Pulmonary hypertension (PH) is an inappropriate elevation of pressure in the pulmonary vascular system attributable to a variety of causes. One subtype of PH is pulmonary arterial hypertension (PAH). PAH is a devastating disease of the pulmonary vasculature that is pathologically characterized by progressive neointimal proliferation, smooth muscle cell hypertrophy, and surrounding adventitial expansion leading to occlusive vascular lesions of the smallest pulmonary arteries.\(^1\) Although there are a variety of methods to classify PAH, the most widely applied is the clinical classification system adopted worldwide and recently updated.\(^2\) In that scheme, Group 1 PAH is divided into disease subgroups that include heritable (HPAH, formerly familial PAH), idiopathic (IPAH), and PAH associated with a variety of other systemic diseases or drug/toxin exposures.\(^3\) Despite advancements in therapy during the past 25 years, PAH remains a devastating disease for incident cases with significantly reduced survival.\(^3\) Unfortunately, no therapies tested to date have demonstrated ability to reverse or cure PAH. Ultimately, an in-depth understanding of the genetic factors relevant to PAH provides the opportunity for improved patient and family counseling about this devastating disease. (Circ Res. 2014;115:189-200.)

Key Words: bone morphogenetic protein receptor, type II ■ genetics ■ hypertension ■ pulmonary

Abstract: Pulmonary arterial hypertension (PAH) is a progressive and fatal disease for which there is an ever-expanding body of genetic and related pathophysiological information on disease pathogenesis. Many germline gene mutations have now been described, including mutations in the gene coding bone morphogenic protein receptor type 2 (BMPR2) and related genes. Recent advanced gene-sequencing methods have facilitated the discovery of additional genes with mutations among those with and those without familial forms of PAH (CAV1, KCNK3, EIF2AK4). The reduced penetrance, variable expressivity, and female predominance of PAH suggest that genetic, genomic, and other factors modify disease expression. These multi-faceted variations are an active area of investigation in the field, including but not limited to common genetic variants and epigenetic processes, and may provide novel opportunities for pharmacological intervention in the near future. They also highlight the need for a systems-oriented multi-level approach to incorporate the multitude of biological variations now associated with PAH. Ultimately, an in-depth understanding of the genetic factors relevant to PAH provides the opportunity for improved patient and family counseling about this devastating disease.
Since its initial descriptions (as primary PH) by Dresdale et al13,14 as a disease that could occur either in isolation or in families in the early 1950s, much has been learned about the molecular and genetic factors that promote PAH. Work in the 1990s and early 2000s led to the discovery that altered bone morphogenic protein receptor type 2 (BMPR2) signaling is the major heritable risk factor for development of PAH, via rare variants (mutations) in the BMPR2 gene.11,12 Since 2000, mutations in other genes related to BMPR2 signaling have been discovered (eg, mutations in ALK1, ENG and SMAD9), and progress has been made in the identification of genetic and epigenetic modifiers of disease expression (Table 1). There has also been a recent explosion in the application of advanced genetic and genomic techniques to uncover novel genomic mechanisms relevant to PAH pathogenesis, making this an exciting time to study the genetic and molecular underpinnings of this devastating disease. The current concepts specific to the genetics of PAH, as well as the ongoing areas of exploration, will be the topic of this review.

**Epidemiological Factors Relevant to the Genetics of PAH**

PH is the elevation of pulmonary vascular pressure as determined by invasive hemodynamic assessment by right heart catheterization. A subgroup of PH (Group 1), PAH is defined as a mean pulmonary artery pressure of $>25$ mm Hg at rest, in the absence of other conditions known to elevate pulmonary vascular pressures, such as left-sided heart disease, hypoxic lung diseases, pulmonary embolism, and various other conditions associated with PH.13,14 Individuals with PAH meet classification for HPAH if they meet any of the following criteria: (1) belong to a family known to have documented PAH in 2 or more individuals (2) possess a rare variant (also known as a mutation) in a gene known to strongly associate with PAH (eg, BMPR2).15 These criteria recognize that many subjects with HPAH do not have a known family history. Important advances in the genetics of PAH during the past 15 years have driven this recognition, as we now know that subjects without a known family history of PAH can have a heritable disease which can be transmitted to their progeny. These subjects would otherwise be considered to have IPAH, but because of reduced penetrance of the known PAH-associated genes, as well as de novo gene changes at conception, these subjects actually have previously unrecognized heritable disease.16 Because IPAH is far more prevalent than familial PAH, the largest number of subjects with PAH that is heritable (HPAH) are actually misclassified IPAH who actually have previously unrecognized heritable disease.16

While familial PAH and IPAH are histopathologically identical, there are differences among subtypes that influence the clinical presentation and progression of disease.15 Independent of mutation status, historical and more recent data support the notion that incident cases of IPAH are $\approx$15 times more frequent than familial PAH.17,18 Although hemodynamics tend to be similar between familial PAH and IPAH cases, BMPR2 mutation PAH patients are diagnosed and die $\approx$10 years earlier than PAH patients without mutation; both IPAH patients and BMPR2 mutation carriers with PAH are exceedingly unlikely to respond to acute vasodilator testing.19-21 However, HPAH associated with mutations in the activin A receptor type II-like kinase-1 (ACVRL1 or ALK1), a receptor in the transforming growth factor-β (TGF-β) receptor family, have even more severe disease, as suggested by a recent French study. Specifically, they found that ALK1 mutation PAH patients had

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### Table 1. Rare Variants (Mutations) Reported to Associate With PAH

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Specialized Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMPR2</td>
<td>TGF-β receptor superfamily member</td>
</tr>
<tr>
<td></td>
<td>≥80% of familial cases</td>
</tr>
<tr>
<td>ALK1</td>
<td>TGF-β receptor superfamily member</td>
</tr>
<tr>
<td></td>
<td>Not known to occur distinct from HHT</td>
</tr>
<tr>
<td>Endoglin</td>
<td>TGF-β receptor superfamily member</td>
</tr>
<tr>
<td></td>
<td>Not known to occur distinct from HHT</td>
</tr>
<tr>
<td>SMAD9</td>
<td>TGF-β superfamily downstream signaling molecule (canonical signaling pathway)</td>
</tr>
<tr>
<td></td>
<td>Encodes SMAD8</td>
</tr>
<tr>
<td>CAV1</td>
<td>Encodes caveolin-1, relevant to caveolar structure as well as nitric oxide signaling</td>
</tr>
<tr>
<td></td>
<td>Exact estimate of frequency among PAH patients yet to be determined</td>
</tr>
<tr>
<td>KCNK3</td>
<td>Encodes TASK-1, a pH sensitive potassium channel</td>
</tr>
<tr>
<td></td>
<td>Exact estimate of frequency among PAH patients yet to be determined</td>
</tr>
<tr>
<td>EIF2AK4 (GCN2)</td>
<td>Association with recessive and sporadic forms of PVOD and PCH</td>
</tr>
<tr>
<td></td>
<td>Encodes a eukaryotic translation initiation factor superfamily member</td>
</tr>
<tr>
<td></td>
<td>Exact estimate of frequency among PVOD and PCH patients yet to be determined</td>
</tr>
</tbody>
</table>

All genes associated with autosomal dominant familial disease except for EIF2AK4, which is associated with autosomal recessive PVOD and PCH. HHT indicates hereditary hemorrhagic telangiectasia; IPAH, idiopathic pulmonary arterial hypertension; PAH, pulmonary arterial hypertension; PCH, pulmonary capillary hemangiomatosis; PVOD, pulmonary veno-occlusive disease; and TGF-β, transforming growth factor-β.
worse survival compared with BMPR2 carriers and with non-carriers with PAH.23

PAH in Families

Well before the discovery of genetic mutations associated with HPAH, it was observed that HPAH is a familial disease transmitted in an autosomal dominant manner. This suggests that heterozygosity for a gene mutation of a major impact is the basis in affected families. But, although an autosomal dominant disease, it does not affect all subjects at risk because of reduced penetrance.24,25 In addition, there is variation in penetrance both within and between families at risk. This reduced penetrance suggests that the presence of a PAH-specific gene mutation is necessary, but insufficient in itself, to cause HPAH.26 While the mechanisms that reduce penetrance are unknown, most investigators agree that it suggests that additional genetic or environmental or both factors modify expression of the disease and may provide clues not only to pathogenesis, but also to potential therapeutic targets.27 Larkin et al28 recently analyzed our HPAH research cohort at Vanderbilt University and calculated the overall penetrance of familial PAH to be 27%. However, consistent with the hypothesis that additional factors modify disease penetrance (such as sex), female penetrance was 42%, while the male penetrance was only 14%.

Several additional interesting features of HPAH highlight the variable expressivity of this disease. While the mean age of diagnosis is the mid-30s, it is highly variable.21 Subjects have been diagnosed at virtually any age in the life course, from early childhood to more than 70 years of age. In addition, while not a feature specific to the heritable form of PAH, HPAH does not attack males and females equally (≈2:1 female: male ratio).15,28

However, it is worth noting that one feature traditionally ascribed to HPAH has recently been refuted. Genetic anticipation, characterized by progressively earlier age of onset of a disease in subsequent generations, was suspected to occur among PAH families despite a lack of biological explanation.29-32 However, because of ascertainment bias, proof of genetic anticipation is difficult to achieve without the benefit of several decades of observation. Thus, in an effort to carefully demonstrate the phenomenon in our research cohort, Larkin et al33 recently performed linear mixed effects models of diagnosis by that occurrence, but observing late PAH onset in the daughter’s generation would require many decades of observation. Thus, current evidence does not support genetic anticipation in HPAH.

The Discovery of BMPR2 Gene Mutations in Familial PAH

Given the rarity of familial PAH, genetics research benefits immensely from collaborative research efforts and centralized centers for the maintenance of phenotypic and biospecimen data. In the 1990s, prior to the advent of next-generation sequencing, 2 teams working independently undertook hunts to test the hypothesis that a single gene was responsible for the majority of HPAH cases. They were successful in part because of the collaborative arrangements and large biorepositories at their disposal, which still exist today. Dr Jane Morse led an effort by Columbia University investigators, while an International PH Consortium (a collaborative composed of investigators from Vanderbilt University, University of Leicester, Cincinnati Children’s Hospital Medical Center, and Indiana University) was led by Drs Richard C. Trembath, Rajiv D. Machado, William C. Nichols, and James E. Loyd. Both groups initially used linkage analysis referenced to short tandem repeats and to other microsatellite markers to identify chromosome 2q31-33 as the region associated with PAH in the families studied.34,35 Within a few years, both groups identified BMPR2 as the gene of interest using different approaches. Unknown to the investigators at the time, heavily affected PAH family was highly genetically informative, and that individual family provided the original evidence suggesting linkage to 2q32 in both groups.36,37 Since these initial reports, BMPR2 gene mutations have been definitively associated with familial PAH, with now more than 400 different mutations in BMPR2 using methods as diverse as direct sequencing, melting curve analysis, DHPLC (denaturing high pressure liquid chromatography), Southern blotting, and multiplex ligation-dependent probe amplification.38 The precise BMPR2 mutation rate in the general population is unknown but thought to be exceedingly low.39 It is now known that mutations in BMPR2 are responsible for >75% of the cases of HPAH. Not surprisingly, the discovery of BMPR2 highlighted the relevance of the TGF-β superfamily of receptors and signaling to PAH. And a small percentage of familial PAH cases are also attributed to mutations in other TGF-β family receptor members or related downstream signaling proteins (eg, ACVR1/ALK1, endoglin/ENG, and SMAD9). While the remaining cases of HPAH that are negative for known mutations may well have as-yet unidentified alterations in genes in the TGF-β pathway such as SMAD9, attention has recently been directed to alternative novel gene mutations.39

As mentioned, mutations at 2 additional loci directly related to the TGF-β superfamily can cause the PAH phenotype in families, although this form of PAH results in conjunction with a broader heritable disease known as hereditary hemorrhagic telangiectasia (HHT). A vascular dysplasia characterized by mucocutaneous telangiectasias, recurrent epistaxis, and gastrointestinal bleeding, HHT is also associated with Group 1 PAH. HHT patients have other vascular abnormalities, however, including arteriovenous malformations of the pulmonary, hepatic, and cerebral circulations, but these findings may be cryptic or develop later in the course. The genes for additional members of the TGF-β signaling superfamily receptor complex, activin receptor-like kinase 1 (ALK1) located on Chromosome 12 and endoglin (ENG) on Chromosome 9, are known to associate strongly with HHT and HHT-associated PAH.40-43 The heterogeneity of loci for mutations in the TGF-β signaling pathway in patients with HPAH suggests that defects in this pathway promote pulmonary vascular disease leading to PAH.
This is not surprising, as the proteins receptors produced signal intracellularly via the Smad family of coactivators, as well as via some noncanonical pathways of signaling.\textsuperscript{44,45} Although the precise mechanisms have yet to be elucidated, it is evident that variations at different steps of signal transduction for 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.

**BMPR2 in Other Forms of PH**

The discovery of \textit{BMPR2} gene mutations in association with PAH in families highlighted the potential importance of the bone morphogenetic protein (BMP)/TGFβ signaling axis in PAH. Given the similarities between PAH in families and IPAH, naturally, investigators immediately evaluated IPAH cases for an association with the \textit{BMPR2} gene despite the absence of familial association. This absence could be explained in many ways, including de novo germline mutations not present in other family members, reduced penetrance, insufficient data for family history, and misdiagnosis.\textsuperscript{46} As expected, a proportion of IPAH cases do have detectable \textit{BMPR2} gene mutations; while the reported proportions are variable, in general =15% of IPAH patients (6–40%) actually have HPAH because of a \textit{BMPR2} gene mutation.\textsuperscript{46–49}

It is important to note that given that truly idiopathic PAH is 10 to 15 times more common than familial PAH, the vast majority of patients with \textit{BMPR2}-associated PAH actually have what would otherwise be classified as IPAH. This point highlights the notion that a significant percentage of IPAH cases actually have a heritable disease for which genetic counseling and family screening should be of consideration. In PAH families, the risk is usually obvious and other members are aware, but PAH patients with a negative family history have no clinical basis to suspect risk to their family members.

Mutations in \textit{BMPR2} and other TGFβ-related genes have not been consistently found in other causes of PH, with some exceptions. For example, some but not all patients with pulmonary veno-occlusive disease (PVOD), a rare form of PH in which the vascular changes also affect small pulmonary veins and venules, possess detectable germline mutations in \textit{BMPR2}.\textsuperscript{48,50,51} The detection of \textit{BMPR2} mutations in PVOD cases may highlight the clinical heterogeneity that may result from a \textit{BMPR2} mutation; that is, perhaps different allelic mutations at a single genetic locus may produce different disease phenotypes. Alternatively, it is possible that PAH and PVOD represent different ends of the same spectra of disease, with the pulmonary blood vessel of primary disease (artery versus vein) influenced by additional genetic or environmental or both modifiers.\textsuperscript{50}

\textit{BMPR2} gene mutations were also detected among those subjects with stimulant-related PAH, such as those with PAH because of exposure to appetite suppressants including fenfluramine and dexfenfluramine.\textsuperscript{52,53} While the precise mechanism remains elusive, these drugs may serve as environmental triggers to promote PAH, possibly in genetically susceptible individuals. Individual factors of susceptibility, or protection, are particularly plausible, given that despite a relatively high exposure rate, the rate of PAH development among stimulant users is quite low (=1 case per 10000 people exposed to fenfluramine, for example).\textsuperscript{18} Investigators in France described nearly 10% of subjects with stimulant-associated PAH positive for a detectable BMPR2 gene mutation; however, because this proportion is similar to that in sporadic IPAH cases, this may suggest that the presence of a gene mutation is unrelated to the drug exposure. But, it is worth noting that compared with other patients with PAH associated with fenfluramine exposure, \textit{BMPR2} mutation carriers expressed disease after a significantly shorter interval of exposure to fenfluramine.\textsuperscript{54}

While a global published assessment of additional groups with PH is lacking, \textit{BMPR2} has been explored in congenital heart disease-associated PAH to some extent. While mutations are present at proportions higher than the population at large, the number of subjects with \textit{BMPR2} mutations remains small. Specifically, Roberts et al\textsuperscript{55} detected \textit{BMPR2} variations in 6% of 106 children (n=66) and adults (n=40), while in a Thai cohort Limsuwan et al\textsuperscript{56} found no \textit{BMPR2} mutations among 30 children. However, no studies have been published using the expanded genetic analyses currently in use including comprehensive assessment for large gene deletions and duplications, which may uncover additional mutations.\textsuperscript{57} Given the importance of the BMP pathway to embryological development of the cardiovascular system, additional studies are needed for both the development of CDH and associated PAH. This may be particularly true for atrioventricular canal or septation defects.\textsuperscript{58}

It seems unlikely that mutations will be found in high proportions for other PH groups. For example, \textit{BMPR2} mutations were not found in small reports of patients with PAH associated with scleroderma or in HIV-infected patients with PAH.\textsuperscript{59,60} No \textit{BMPR2} mutations were detected in a larger series of 103 patients with chronic thromboembolic PAH.\textsuperscript{61}

**Molecular Ramifications of a \textit{BMPR2} Gene Mutation**

While the association of \textit{BMPR2} gene mutations with HPAH is no longer of dispute because of the genetic epidemiology available, it is surprising that to date we still do not understand why \textit{BMPR2} mutation carriers develop PAH. For example, \textit{BMPR2} mutations do not all have the same impact on cell signaling, and there are cell-specific variations even within the pulmonary vasculature. Pulmonary vascular endothelial cells seem dysfunctional and more susceptible to apoptosis in the presence of a \textit{BMPR2} mutation.\textsuperscript{62} However, pulmonary vascular smooth muscle cells with \textit{BMPR2} mutations have a failure of growth suppression. It is unclear whether this proliferative phenotype is attributable to a \textit{BMPR2} mutation, or more generically attributable to an increased release of growth factors that promote exuberant smooth muscle cell proliferation by dysfunctional endothelial cells.\textsuperscript{63}

In addition, each mutation type is different and may promote a state of either haploinsufficiency (insufficient protein product and function) or a dominant negative (overly deleterious protein action) effect. Of note, Bmpr2 knockout rodents do not develop PAH, and dominant negative Bmpr2 mutations knocked in to rodents required an additional insult to improve disease penetrance.\textsuperscript{64} While some data suggest that
dominant negative mutations cause a more severe phenotype, whether reproducible phenotypic differences will emerge is unknown.\textsuperscript{21,65} A true and comprehensive understanding of the functional impact of \textit{BMPR2} (and other gene) mutations on the pulmonary vasculature remains a work in progress.

While \textit{BMPR2} mutations associated with HPAH are germline and thus presumably present in every cell in the body, the pulmonary vasculature is the site of clinically manifest pathology—this has prompted a long-standing question of why is only the pulmonary vasculature abnormal? This question remains unclear, as there are no consistently reported obviously vascular or other anatomic abnormalities associated with a \textit{BMPR2} gene mutation. This is particularly striking, given the known systemic vascular lesions associated with mutations in other TGF\(\beta\) superfamily genes, such as SMAD3 mutations with aortic aneurysms, as well as fibrillin 1 and other mutations that cause excessive signaling by the TGF\(\beta\) family of cytokines associated with marfan syndrome.\textsuperscript{66,67} One possibility is the existence of a lung-specific susceptibility to a disturbance of the presumed balance between canonical TGF\(\beta\) signaling and BMP signaling—reduced BMP signaling in the setting of normal or enhanced TGF\(\beta\) signaling promotes PAH pathogenesis.\textsuperscript{68,69}

However, there is now a growing body of data to suggest that while the pulmonary circulation is the site of primary pathology, \textit{BMPR2} mutation carriers do have systemic irregularities that may contribute to PAH pathogenesis or maladaptation to ventricular stress. For example, insulin resistance is present as an early feature of Bmpr2 mutation in a dominant negative murine model of PAH, and impaired right ventricular hypertrophy with abundant triglyceride deposition is present in those same mice.\textsuperscript{70,71} Consistent with this in human patients, Hemnes et al\textsuperscript{71} recently demonstrated enhanced right ventricular lipid deposition as well as right ventricular defects in fatty acid oxidation. Intriguingly, this may represent a more generalizable manner in which insulin resistance, BMP insufficiency, and PAH intersect.\textsuperscript{72,73}

Current Food and Drug Administration–approved therapeutics for PH do not intentionally include agents that directly modify genetic variants, or their consequences, such as \textit{BMPR2} gene mutations. However, such interactions may exist. Also, there is some evidence to suggest that \textit{BMPR2} gene mutations result in disruption of pathways related to currently available therapeutics. For example, patients with \textit{BMPR2}-PAH may have alterations in the endothelin receptor cascade inherent to the presence of the mutations; this could have ramification not only for disease susceptibility but also for pharmacological susceptibility to endothelin receptor antagonists currently used.\textsuperscript{74} Conversely, treprostinil, a stable prostacyclin analog, inhibits the TGF-\(\beta\) pathway by reducing SMAD3 phosphorylation; this is relevant because exuberant SMAD3 phosphorylation is thought to enhance PAH susceptibility in those with a \textit{BMPR2} gene mutation or those with insufficient BMPR2 activity.\textsuperscript{65,68} Meanwhile, sildenafil enhances BMP signaling and partly restores deficient BMP signaling in the setting of a \textit{BMPR2} gene mutation in vitro.\textsuperscript{76} These findings demonstrate that deficient BMP signaling may be related to current PH-specific pharmaceuticals and suggest that more work to target BMP signaling may reveal more beneficial compounds.

### HPAH Not Attributable to Mutations in the TGF\(\beta\) Superfamily Related Genes

As stated, \(\approx\)20\% of families lack detectable mutations but clearly demonstrate familial PAH characterized by autosomal dominant transmission. Recent progress in the development of next-generation sequencing platforms has facilitated the opportunity to perform broad, unbiased, evaluations of the exome (and genome) to search for additional mutations which strongly associate with human disease.\textsuperscript{77} The application of this approach to detect rare variants (mutations) of large impact has prompted the recent discovery of several novel, but biologically plausible, loci which associate with HPAH and may contribute to disease pathogenesis more broadly. In both cases, whole-exome sequencing (WES) was used successfully, with the identification of 2 new PAH-associated genes: \textit{KCNK3} and \textit{CAV1}.

Mutations in the gene \textit{KCNK3} (Potassium Channel, Subfamily K, Member 3), which encodes the human TASK-1 protein, seem to be the more frequent of the 2 new genetic associations. \textit{KCNK3} was reported recently by Ma et al\textsuperscript{78} based on a collaborative WES study of unrelated PAH families without known PAH gene mutations. Ultimately, 3 PAH families possessed a deleterious missense mutation in \textit{KCNK3}. After screening a large number of IPAH cases, 3 unrelated IPAH patients were found to possess different missense mutations predicted to have damaging consequences. Each mutation discovered occurred in a highly conserved region of the gene and resulted in loss of function according to electrophysiological studies. Intriguingly, function was partially restored by pharmacological manipulation in vitro.

The discovery of \textit{KCNK3} was buoyed by strong biological plausibility because it encodes TASK-1, which is a pH-sensitive potassium channel in the 2 pore domain superfamily.\textsuperscript{79} Ion channels have long been of interest in the pulmonary vascular field, given their potential role not only in vasoconstriction but also in vascular remodeling. While work continues in this area to clarify the biology, there is likely a complicated interplay among ion channels to regulate membrane depolarization via calcium. For example, it seems that reduced potassium channel activity may facilitate calcium-mediated vasoconstriction, which may provide one explanation for the association between \textit{KCNK3} mutations and PAH.\textsuperscript{80} Not surprisingly, for a variety of reasons including the potential channelopathy, most \textit{KCNK3} mutants described to date lack response to vasodilator testing. While an independent replication has yet to be published, the \textit{KCNK3} discovery, in concert with considerable prior background ion channel research, may propel novel therapeutics because pharmacological manipulation of currents through TASK-1 channels is possible.\textsuperscript{81}

A virtually identical approach was taken by the same collaborative team to study 4 PAH patients from another large family without detectable genetic mutations in the TGF-\(\beta\) pathway, again using WES. In this family the mutation of relevance was ultimately determined to be a rare variant in the caveolin-1 (\textit{CAV1}) gene, at a highly conserved region...
with a high likelihood of detrimental functional consequences. Subsequent evaluation of an additional 62 unrelated PAH families and 198 IPAH patients (all without detectable BMPR2 mutations) uncovered the independent finding of a de novo CAV1 mutation in an unrelated child with IPAH (both mutations described were frameshift mutations in exon 3: c.474delA [P158P fsX22] and c.473delC [P158H fsX22]). As with the KCNK3 discovery, the variants were genotyped in more than 1000 ethnically matched white, European controls and were not identified in any healthy individuals, supporting the association with PAH.82

While the number of known CAV1 mutants with PAH is low, as with families with TGF-β receptor mutations and KCNK3 mutations, CAV1 mutations seem to associate with PAH with reduced penetrance and variable expressivity. As with TGF-β and KCNK3, biological plausibility for CAV1 is high. Its protein product, caveolin-1, is a membrane protein required for 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.83–89 Caveolae are abundant in endothelial, adipocyte, mesenchymal, and other cell types.90,91 As with KCNK3, prior research had implicated CAV1, as haploinsufficient mice have airway and pulmonary vascular abnormalities, and expression of caveolin-1 in endothelial cells of the mice rescues many of these defects.85,87 In addition, reduced caveolin-1 endothelial cell staining and expression had been previously reported to occur in the lungs of PAH patients.96,100 But while the finding that CAV1 is mutated in human PAH does not come as a surprise, its specific role in PAH pathogenesis remains incompletely understood. Intriguingly, caveolin-1 seems to modify TGF-β signaling including a reduction in BMP signaling and, separately, reduction in caveolin-1 is associated with hyperactivation of STAT3 which can directly dampen BMP signaling—both these findings provide a mechanistic link between CAV1 and BMPR2 mutations in the pathogenesis of PAH.73,94 In addition, caveolin-1 directly reduces endothelial nitric oxide synthase activity, and loss of caveolin-1 prompts pathological exuberant endothelial nitric oxide synthase activity such that mice null for caveolin-1 develop PH.102 Recent human and rodent investigations suggest that activated CD47 suppresses caveolin-1, which allows uncoupled endothelial nitric oxide synthase to produce pathological reactive oxygen species that promote PAH.93,96,100,101,105 As with KCNK3, the distinct but potentially interwoven mechanisms by which caveolin-1 deficiency alters cell function may be a prime target for novel therapeutic development.

Novel Gene Discoveries Related to the PAH Phenotype

The clinical presentation of rare subsets may be identical to that of PAH. PVOD and pulmonary capillary hemangiomatosis (PCH) have PH which may be difficult to distinguish from each other and from PAH. As such, the current classification scheme contains these diagnoses together in a single subcategory of Group 1 PAH. This subcategory is labeled as 1′: PVOD or PCH or both.12 Recent findings further validate their inclusion together.

Two separate investigative groups recently performed WES to identify novel mutations successfully in the same gene associated with PVOD and PCH. By studying different unrelated families, both groups reported that EIF2AK4 (also known as GCN2) gene mutations associate with familial disease transmitted in an autosomal recessive form. EIF2AK4 encodes Eukaryotic Translation Initiation Factor 2 Alpha Kinase, a serine-threonine kinase. In the recessive form, unlike BMPR2/KCNK3/CAV1 mutations and familial PAH (an autosomal dominant disease), patients possessed deleterious gene mutations for both alleles of the EIF2AK4 gene. In addition, PVOD and PCD patients with negative family history were also associated with mutations in this gene, suggesting previously unrecognized heritable disease.

Investigators in France focused their study on recessive PVOD and found biallelic mutations in EIF2AK4 present in all 13 families studied. In addition, 5/20 (25%) of sporadic PVOD cases had biallelic mutations, similar to the percentage of IPAH cases with a single-allele BMPR2 mutation to explain their PAH. In total, 22 distinct EIF2AK2 mutations were detected, all of which seemed detrimental to gene function, and strong background evidence was provided to support the notion that EIF2AK2 mutations are a common basis for PVOD. While penetrance of the gene mutation was difficult to assess because of low numbers, as with BMPR2 (and ALK1) and autosomal dominant PAH, patients with EIF2AK4 mutations had variable age at diagnosis but were significantly younger than PVOD patients without mutation.104 Not surprisingly, 2 patients were initially diagnosed with PCH, which can be difficult to distinguish clinically and histopathologically from PVOD.105 In fact, the authors further support their contention that PCH and PVOD represent the same disease.

Independently, a US collaborative team was focused on autosomal recessive PCH cases and discovered biallelic EIF2AK4 gene mutations, again using a WES approach. In addition to a family with recessive PCH, EIF2AK4 gene mutations were detected in 2/10 (20%) sporadic PCH cases. Not surprisingly, for some of these patients, distinction from PCH and PVOD was challenging at the time of phenotypic determination.106

The discovery of this novel genetic association for PVOD and PCH supports the assertion that these 2 disease entities represent a single disease spectrum. This role for genetic findings to assist with complicated phenotypic classification is relatively new to the field of PH. EIF2AK4 mutations also confirm the heritable nature of these diseases and may provide critical consideration for genetic counseling of these patients in the future. Hopefully, novel molecular and therapeutic advances will rapidly emerge, as well as extension of these studies to additional patients with PVOD and PCH.

Beyond Heritable Gene Mutations: Genetic Modifiers and Novel Associations With Human PAH

It is clear that no genetic variation, be it rare mutation or common variation, will occur in isolation in a given person. There is growing information to suggest that additional genetic and nongenetic factors exist and modify the development (or not)
of PAH among susceptible individuals (Figure 1). Among those with a BMPR2 gene mutation, the lack of complete penetrance implies that a mutation in the BMPR2 gene is required but insufficient alone for phenotypic expression. Among those without a single PAH gene mutation, alternative genetic risk factors likely exist. During the past 15 years, multiple candidate genes and genetic factors have emerged although all require additional investigation or have not yet been convincingly replicated. Here we briefly present several promising areas of investigation which may ultimately shed light on PAH pathogenesis.

**Activity of the Wild-Type BMPR2 Allele**

There is an accepted reduction in BMPR2 immunostaining from the lungs of patients with familial and idiopathic PAH regardless of BMPR2 mutation status. Thus, factors that regulate the production of BMPR2 protein by mutated and wild-type BMPR2 alleles may be relevant. Examining subjects with BMPR2 mutations, Hamid recently demonstrated that the level of production of BMPR2 transcript and protein by the wild-type allele was associated with disease penetrance in the setting of a haploinsufficient BMPR2 mutation. In that situation, the wild-type BMPR2 allele was the major determinant transcript and protein production. Mutation carriers with HPAH had lower wild-type BMPR2 transcript levels compared with unaffected mutation carriers with the same mutation PAH. Thus, BMPR2 production by the wild-type allele seems to modify disease penetrance among genetically susceptible individuals and might be a novel therapeutic target for disease prevention. It may also explain the virtual absence of BMPR2 protein detectable by immunohistochemistry of the lungs from HPAH patients with a BMPR2 mutation. Work is currently being done to evaluate this finding more broadly among BMPR2 mutation carriers and other subjects at risk of PAH.

**Somatic Lung Mutations**

While the traditional approach in the PAH field is to assess for inherited germline mutations in BMPR2 and other genes, Aldred et al recently studied the lungs from 2 BMPR2 mutation carriers with HPAH in the search for a second hit which may occur de novo in the lungs. They found a somatic mutation within chromosome 13 in a location that includes the SMAD9 gene in 1 subject, suggesting an additional insult to BMP signaling. While this novel finding has yet to be replicated, it supports the concept that somatic mutations in the lungs could promote or modify disease penetrance among susceptible individuals, which is a concept well described in other fields, such as cancer biology.

**Common Genetic Variations: CBLN2**

Perhaps the most promising common genetic variation described to date with regard to PAH pathogenesis may be that which emerged from a recent multinational genome-wide association study (GWAS) of familial and idiopathic PAH cases without BMPR2 gene mutations. In this search for common genetic variations associated with PAH led by French investigators, 2 independent case-control studies were undertaken including 625 PAH cases and 1525 healthy control subjects. Germain et al identified a significant association at the 18q22.3 locus, with an odds ratio for PAH of nearly 2.0. They focused their finding on the CBLN2 gene, which belongs to the cerebellin gene family related to secreted neuronal glycoproteins. While not previously associated with lung disease, they found that mRNA levels of CBLN2 were significantly higher in PAH lungs compared with controls, as well as from cultured PAH-derived endothelial cells. While considerable additional work will be needed to determine the precise role of CBLN2 in PAH, it is believed that it may modify cellular proliferation locally within the lung.

**Common Genetic Variations: Sex Hormones**

PAH has long been known to preferentially affect females more than males, which suggests that factors associated with sex contribute to pathogenesis. While chromosomal differences (XX versus XY) or aberrant X-inactivation may contribute, there is a paucity of supportive data. However, there is a growing body of literature to implicate sex hormones in PAH pathogenesis epidemiologically as well as based on in vitro, in vivo, and human data. For example, MacLean et al and White et al used a genetic-based model of rodent PAH, using manipulation of the serotonin transporter (SERT), to develop a model of PAH which demonstrated female excess. They used hypoxia to show that female mice that overexpress the SERT (SERT+ mice) exhibit PAH and exaggerated hypoxia-induced PAH, while male SERT+ mice do not. Furthermore, ovarian removal abolished the PAH in the female mice, while estradiol re-established the PAH phenotype. This model’s link of female sex hormones with enhanced serotonin activity...
presents an intriguing biological and epidemiological connection, in part because common genetic variations in SERT have been investigated previously in PAH with mixed conclusions.109–111,120,121

Aberrant sex hormone metabolism has been recently implicated in the pathogenesis of human PAH. Using expression arrays from BMPR2 mutants, West et al122 found a major difference in the gene CYP1B1. CYP1B1, which encodes an estrogen metabolizing enzyme, had 10-fold lower expression levels in female mutation carriers with PAH compared with those without PAH. CYP1B1 metabolizes parent compound estrogens to 2-hydroxy and 4-hydroxy metabolites.123 These metabolites are less estrogenic than the 16α-hydroxy metabolites, which seem to be mitogenic and proproliferative.124,125

We followed up this work to investigate common genetic polymorphisms in CYP1B1 linked to estrogen metabolite levels. While a larger replication study is underway, the results were consistent with West’s array study. BMPR2 mutation penetrance was 4-fold higher for those homozygous for the less active N/N CYP1B1 allele compared with those who were heterozygous (N/S) or homozygous (S/S) for the Asn453Ser polymorphism. In the nested case–control portion of the study, the 2-OHE/16α-OHE, ratio was 2.3-fold lower among female HPAH patients compared with the healthy mutation carriers.126 Of course, the influence of female and male sex hormones and their metabolites is likely much more complicated than this study could adequately represent. But there are now data to suggest that sex hormones and their metabolites themselves contribute to BMPR2-mediated PAH.

Other work by Roberts et al127 simultaneously supported the role of sex hormones in the pathogenesis of portopulmonary hypertension, another form of PAH. They found that common variations in genes related to both estrogen signaling and cell growth regulators associated with portopulmonary hypertension, including the gene coding for estrogen receptor 1. In addition, for the gene that encodes aromatase (CYP19A1), which is the rate-limiting step in the conversion of androgens to estradiol, common polymorphic variation was associated with increasing levels of estradiol in a dose-dependent fashion, regardless of sex.

**Epigenetics in the Pathogenesis of PAH**

The wide variety of causes of PAH, and the lack of unifying DNA variants across all causes, even among those with familial PAH, suggest that non–DNA based cellular memory contributes to PAH pathogenesis. There is thus growing interest in the contribution of epigenetic mechanisms. These heritable, often self-perpetuating yet reversible variations may be of a variety of types such as CpG island methylation by DNA methyltransferases, noncoding RNAs, and perhaps histone modification. For example, Archer et al128 recently identified CpG island hypermethylation as an epigenetic cause of mitochondrial superoxide dismutase-2 deficiency in experimental PH, consistent with prior human lung data with superoxide dismutase-2 reduction. In addition, there is tremendous current interest in the contribution of microRNAs (miRs) to the pathogenesis of PAH. Many miRs have been implicated in human PAH to date (eg, miR-17/92 cluster, miR-26a, miR-27a, miR-124, miR-145, miR-150, miR-204, miR-206) with some but not all related to alterations in BMP signaling or other pathways such as DNA damage and repair, although additional studies are needed.129–131

These and other avenues of progress in understanding the genetic and genomic factors that promote PAH in the susceptible, and theoretically not susceptible, individual provide tremendous opportunities for discovery and progress in the PAH field. The current era of next-generation sequencing provides tremendous opportunity to expand our understanding of PAH pathogenesis and hopefully lead to therapeutic and curative therapies. For example, the evaluation of large numbers of subjects by whole-exome, whole-genome, and RNA sequencing techniques offers the promise that one day very soon comprehensive systems biology approaches will provide new breakthroughs. We should
have the capacity to overlay complicated layers of informative data together to provide impactful understanding of all types of PAH, regardless of subtype.

Genetic Testing for PAH

Genetic testing for known mutations in PAH-associated autosomal dominant genes is available in North America and Europe for the BMPR2, ALK1, ENG, SMAD9, CAV1, and now KCNK3 genes (Figure 2). There currently is no unified PAH mutation panel incorporating all genes in North America, but one may emerge soon. Unless there is a known family history of HHT or a strong clinical suspicion of HHT, clinical mutation testing specific to PAH should begin with testing for BMPR2 mutations given the higher prevalence. Aside from familial and idiopathic PAH, no other forms of PH justify clinical mutation testing at this time. Incorporation of testing for common genetic variants into the clinical testing approach is also not recommended at present.

Provision of genetic counseling by trained professionals is vital before and after undertaking clinical genetic testing. Pre-test informed consent and counseling, supported by counseling at the time of result provision, should ensure that all involved understand the possible results of the testing and what these results might imply for both the patient and family members. Reduced penetrance is one of the many reasons why this is crucial. Furthermore, current mutation testing does not account for the contribution of alternative genetic and nongenetic modifiers of disease expression.

The pediatric PAH patient presents similar challenges with regard to clinical genetic testing, with some additional issues of consideration. In general, the notion of genetic testing is more prevalent within the broader context of complex pediatric disease, and thus testing for PAH-associated genetic variants may actually be more common in pediatric PAH although data in this regard are lacking. Overall, the same principles of genetic testing in pediatrics apply as for adults. However, it is critical to keep in mind that clinical genetic testing should be used for the evaluation of the patient and not strictly for the purposes of familial-based risk assessment. Because, while the detection of a PAH-associated gene mutation can be highly informative to assess familial risk for siblings, parents, etc, given the lack of autonomy of the child, this should not be the primary reason for genetic testing of the pediatric patient without extensive pre- and post-test counseling. More typically, mutation testing may be incorporated as part of a broader evaluation as to the pathogenesis of the PAH, although this is not a mandatory component of PAH evaluation.

For the asymptomatic offspring of a BMPR2 mutation carrier, there is a 50% chance of inheriting the BMPR2 gene mutation from the parent. A negative genetic screen is extremely reassuring—the absence of the implicated mutation takes the subject’s PAH risk to near zero. But the presence of a detectable mutation doubles lifetime pretest probability risk. Because the pretest probability of PAH is higher for a female (0.5x0.42=0.21) than a male (0.5x0.14=0.07), disease risk is not equal. For a male the detection of the family BMPR2 mutation changes the risk from ≈7% to 14% in a lifetime. For a female, the detection of the family BMPR2 mutation changes the risk from ≈21% to 42% in a lifetime. While additional studies are needed, many investigators suspect that the presence of a BMPR2 mutation associates with at least subtle pulmonary vascular developmental and functional abnormalities such as an enhanced pressure response to hypoxia.

Genetic testing for IPAH cases deserves consideration, although this topic is controversial in the field. For incident IPAH cases, the detection of a BMPR2 mutation could prompt substantial anxiety because of the concern for one’s family. The significant emotional stress both for the patient, who can experience what has been termed the guilt of heritability, and for informed family members can be a significant burden. In contrast, the discovery of a heritable disease in theory could provide the opportunity for family screening and closer observation in the hope of earlier disease detection.

However, there is no practice guideline for the management of individuals who have tested positive for a mutation but are currently without signs or symptoms of PAH. While needed, there have been no studies to determine the best strategy for screening and early detection of clinically significant disease. At a minimum, such individuals should have clinical noninvasive echocardiographic screening every 3 to 5 years. Compliance with this recommendation is unknown and has not been reported. There is a growing list of PH-specific therapies, some of which have been used extensively in subjects without PH (eg, sildenafil and tadalafil for erectile dysfunction), which one day could be used as a means of primary prevention for those at significant risk of developing PAH (eg, healthy BMPR2 mutation carriers in families with known familial PAH). However, there are no current data to support or refute such an approach. Primary prevention trials for PH-specific therapies are needed to determine what drugs to select, if they are helpful, and the optimal time to initiate. This is crucial because PH-specific therapies are expensive, complicated, disruptive to normal routines, and can have significant side effects.

Global Conclusions

Tremendous progress has been made to mature our understanding of the genetic basis of PAH since the initial descriptions of BMPR2 gene mutations in familial PAH. In particular, the next-generation sequencing and genomics revolutions currently underway have propelled progress during the past 5 to 10 years. However, fundamental questions remain to be answered. The future of PAH research must blend all data sources to provide a more comprehensive understanding of the complex biological networks and events that promote PAH in the susceptible individual. Such a systems-oriented multi-level framework will be critical as we recognize that genetic and other types of variations rarely occur in isolation. The inherent complexity of molecular events over time, in concert with environmental exposures, must be understood to ultimately determine the critical major and minor variations which intersect to promote a PAH phenotype. Only then can we optimally harness the genetic, and growing genomic, progress to modify this devastating human disease.
A Patient Asks Questions…

Why me? I am not aware of anybody else in my family ever being diagnosed with this disease, dying prematurely, or having similar symptoms.

Our patient is understandably concerned about the implications of her recent diagnosis of idiopathic pulmonary arterial hypertension (PAH). Despite a lack of family history, ≈20% of idiopathic PAH patients have a detectable mutation in a gene known to associate with PAH. This is most commonly a mutation in the gene bone morphogenic protein receptor type 2 (BMPR2). While the therapeutic implications of the detection of a BMPR2 mutation are not currently relevant to a patient with PAH, researchers are actively pursuing this issue for future therapeutic development.

I wish to have children. Should I be concerned about passing this disease to my children and are there any tests that I can take to know for sure?

Our patient wisely raises the issue of reproduction. There is an option to consider gene mutation testing, including BMPR2. This should be performed in concert with the patient’s PH physician and include informed genetic counseling perhaps with a genetic counselor to facilitate the discussion. If the patient elects to pursue mutation testing, and she does turn out to possess a mutation in BMPR2, this could be of concern for her children. Because BMPR2-associated PAH is an autosomal dominant disease, theoretically the inheritance of 1 BMPR2 mutation dramatically increases PAH susceptibility. However, because of reduced penetrance, possession of a BMPR2 mutation is not a guarantee that a person will ever develop PAH in their lifetime. The risk of developing PAH for those who have a pathogenic BMPR2 gene mutation is ≈20% (this risk is not equal for males and females, but we will use 20% for simplicity). Thus, a rough calculation of the risk of a person’s child to develop PAH because of a parent with BMPR2-associated PAH is as follows:

**Issue One:** 50% chance a parent with a BMPR2 mutation will pass that mutation to her child.

**Issue Two:** 20% chance a person with a BMPR2 mutation will develop PAH in their lifetime

**Mathematical Estimation:** 0.50×0.20=0.10=10% risk that the patient’s child will ever develop PAH.

Mutation testing for PAH-related genes should occur in a clinical laboratory experienced in the assays to detect mutations in these genes. Several such laboratories are now available on each continent for clinical genetic testing. Pre-test and postgenetic counseling is an important component of the testing for patients and their families.

It is also important to know that pregnancy by itself poses a risk for the mother with PAH, for which there are many reasons, including the fact that the retention of fluids in the body caused by pregnancy poses additional stress on the function of the right heart chambers, which is often already compromised at that point. The worsening in the heart function may put in danger the life of the mother and the fetus. While it is possible that a pregnancy in a patient with PAH can be completed successfully, it is considered a high-risk pregnancy and requires very careful management by many specialists, prolonged monitoring, and hospitalization.

For the case description, see introductory article by E.D. Michelakis, page 109.

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Disclosures

None.

References


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