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. *J Med Genet*. 2003; 40:865–71. [PubMed: 14684682]
42. Trembath RC, Thomson JR, Machado RD, et al. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med*. 2001; 345:325–34. [PubMed: 11484689]
43. Chaouat A, Coulet F, Favre C, et al. Endoglin germline mutation in a patient with hereditary haemorrhagic telangiectasia and dexfenfluramine associated pulmonary arterial hypertension. *Thorax*. 2004; 59:446–8. [PubMed: 15115879]
44. Harrison RE, Berger R, Haworth SG, et al. Transforming growth factor-beta receptor mutations and pulmonary arterial hypertension in childhood. *Circulation*. 2005; 111:435–41. [PubMed: 15687131]
45. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. 2003; 113:685–700. [PubMed: 12809600]

46. Fernandez LA, Sanz-Rodriguez F, Blanco FJ, Bernabeu C, Botella LM. Hereditary hemorrhagic telangiectasia, a vascular dysplasia affecting the TGF-beta signaling pathway. *Clin Med Res*. 2006; 4:66–78. [PubMed: 16595794]
47. Bamshad MJ, Ng SB, Bigham AW, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet*. 2011; 12:745–55. [PubMed: 21946919]
48. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009; 25:1754–60. [PubMed: 19451168]
49. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009; 25:2078–9. [PubMed: 19505943]
50. Austin ED, Ma L, LeDuc C, et al. Whole exome sequencing to identify a novel gene (caveolin-1) associated with human pulmonary arterial hypertension. *Circ Cardiovasc Genet*. 2012; 5:336–43. [PubMed: 22474227]
51. Chidlow JH Jr, Sessa WC. Caveolae, caveolins, and cavins: complex control of cellular signalling and inflammation. *Cardiovasc Res*. 2010; 86:219–25. [PubMed: 20202978]
52. Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev*. 2004; 84:1341–79. [PubMed: 15383654]
53. Frank PG, Cheung MW, Pavlides S, Llaverias G, Park DS, Lisanti MP. Caveolin-1 and regulation of cellular cholesterol homeostasis. *Am J Physiol Heart Circ Physiol*. 2006; 291:H677–86. [PubMed: 16603689]
54. Mercier I, Jasmin JF, Pavlides S, et al. Clinical and translational implications of the caveolin gene family: lessons from mouse models and human genetic disorders. *Laboratory investigation; a journal of technical methods and pathology*. 2009; 89:614–23.
55. Parton RG, Simons K. The multiple faces of caveolae. *Nature reviews Molecular cell biology*. 2007; 8:185–94.
56. Patel HH, Murray F, Insel PA. Caveolae as organizers of pharmacologically relevant signal transduction molecules. *Annual review of pharmacology and toxicology*. 2008; 48:359–91.
57. Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG. Caveolin, a protein component of caveolae membrane coats. *Cell*. 1992; 68:673–82. [PubMed: 1739974]
58. Minshall RD, Sessa WC, Stan RV, Anderson RG, Malik AB. Caveolin regulation of endothelial function. *Am J Physiol Lung Cell Mol Physiol*. 2003; 285:L1179–83. [PubMed: 14604847]
59. Xu Y, Buikema H, van Gilst WH, Henning RH. Caveolae and endothelial dysfunction: filling the caves in cardiovascular disease. *Eur J Pharmacol*. 2008; 585:256–60. [PubMed: 18423600]
60. Razani B, Engelman JA, Wang XB, et al. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem*. 2001; 276:38121–38. [PubMed: 11457855]
61. Zhao YY, Liu Y, Stan RV, et al. Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice. *Proc Natl Acad Sci U S A*. 2002; 99:11375–80. [PubMed: 12177436]
62. Maniatis NA, Shinin V, Schraufnagel DE, et al. Increased pulmonary vascular resistance and defective pulmonary artery filling in caveolin-1^{-/-} mice. *Am J Physiol Lung Cell Mol Physiol*. 2008; 294:L865–73. [PubMed: 18192592]
63. Frank PG, Hassan GS, Rodriguez-Feo JA, Lisanti MP. Caveolae and caveolin-1: novel potential targets for the treatment of cardiovascular disease. *Current pharmaceutical design*. 2007; 13:1761–9. [PubMed: 17584106]
64. Drab M, Verkade P, Elger M, et al. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science*. 2001; 293:2449–52. [PubMed: 11498544]
65. Murata T, Lin MI, Huang Y, et al. Reexpression of caveolin-1 in endothelium rescues the vascular, cardiac, and pulmonary defects in global caveolin-1 knockout mice. *J Exp Med*. 2007; 204:2373–82. [PubMed: 17893196]
66. Wertz JW, Bauer PM. Caveolin-1 regulates BMPRII localization and signaling in vascular smooth muscle cells. *Biochem Biophys Res Commun*. 2008; 375:557–61. [PubMed: 18725205]
67. Nohe A, Keating E, Underhill TM, Knaus P, Petersen NO. Dynamics and interaction of caveolin-1 isoforms with BMP-receptors. *J Cell Sci*. 2005; 118:643–50. [PubMed: 15657086]

68. Ramos M, Lame MW, Segall HJ, Wilson DW. The BMP type II receptor is located in lipid rafts, including caveolae, of pulmonary endothelium in vivo and in vitro. *Vascul Pharmacol.* 2006; 44:50–9. [PubMed: 16271518]
69. Hartung A, Bitton-Worms K, Rechtman MM, et al. Different routes of bone morphogenic protein (BMP) receptor endocytosis influence BMP signaling. *Mol Cell Biol.* 2006; 26:7791–805. [PubMed: 16923969]
70. Garcia-Cardena G, Oh P, Liu J, Schnitzer JE, Sessa WC. Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling. *Proc Natl Acad Sci USA.* 1996; 93:6448–53. [PubMed: 8692835]
71. Garcia-Cardena G, Martasek P, Masters BS, et al. Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. *J Biol Chem.* 1997; 272:25437–40. [PubMed: 9325253]
72. Sbaa E, Frerart F, Feron O. The double regulation of endothelial nitric oxide synthase by caveolae and caveolin: a paradox solved through the study of angiogenesis. *Trends Cardiovasc Med.* 2005; 15:157–62. [PubMed: 16165011]
73. Feron O, Balligand JL. Caveolins and the regulation of endothelial nitric oxide synthase in the heart. *Cardiovasc Res.* 2006; 69:788–97. [PubMed: 16483868]
74. Li XA, Everson W, Smart EJ. Nitric oxide, caveolae, and vascular pathology. *Cardiovasc Toxicol.* 2006; 6:1–13. [PubMed: 16845178]
75. Maniatis NA, Brovkovich V, Allen SE, et al. Novel mechanism of endothelial nitric oxide synthase activation mediated by caveolae internalization in endothelial cells. *Circ Res.* 2006; 99:870–7. [PubMed: 16973909]
76. Rath G, Dessy C, Feron O. Caveolae, caveolin and control of vascular tone: nitric oxide (NO) and endothelium derived hyperpolarizing factor (EDHF) regulation. *J Physiol Pharmacol.* 2009; 60 (Suppl 4):105–9. [PubMed: 20083858]
77. Dessy C, Feron O, Balligand JL. The regulation of endothelial nitric oxide synthase by caveolin: a paradigm validated in vivo and shared by the 'endothelium-derived hyperpolarizing factor'. *Pflugers Arch.* 2010; 459:817–27. [PubMed: 20339866]
78. Rahman A, Sward K. The role of caveolin-1 in cardiovascular regulation. *Acta Physiol (Oxf).* 2009; 195:231–45. [PubMed: 18826501]
79. Patel HH, Zhang S, Murray F, et al. Increased smooth muscle cell expression of caveolin-1 and caveolae contribute to the pathophysiology of idiopathic pulmonary arterial hypertension. *FASEB J.* 2007; 21:2970–9. [PubMed: 17470567]
80. Mathew R, Huang J, Gewitz MH. Pulmonary artery hypertension: caveolin-1 and eNOS interrelationship: a new perspective. *Cardiol Rev.* 2007; 15:143–9. [PubMed: 17438380]
81. Zhao YY, Malik AB. A novel insight into the mechanism of pulmonary hypertension involving caveolin-1 deficiency and endothelial nitric oxide synthase activation. *Trends Cardiovasc Med.* 2009; 19:238–42. [PubMed: 20382348]
82. Zhao YY, Zhao YD, Mirza MK, et al. Persistent eNOS activation secondary to caveolin-1 deficiency induces pulmonary hypertension in mice and humans through PKG nitration. *J Clin Invest.* 2009; 119:2009–18. [PubMed: 19487814]
83. Ma L, Roman-Campos D, Austin ED, et al. A Novel Channelopathy in Pulmonary Arterial Hypertension. *N Engl J Med.* 2013 in press.
84. Olschewski A, Li Y, Tang B, et al. Impact of TASK-1 in human pulmonary artery smooth muscle cells. *Circ Res.* 2006; 98:1072–80. [PubMed: 16574908]
85. Kuhr FK, Smith KA, Song MY, Levitan I, Yuan JX. New mechanisms of pulmonary arterial hypertension: role of Ca(2)(+) signaling. *Am J Physiol Heart Circ Physiol.* 2012; 302:H1546–62. [PubMed: 22245772]
86. Streit AK, Netter MF, Kempf F, et al. A specific two-pore domain potassium channel blocker defines the structure of the TASK-1 open pore. *J Biol Chem.* 2011; 286:13977–84. [PubMed: 21362619]
87. De Caestecker M, Meyrick B. Bone morphogenetic proteins, genetics and the pathophysiology of primary pulmonary hypertension. *Respir Res.* 2001; 2:193–7. [PubMed: 11686884]

88. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature*. 2003; 425:577–84. [PubMed: 14534577]
89. Davies RJ, Morrell NW. Molecular mechanisms of pulmonary arterial hypertension: role of mutations in the bone morphogenetic protein type II receptor. *Chest*. 2008; 134:1271–7. [PubMed: 19059957]
90. Machado RD, Pauciulo MW, Thomson JR, et al. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. *Am J Hum Genet*. 2001; 68:92–102. [PubMed: 11115378]
91. Koehler R, Grunig E, Pauciulo MW, et al. Low frequency of BMPR2 mutations in a German cohort of patients with sporadic idiopathic pulmonary arterial hypertension. *J Med Genet*. 2004; 41:e127. [PubMed: 15591269]
92. Morisaki H, Nakanishi N, Kyotani S, Takashima A, Tomoike H, Morisaki T. BMPR2 mutations found in Japanese patients with familial and sporadic primary pulmonary hypertension. *Hum Mutat*. 2004; 23:632. [PubMed: 15146475]
93. Elliott CG. Genetics of pulmonary arterial hypertension: current and future implications. *Semin Respir Crit Care Med*. 2005; 26:365–71. [PubMed: 16121312]
94. Cogan JD, Pauciulo MW, Batchman AP, et al. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2006; 174:590–8. [PubMed: 16728714]
95. Aldred MA, Vijayakrishnan J, James V, et al. BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. *Hum Mutat*. 2006; 27:212–3. [PubMed: 16429403]
96. Fujiwara M, Yagi H, Matsuoka R, et al. Implications of mutations of activin receptor-like kinase 1 gene (ALK1) in addition to bone morphogenetic protein receptor II gene (BMPR2) in children with pulmonary arterial hypertension. *Circulation journal: official journal of the Japanese Circulation Society*. 2008; 72:127–33. [PubMed: 18159113]
97. Rudarakanchana N, Flanagan JA, Chen H, et al. Functional analysis of bone morphogenetic protein type II receptor mutations underlying primary pulmonary hypertension. *Hum Mol Genet*. 2002; 11:1517–25. [PubMed: 12045205]
98. Nishihara A, Watabe T, Imamura T, Miyazono K. Functional heterogeneity of bone morphogenetic protein receptor-II mutants found in patients with primary pulmonary hypertension. *Molecular biology of the cell*. 2002; 13:3055–63. [PubMed: 12221115]
99. Humbert M, Deng Z, Simonneau G, et al. BMPR2 germline mutations in pulmonary hypertension associated with fenfluramine derivatives. *Eur Respir J*. 2002; 20:518–23. [PubMed: 12358323]
100. Khajavi M, Inoue K, Lupski JR. Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *Eur J Hum Genet*. 2006; 14:1074–81. [PubMed: 16757948]
101. Yang X, Long L, Southwood M, et al. Dysfunctional Smad signaling contributes to abnormal smooth muscle cell proliferation in familial pulmonary arterial hypertension. *Circ Res*. 2005; 96:1053–63. [PubMed: 15845886]
102. Phillips JA 3rd, Poling JS, Phillips CA, et al. Synergistic heterozygosity for TGFbeta1 SNPs and BMPR2 mutations modulates the age at diagnosis and penetrance of familial pulmonary arterial hypertension. *Genet Med*. 2008; 10:359–65. [PubMed: 18496036]
103. Hamid R, Cogan JD, Hedges LK, et al. Penetrance of pulmonary arterial hypertension is modulated by the expression of normal BMPR2 allele. *Hum Mutat*. 2009; 30:649–54. [PubMed: 19206171]
104. Atkinson C, Stewart S, Upton PD, et al. Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. *Circulation*. 2002; 105:1672–8. [PubMed: 11940546]
105. Aldred MA, Comhair SA, Varella-Garcia M, et al. Somatic chromosome abnormalities in the lungs of patients with pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2010; 182:1153–60. [PubMed: 20581168]
106. Abraham WT, Raynolds MV, Badesch DB, et al. Angiotensin-converting enzyme DD genotype in patients with primary pulmonary hypertension: increased frequency and association with

- preserved haemodynamics. *J Renin Angiotensin Aldosterone Syst.* 2003; 4:27–30. [PubMed: 12692750]
107. Hoepfer MM, Tacacs A, Stellmacher U, Lichtinghagen R. Lack of association between angiotensin converting enzyme (ACE) genotype, serum ACE activity, and haemodynamics in patients with primary pulmonary hypertension. *Heart.* 2003; 89:445–6. [PubMed: 12639879]
 108. Koehler R, Olschewski H, Hoepfer M, Janssen B, Grunig E. Serotonin transporter gene polymorphism in a cohort of German patients with idiopathic pulmonary arterial hypertension or chronic thromboembolic pulmonary hypertension. *Chest.* 2005; 128:619S. [PubMed: 16373876]
 109. Machado RD, Koehler R, Glissmeyer E, et al. Genetic association of the serotonin transporter in pulmonary arterial hypertension. *Am J Respir Crit Care Med.* 2006; 173:793–7. [PubMed: 16399993]
 110. Willers ED, Newman JH, Loyd JE, et al. Serotonin Transporter Polymorphisms in Familial and Idiopathic Pulmonary Arterial Hypertension. *Am J Respir Crit Care Med.* 2006; 173:798–802. [PubMed: 16339917]
 111. Remillard CV, Tigno DD, Platoshyn O, et al. Function of Kv1. 5 channels and genetic variations of KCNA5 in patients with idiopathic pulmonary arterial hypertension. *Am J Physiol Cell Physiol.* 2007; 292:C1837–53. [PubMed: 17267549]
 112. Morrell NW. Pulmonary hypertension due to BMPR2 mutation: a new paradigm for tissue remodeling? *Proc Am Thorac Soc.* 2006; 3:680–6. [PubMed: 17065373]
 113. Morrell NW, Yang X, Upton PD, et al. Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-beta(1) and bone morphogenetic proteins. *Circulation.* 2001; 104:790–5. [PubMed: 11502704]
 114. Zaiman AL, Podowski M, Medicherla S, et al. Role of TGF- β /ALK5 kinase in Monocrotaline-Induced Pulmonary Hypertension. *Am J Respir Crit Care Med.* 2008
 115. Germain M, Eyries M, Montani D, et al. Genome-wide association analysis identifies a susceptibility locus for pulmonary arterial hypertension. *Nat Genet.* 2013; 45:518–21. [PubMed: 23502781]
 116. Yiangou Y, Burnet P, Nikou G, Chrysanthou BJ, Bloom SR. Purification and characterisation of cerebellins from human and porcine cerebellum. *Journal of neurochemistry.* 1989; 53:886–9. [PubMed: 2760624]
 117. Runo JR, Vnencak-Jones CL, Prince M, et al. Pulmonary veno-occlusive disease caused by an inherited mutation in bone morphogenetic protein receptor II. *Am J Respir Crit Care Med.* 2003; 167:889–94. [PubMed: 12446270]
 118. Montani D, Achouh L, Dorfmueller P, et al. Pulmonary veno-occlusive disease: clinical, functional, radiologic, and hemodynamic characteristics and outcome of 24 cases confirmed by histology. *Medicine (Baltimore).* 2008; 87:220–33. [PubMed: 18626305]
 119. Simonneau G, Galie N, Rubin LJ, et al. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol.* 2004; 43:5S–12S. [PubMed: 15194173]
 120. Abenham L, Moride Y, Brenot F, et al. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N Engl J Med.* 1996; 335:609–16. [PubMed: 8692238]
 121. Sztrymf B, Yaici A, Jais X, Sitbon O, Simonneau G, Humbert M. Idiopathic pulmonary hypertension: what did we learn from genes? *Sarcoidosis Vasc Diffuse Lung Dis.* 2005; 22 (Suppl 1):S91–100. [PubMed: 16457021]
 122. Roberts KE, McElroy JJ, Wong WP, et al. BMPR2 mutations in pulmonary arterial hypertension with congenital heart disease. *Eur Respir J.* 2004; 24:371–4. [PubMed: 15358693]
 123. Rubin LJ. BMPR2 mutation and outcome in pulmonary arterial hypertension: clinical relevance to physicians and patients. *Am J Respir Crit Care Med.* 2008; 177:1300–1. [PubMed: 18522952]
 124. McGoon M, Guterman D, Steen V, et al. Screening, early detection, and diagnosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. *Chest.* 2004; 126:14S–34S. [PubMed: 15249493]
 125. Jones DL, Sandberg JC, Rosenthal MJ, Saunders RC, Hannig VL, Clayton EW. What patients and their relatives think about testing for BMPR2. *J Genet Couns.* 2008; 17:452–8. [PubMed: 18791814]

126. Grunig E, Dehnert C, Mereles D, et al. Enhanced hypoxic pulmonary vasoconstriction in families of adults or children with idiopathic pulmonary arterial hypertension. *Chest*. 2005; 128:630S–3S. [PubMed: 16373883]
127. Frydman N, Steffann J, Girerd B, et al. Pre-implantation genetic diagnosis in pulmonary arterial hypertension due to BMPR2 mutation. *Eur Respir J*. 2012; 39:1534–5. [PubMed: 22654007]
128. Hamid R, Loyd J. Pre-implantation genetic testing for hereditary pulmonary arterial hypertension: promise and caution. *Eur Respir J*. 2012; 39:1292–3. [PubMed: 22654004]

Figure 1. Pedigree of an extended kindred with heritable pulmonary arterial hypertension (HPAH) due to a *BMPR2* mutation with reduced penetrance

This pedigree contains 16 total patients (11 females, 5 males) with HPAH, as well as 10 known *BMPR2* mutation carriers without HPAH. Solid symbols represent individuals with disease. Circles represent women, squares represent men. Line through symbol represents death. Dot inside symbol represents obligate carrier of the *BMPR2* mutation. Numbers below symbols represent age at death or current living age. Numbers inside symbols represent numbers of unaffected siblings of each gender. S inside symbol represents stillbirth. Diamond symbol represents sex unknown.

Figure 2. Age at diagnosis and death from pulmonary arterial hypertension in a French series of *BMPR2* mutation carriers and noncarriers; adapted from Sztrymf et al.²⁸

Age at diagnosis ($P < 0.001$) and age at death ($P = 0.003$) are lower in *BMPR2* mutation carriers, as compared to noncarriers.

Figure 3. Wild-type *BMPR2* transcript levels, as measured by relative real-time PCR analysis, in affected (gray) and unaffected (black) *BMPR2* mutation carriers; adapted from Hamid et al.¹⁰³

This study includes four distinct families with four different types of *BMPR2* mutations predicted to have a haploinsufficient effect due to the activation of the nonsense-mediated decay pathway. Note the lower relative *BMPR2* expression among affected carriers, as compared to unaffected carriers.

Figure 4. Simplified schematic of the proposed relationship between BMPR2 signaling, canonical TGFβ signaling, and caveolae

Caveolae are receptor-rich regions of the plasma membrane, populated by multiple receptor types including BMPR2 and TGFβ receptors. A delicate balance between BMPR2 pathway and TGFβ pathway signaling modulates pro-proliferative and apoptotic forces. A *BMPR2* mutation, or reduced wild-type *BMPR2* gene expression, may perturb this balance—perturbation may promote PAH pathogenesis. Disruption of caveolae structure or function could also disrupt cell signaling by reducing receptor density.

Table 1

Rare variants (mutations) associated with PAH in families and in IPAH patients; all PAH patients with detectable germline mutations in these genes have heritable PAH (HPAH) by definition.

GENE	SPECIALIZED INFORMATION
<i>BMPR2</i>	TGF- β Superfamily Member 80% of familial cases ~20% of IPAH cases
<i>ALK1</i>	TGF- β Superfamily Member Hereditary Hemorrhagic Telangiectasia families
<i>Endoglin</i>	TGF- β Superfamily Member Hereditary Hemorrhagic Telangiectasia families
<i>SMAD9</i>	TGF- β Superfamily Member
<i>CAV1</i>	Accurate estimate of frequency among PAH patients yet to be determined.
<i>KCNK3</i>	Accurate estimate of frequency among PAH patients yet to be determined.