

# Identifying Potential Mutations Responsible for Cases of Pulmonary Arterial Hypertension

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**Abstract:** Pulmonary Arterial Hypertension (PAH) is a progressive and devastating disease for which there is an escalating body of genetic and related pathophysiological information on disease pathobiology. Nevertheless, the success to date in identifying susceptibility genes, genetic variants and epigenetic processes has been limited due to PAH clinical multi-faceted variations. A number of germline gene candidates have been proposed but demonstrating consistently the association with PAH has been problematic, at least partly due to the reduced penetrance and variable expressivity. Although the data for bone morphogenetic protein receptor type 2 (BMPR2) and related genes remains undoubtedly the most extensive, recent advanced gene sequencing technologies have facilitated the discovery of further gene candidates with mutations among those with and without familial forms of PAH. An in depth understanding of the multitude of biologic variations associated with PAH may provide novel opportunities for therapeutic intervention in the coming years. This knowledge will irrevocably provide the opportunity for improved patient and family counseling as well as improved PAH diagnosis, risk assessment, and personalized treatment.

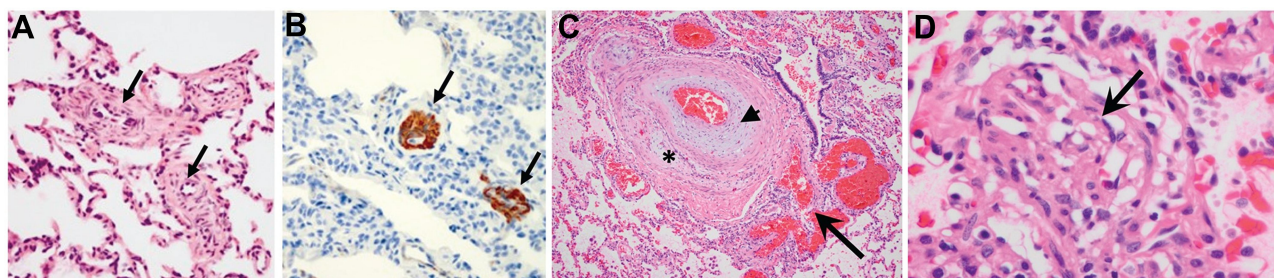
**Keywords:** PAH, pulmonary arterial hypertension, genes, mutations, BMPR2, bone morphogenetic protein receptor type 2

## Introduction

Pulmonary arterial hypertension (PAH) is a severe and life-threatening disorder of the pulmonary vasculature that is pathobiologically characterized by abnormal proliferation of endothelial and smooth muscle cells, and surrounding adventitial expansion leading to an increase in pulmonary vascular resistance which in turn increases afterload of the right ventricle (Figure 1).<sup>1-4</sup> Among the various groups of PH, Group 1 PAH includes idiopathic (IPAH), heritable (HPAH, formerly familial PAH) and PAH associated with a variety of other systemic disorders or drug/toxin exposures.<sup>3,5</sup> Despite remarkable advancements in the treatment over the past 30 years, PAH remains a fatal disease for incident cases characterized by increased morbidity and mortality.<sup>3,6</sup> Unfortunately, no single pharmacological agent tested to date has demonstrated the ability to reverse or least halt PAH, and there is as yet no prospect of cure of this devastating disease. Therefore, there is an urgent unmet need to further our pathobiological mechanisms and understanding to promote new therapeutic strategies and clinical practice.<sup>3,6,7</sup>

Since its initial descriptions (hemodynamically) in 1951 by David Dresdale et al as a clinical entity that could occur in either isolation (IPAH) or in families (HPAH), there has been significant progress in our understanding of the molecular

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**Figure 1** Typical histopathological characteristics of PAH.

**Notes:** (A, B) Lung tissue from a HPAH patient with a mutation in the *CAV1* gene (grades I and II). Hematoxylin and eosin staining may show pulmonary vascular smooth muscle cell proliferation, medial thickening of small pulmonary arteries (A, arrows), as confirmed by immunohistochemical staining of  $\alpha$ -smooth muscle actin (B, arrows).<sup>117</sup> (C, D) Lung biopsy from a HPAH patient with a mutation in the *KCNK3* gene. (C) Fibrosis (arrowhead), intimal proliferation, and recanalization (asterisk), with an adjacent angiomatoid lesion (arrow) typical of HPAH/IPAH (grade III). (D) Grade IV PAH disease may include plexiform lesions characterized by intimal and endothelial proliferation (arrow).<sup>118</sup> Copyright ©2017. John Wiley and Sons. Reproduced from Ma L, Chung WK. The role of genetics in pulmonary arterial hypertension. *J Pathol*. 2017;241(2):273–280.<sup>4</sup>

and genetic factors that promote PAH.<sup>3,8,9</sup> Although the exact pathobiological mechanisms responsible for both idiopathic and heritable forms of PAH (IPAH and HPAH) are still not fully understood, a number of potentially causative mutations in genes primarily related to PAH as well as genetic and epigenetic modifiers of disease expression have been discovered via advanced genetic and genomic techniques including but not limited to conventional linkage analysis and next-generation sequencing technologies.<sup>10</sup> The effects of these genetic risk factors may interplay with those of environmental factors and other signaling molecule to alter pulmonary vascular structure and function.<sup>4,6</sup> Understanding the genetic etiology of PAH as well as the molecular variants that modulate pulmonary vascular resistance should facilitate better diagnosis and development of novel therapeutic strategies and clinical practice in the future.

## Genetics of PAH

Familial cases of PAH have long been reported and are transmitted in an autosomal dominant manner, but HPAH does not affect all individuals at risk due to reduced penetrance.<sup>9,11,12</sup> Although the mechanisms which reduce penetrance are unknown, several features of HPAH highlight the variable expressivity of this disorder, and these include the fact that females are preferentially affected (~2:1 female: male ratio) but also the highly variable age of diagnosis.<sup>3,13,14</sup> HPAH accounts for about 6% of PAH.<sup>15</sup> This is likely an underestimate as a significant number of individuals with PAH that is heritable (HPAH) may actually be misclassified as IPAH due to reduced penetrance of the known PAH-associated genes, as well as de novo genetic mutations at conception.<sup>3,16</sup> In fact,

evidence suggests that up to 20% of cases previously classified as IPAH harbor identifiable mutations in PAH-associated genes and thus pose a hereditary risk to other family members.<sup>17</sup>

The first evidence of genetic contributions to PAH was identified following linkage analysis in which mutations in the gene encoding bone morphogenetic protein receptor type 2 (*BMPR2*), a member of the transforming growth factor-beta (*TGF- $\beta$* ) receptor superfamily, were responsible for approximately 75% of cases of HPAH and ~20% of patients with IPAH.<sup>15,18</sup> The estimated penetrance of *BMPR2* mutations is approximately 20% and may be regulated by the level of expression of the normal *BMPR2* allele,<sup>19</sup> which appears to be lower in female subjects compared with male with PAH.<sup>20</sup> In addition, patients with PAH and disease-causing *BMPR2* mutations are diagnosed and tend to die approximately 10 years earlier than those without mutation.<sup>3</sup> *BMPR2* mutation PAH subjects are also unlikely to respond to acute vasodilator testing during right heart catheterization and are thus less likely to benefit from therapy with calcium channel blockade.<sup>17,21</sup>

Since its initial descriptions, over 400 different mutations in *BMPR2* have been definitively associated with HPAH, highlighting the relevance of the *TGF- $\beta$*  superfamily of receptors and signaling to PAH.<sup>3,4,17</sup> Further genes related to *TGF- $\beta$*  superfamily receptor members or related downstream signaling molecules have been identified as uncommon causes of PAH in families, including but not limited to activin receptor-like type 1 (*ACVRL-1*), SMAD family member 4 (*SMAD4*) SMAD family member 8 (*SMAD8*; also known as *SMAD9*), and endoglin (*ENG*).<sup>4,22–28</sup> The *TGF- $\beta$*  family comprises a large series

of cytokine growth factors that are involved in the regulation of multiple cellular functions and homeostasis, among them endothelial mesenchymal transition, proliferation, differentiation, migration, apoptosis, and extracellular matrix secretion and deposition.<sup>17,29</sup> The implication of BMPR2, ALK-1, and ENG as causal genetic factors in HPAH has emphasized the vital role of this signaling pathway to the integrity of the pulmonary vascular bed.<sup>17,30</sup>

## HPAH Not Due to Mutations in the TGF $\beta$ Superfamily-Related Genes

Approximately 20% of families with demonstrable HPAH lack detectable mutations in the TGF- $\beta$  pathway.<sup>3</sup> This has led the scientific community to search for additional mutations which may contribute to PAH pathobiology. Recent application of whole-exome sequencing (WES) has allowed the discovery of several other novel, but biologically plausible PAH-associated genes, including but not limited to *CAVI* (involved in BMPR2 membrane localization and signaling) and *KCNK3* (a potassium channel that regulates resting membrane potential).<sup>31,32</sup>

## PAH Due to *KCNK3* Mutations

Mutations in the gene *KCNK3* (Potassium two-pore-domain channel, subfamily K member 3), which encodes the human pH-sensitive outwardly rectifying potassium channel, appear to be the more frequent of the two new biologically plausible PAH-associated genes.<sup>3</sup> Although genetic and electrophysiological data suggest that *KCNK3* (also known as *TASK-1*) mutation may be a rare genetic cause of HPAH and IPAH, its specific role in PAH pathobiology remains incompletely understood.<sup>4,32</sup> *KCNK3* is ubiquitous and highly expressed in animal and human pulmonary artery smooth muscle cells.<sup>4</sup> Regulation of ion channels is a hot topic in vascular physiology, given its crucial role in not only vasoconstriction but also vascular remodeling.<sup>3</sup> The function of *KCNK3* is to conduct leak  $K^+$  current, regulate pulmonary vascular tone and maintain the resting membrane potential.<sup>4</sup> Activation of *KCNK3* may cause  $K^+$  efflux, membrane hyperpolarization and vasodilatation.<sup>4,33–36</sup> A loss of function of *KCNK3* may thus promote calcium-mediated vasoconstriction, which may, at least in part explain to date lack of response to vasodilator testing.<sup>3</sup> Single nucleotide polymorphisms in another gene in the potassium channel family (*KCNA5*, potassium voltage-

gated channel subfamily A member 5) have also been identified in individuals with HPAH and IPAH.<sup>4</sup> Whether *KCNA5* may be a genetic risk factor for PAH and thus may play important roles in determining pulmonary vascular tone, cell proliferation, apoptosis and oxygen sensing remains unclear.<sup>4,37,38</sup> A recent meta-analysis including 7583 subjects indicates that *KCNA5* mutation may not represent a genetic susceptibility factor, at least for systemic sclerosis-associated PAH.<sup>39</sup>

## PAH Due to Caveolin 1 Mutations

Mutations in caveolin 1 (*CAVI*) are a rare cause of HPAH and IPAH.<sup>31</sup> *CAV1* encodes a membrane protein, which is required to form flask-shaped invaginations of the plasma membrane (known as caveolae) and plays a crucial role in mediating TGF- $\beta$ , G-protein and nitric oxide signaling in PAH.<sup>4</sup> Caveolae are ubiquitous and highly expressed in adipocytes, endothelial cells, and fibroblasts.<sup>40</sup> Mechanisms of *CAVI* mutation in HPAH and IPAH have been extensively evaluated. In experimental models, *Cav1* may be expressed in endothelial and epithelial cells of the septum which is located between the alveolar space and the pulmonary blood capillaries.<sup>41</sup> In humans, *CAVI* may be detected in the endothelium of arteries in the lungs.<sup>31</sup> Although heterozygous *CAVI* mutations have been identified in isolated PAH or PAH associated with lipodystrophy, its specific role in PAH pathobiology remains incompletely understood.<sup>40,42</sup> Evidence suggests that *CAV1* may modify TGF- $\beta$  signaling including an inhibition of BMP signaling pathway in various cell types, such as vascular smooth muscle cells;<sup>43,44</sup> and separately, reduction in *CAV1* may be associated with an up-regulation of STAT3 which may in turn, directly reduce BMP signal transduction—both these observations suggest a mechanistic link between *CAVI* and *BMPR2* mutations in the pathobiology of PAH.<sup>3,45,46</sup> Moreover, *CAV1* may inhibit endothelial nitric oxide synthase (eNOS) activity, and loss of *CAV1* may allow uncoupled eNOS to produce pathological reactive oxygen species that promote PAH.<sup>3,41,47–50</sup>

## PAH Due to Other Rare Gene Mutations

Several other new genes predisposing to PAH have been identified during the last decade. Eyries et al found that a loss-of-function mutation in the *KDR* gene may cause a particular form of PAH characterized by low diffusing capacity for carbon monoxide and radiological evidence of interstitial lung disease.<sup>51</sup> Chida et al identified two missense mutations in *NOTCH3* (which encode a group of 300-kD single-pass transmembrane receptors) in IPAH

patients.<sup>52</sup> The authors found that these mutations may be involved in cell proliferation and viability.<sup>52</sup>

## Rare Disease Alleles Underlying PAH

### Hereditary Hemorrhagic Telangiectasia

Hereditary hemorrhagic telangiectasia (HHT) is a rare autosomal dominantly inherited vascular dysplasia characterized by the appearance of mucocutaneous telangiectasias and arteriovenous malformations (AVMs), including AVMs of the pulmonary, hepatic, and cerebral circulations, but these lesions may be cryptic or develop later in the course.<sup>3</sup> The disease is caused by pathogenic mutations in *ENG* located on Chromosome 9 or *ACVRL1* located on Chromosome 12, which are identified in 80–85% of HHT patients; while *SMAD4* mutations, which are also associated with juvenile polyposis, are found in 1–2% of HHT.<sup>53,54</sup> Another genetic cause for HHT is mutations in Growth differentiation factor 2 (*GDF2*, previously known as bone morphogenetic protein 9, *BMP9*).<sup>55,56</sup> Mutations in *GDF2/BMP9* have been identified in HHT-associated PAH as well as isolated PAH.<sup>4,57</sup> Wang et al performed an exome-wide gene-based burden analysis on two independent case-control studies, including a total of 331 IPAH cases and 10508 controls, and identified rare bone morphogenetic protein 9 (*BMP9*) mutations in 6.7% of the cases, ranking this gene second to *BMPR2*.<sup>58</sup> The authors also demonstrated that the *BMP9* mutations led to impaired *BMP9* secretion and reduced anti-apoptosis ability in pulmonary vascular endothelial cells.<sup>58</sup> It is estimated that roughly one-third of HHT patients may have pulmonary AVMs, and a small proportion (<1%) of HHT subjects may have PAH that is clinically and histopathobiologically indistinguishable from other HPAH, while others have PAH secondary to pulmonary arteriovenous fistulas.<sup>4,30</sup> Mutations of *ACVRL1* appear to be the most likely underlying causative factor in these individuals.<sup>4</sup> Up to 20% of all detected mutations in *ACVRL1* may be associated with the development of PAH, and, of these, 81% may have PAH.<sup>4,59,60</sup> In rare instances, mutations of *ACVRL1* may cause PAH without HHT.<sup>61,62</sup>

### Pulmonary Veno-Occlusive Disease and Pulmonary Capillary Hemangiomatosis

Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are rare causes of PAH with clinical presentation which may be

indistinguishable from each other, and from PAH.<sup>3</sup> As a result, the current WHO clinical classification combines these diagnoses in a single subcategory of Group 1 PAH, labeled as 1': PVOD and/or PCH.<sup>2,3,63</sup>

Eyries et al performed whole-exome sequencing and identified recessive mutations in *EIF2AK4* that may cosegregate with PVOD in all the 13 families evaluated.<sup>64</sup> *EIF2AK4* (also called *GCN2*) encodes Eukaryotic Translation Initiation Factor 2 Alpha Kinase, a serine-threonine kinase that belongs to a family of kinases that modulate angiogenesis in response to cellular stress.<sup>3</sup> The authors also reported biallelic *EIF2AK4* mutations in 25% of histologically confirmed sporadic cases of PVOD.<sup>64</sup> All identified *EIF2AK2* mutations disrupted the function of the gene, thus supporting the notion that *EIF2AK2* mutations may be the major gene that is linked to the development of PVOD.<sup>64</sup> Interestingly, the authors found that subjects with *EIF2AK4* mutations had variable age at diagnosis and were more likely to be younger than PVOD patients without the mutation.<sup>64</sup> Independently, Best et al performed an exome sequencing to identify a candidate gene for PCH and discovered biallelic *EIF2AK4* gene mutations as the likely cause of autosomal-recessive PCH in familial and some nonfamilial cases.<sup>65</sup>

The above suggested genetic association for PVOD and PCH further supports the notion that these two clinical entities may represent a single disease spectrum.<sup>3</sup> *EIF2AK4* mutations also indicate the potential heritable nature of these clinical entities.

### Pediatric PAH

Pediatric PAH includes IPAH, HPAH and PAH associated with other conditions such as congenital heart disease and abnormal lung development.<sup>4</sup> Although the genetics of pediatric PAH has not been extensively evaluated, evidence suggests that PAH in children may have a different genetic pathobiology from that in adults.<sup>4</sup> Grünig et al performed a systematic genetic and clinical family screening in 13 children with IPAH and found that none of the assessed children had mutations in the *BMPR2* gene or a history of HHT.<sup>66</sup> In a cohort of 54 patients with IPAH or HPAH whose onset of disease was at <16 years of age, 18 *BMPR2* and 7 *ACVRL1* mutation carriers were identified, and these had a worse outcome than mutation noncarriers.<sup>67</sup> A study of 50 patients with PAH associated with congenital heart disease (CHD) demonstrated that 26% of them may have *BMPR2* mutations.<sup>68</sup> In a mixed cohort of 40 pediatric PAH cases, including 29 IPAH/



HPAH and 11 PAH-CHD, mutations of the *BMPR2*, *ACVRL1*, and *ENG* gene, respectively, occurred in 27.5% of IPAH/HPAH patients and even in 18.2% with PAH-CHD.<sup>69</sup> The differences in genetic results in various PAH studies in children may be the result of a combination of factors, including but not limited to different genetic backgrounds, small sample sizes and selection criteria such as whether the PAH was associated with other diseases.<sup>4</sup> *Pediatric patients with BMPR2* mutations appear less likely to respond to acute vasodilator testing (thus unlikely to benefit from calcium channel blockade therapy) than mutation-negative subjects and may have more severe disease at diagnosis.<sup>70</sup>

TBX4 is a transcription factor in the T-box gene family, expressed in the mesenchyme of the lung, the limbs, and the atrium of the heart.<sup>4</sup> In a cohort of 20 consecutive pediatric cases with IPAH or HPAH and 49 adults with PAH, Kerstjens-Frederikse et al performed an array-comparative genomic hybridization analysis and found that *TBX4* mutations were associated with pediatric PAH, but that the prevalence of PAH in adult *TBX4* mutation carriers was low.<sup>71</sup> Zhu et al performed a WES screen of 155 paediatric- and 257 adult-onset PAH patients and found that in addition to *BMPR2*, there was significant enrichment of *TBX4* (a gene linked to small patella syndrome) mutations in paediatric- compared with adult-onset subjects, and *TBX4* carriers had younger mean age-of-onset compared with *BMPR2* carriers.<sup>72</sup> Haarman et al performed a targeted next-generation sequencing to explore genotype-phenotype associations and outcomes in 70 children with HPAH/IPAH from the Dutch National registry and to explore genotype-phenotype associations and outcome.<sup>73</sup> The authors found *TBX4* variant carriers may have favorable outcome (higher survival rate) as compared with individuals carrying *BMPR2/ACVRL1/KCNK3*, or *EIF2AK4* mutations.<sup>73</sup> In a Spanish cohort of 165 adult-onset PAH, *TBX4*-related forms of PAH appeared to have a more benign course and late diagnosis was the only predictor of worse outcomes in HPAH.<sup>74</sup>

Several other new genes predisposing to pediatric PAH have been identified during the last decade. Bohnen et al performed an exome sequencing to identify novel genes in a cohort of 99 pediatric and 134 adult-onset group I PAH patients and discovered novel and rare missense variants in *ABCC8*, which encodes SUR1 (sulfonylurea receptor 1)-a regulatory subunit of the ATP-sensitive potassium channel.<sup>75</sup> Gräf et al perform whole-genome sequencing in 1038 PAH index cases and 6385 PAH-negative control

subjects and found novel mutations in *GDF2* (which codes the ligand for the endothelial *BMPR2/ACVRL1* receptor complex) and identified significant overrepresentation of rare variants in *ATP13A3* (a poorly characterised P-type ATPase of the P5 subfamily which loss of function may inhibit proliferation and increase apoptosis of endothelial cells), *AQP1* (codes for the Aquaporin-1 known to promote endothelial cell migration and angiogenesis, while its inhibition may ameliorate hypoxia-induced PH), and *SOX17* (which encodes the SRY-box containing transcription factor 17, known to promote angiogenesis and arteriovenous differentiation while its deletion may lead to impaired formation of pulmonary vasculature).<sup>76</sup> The majority of the causal *GDF2* variants detected in Gräf et al's cohort was associated with reduced production of GDF2 from cells.<sup>76</sup> As already hinted, *GDF2* gene encodes the circulating BMP9, which is a ligand for the BMP2 receptor.<sup>77</sup> *GDF2* mutations may result in BMP9 loss of function and are likely causal.<sup>77</sup> These observations raise the intriguing question of whether GDF2 replacement may be a therapeutic strategy in the management of, at least, some patients with HPAH/IPAH.<sup>78</sup>

Other genes may play an important role in pediatric PAH, including mutations in *BMPR1B*, which is one of the BMP type I receptors that interact with BMP type II receptors and mediates BMP signaling;<sup>79,80</sup> mutations in *NOTCH3*, which may be involved in vascular homeostasis and in the TGF- $\beta$  signaling network.<sup>52,81,82</sup>

## Npr3 as a Novel Gene for HPAH/IPAH

Despite advances in the science of genetics, there are still some patients with HPAH but without any known PAH-causing mutations, indicating there may be other physiologic candidate genes.<sup>17</sup> Evidence suggests that the *NPR3* gene encoding for the Natriuretic Peptide Receptor type C (NPR-C) may have an important role in the genetics of HPAH.<sup>83–86</sup> Although still commonly called a natriuretic peptide clearance receptor (and thus largely ignored),<sup>87</sup> evidence suggests that the *NPR3* gene may be a causative factor for skeletal abnormalities.<sup>83–86</sup> Mice with inactivated *NPR3* may exhibit striking skeletal deformities similar to those observed mice with *BMPR2*.<sup>88–92</sup> We have recently shown that mice lacking *NPR-C* exhibit echocardiographic and hemodynamic findings that are similar to those typically seen in humans with PAH.<sup>93,94</sup> Although the above data are intriguing, there is, of course,

no guarantee that identifying the causative genes for rodent will be relevant to human PAH.

## Genetic and Non-Genetic Modifiers of Risk for PAH

The complex clinical and epidemiological features of HPAH and IPAHA, such as variable age of disease onset both within and between families, female predominance and incomplete penetrance of dominantly inherited mutations, imply the existence of additional genetic and non-genetic factors capable of modulating the likelihood of developing PAH among susceptible subjects.<sup>95</sup> The lack of complete penetrance suggests that *BMPR2* gene mutation may be required but not sufficient to ensure phenotypic expression.<sup>3</sup> Hamid et al demonstrated that disease penetrance and phenotypic expression may be inversely proportional to the levels of expression of wild-type allele *BMPR2* transcript and proteins.<sup>19</sup> Although the traditional strategy in the PAH field is to evaluate for inherited germline mutations in *BMPR2* and other genes, Aldred et al performed genome-wide microarray copy number analysis on pulmonary artery endothelial cells and smooth muscle cells isolated from the lungs of two *BMPR2* mutation carriers with HPAH, in the search for a “second (somatic) genetic hit” which may occur *de novo* in the lungs.<sup>96</sup> The authors found a somatic mutation within chromosome 13 in a location that includes the *SMAD9* gene (which encodes the protein Smad-8, a downstream mediator of BMPR-II signaling) in one subject, suggesting an additional insult that may represent a second hit that further dysregulates the BMP signaling pathway.<sup>96</sup> This observation supports the notion that somatic mutations in the lungs may promote or at least modify PAH penetrance among susceptible subjects, which is a concept well described in cancer pathobiology.<sup>97</sup>

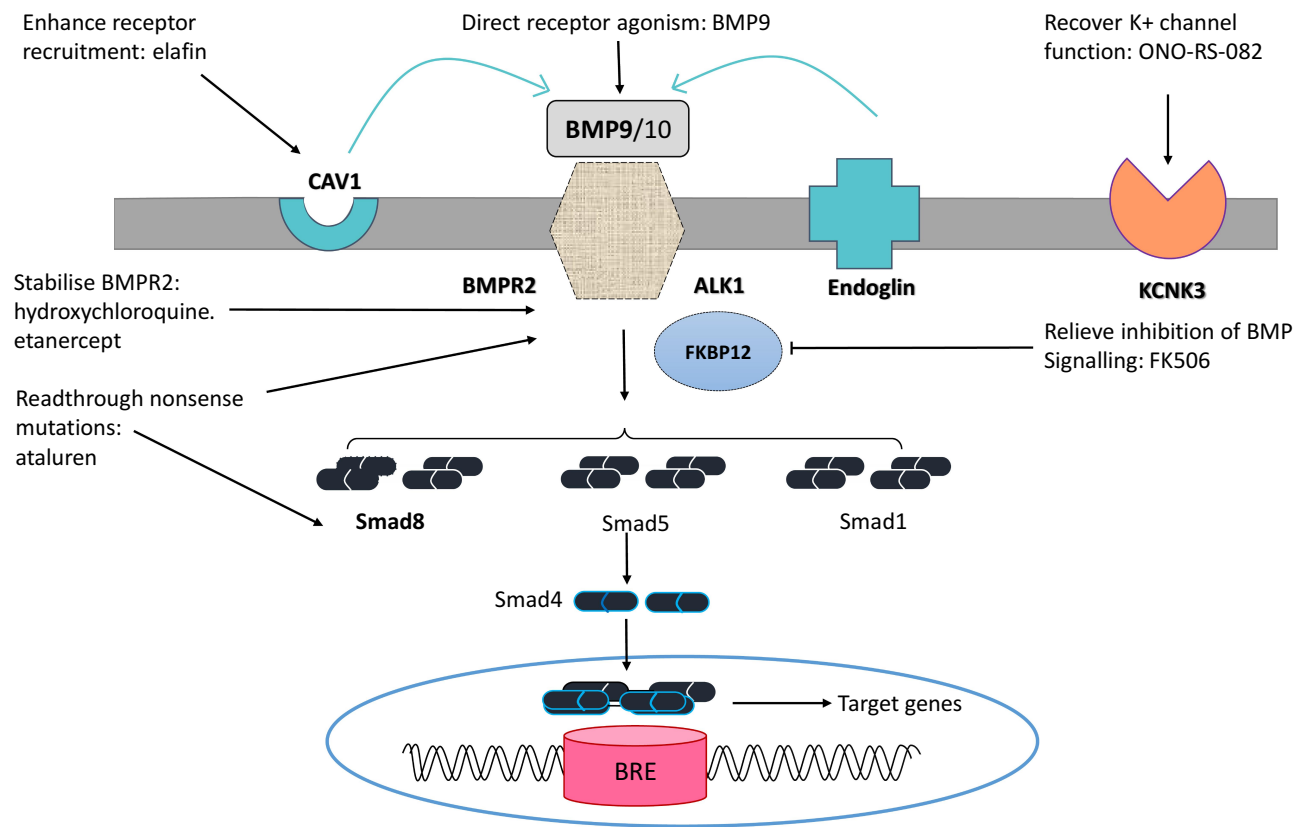
There are also common genetic variations that have been associated with PAH pathobiology. Germain et al conducted a genome-wide association study (GWAS) based on 2 independent case-control studies for *BMPR2* mutation-negative HPAH and IPAHA, including a total of 625 cases and 1525 healthy subjects.<sup>98</sup> The authors found a striking association at the *CBLN2* locus mapping to 18q22.3, with the risk allele conferring two-fold increased risk.<sup>98</sup> Interestingly, the authors found that mRNA levels of *CBLN2*, which belongs to the cerebellin gene family related to secreted neuronal glycoproteins, were significantly higher in explanted lungs from subjects with PAH and PAH-derived endothelial cells.<sup>98</sup>

As already hinted, HPAH and IPAHA preferentially affect females more than males, which suggests that sex hormones may modify and influence penetrance of PAH. White et al examined the influence of gender on the development of PAH as well as investigating how this is modulated by female hormones, using a genetic-based model of rodent PAH, which was developed by overexpressing the serotonin transporter (SERT).<sup>99</sup> The authors found that only female mice that overexpress SERT (SERT+ mice) developed PAH features, which were abolished by ovarian removal.<sup>99</sup> Following hypoxia exposure, only female SERT+ mice developed severe PAH features, which were also attenuated by ovarian removal.<sup>99</sup> Interestingly, chronic administration of estradiol re-established the PAH phenotype.<sup>99</sup> Consistently, West et al demonstrated that female but not male *BMPR2* mutation-positive PAH patients had tenfold lower expression of the estrogen metabolizing Cytochrome P450 1B1 (*CYP1B1*) gene than carriers unaffected by the disease.<sup>100</sup> In fact, lowered levels of *CYP1B1* may result in increased local estrogen level, which in turn may increase the risk of PAH phenotype.<sup>100</sup> Other work supported the role of common variations in genes and genetic polymorphisms related to both estrogen signaling and metabolism in the pathobiology of PAH.<sup>101,102</sup>

There is tremendous current interest in the role of epigenetics in PAH pathobiology. The first epigenetic basis for PAH was demonstrated by Archer et al.<sup>3,103</sup> The expression and activity of mitochondrial superoxide dismutase 2 (*SOD2*) are known to be reduced in the pulmonary artery smooth muscle cells of experimental PAH and humans with PAH.<sup>3,103</sup> The authors elegantly demonstrated that *SOD2* deficiency was not due to gene mutation, rather the *SOD2* gene was epigenetically silenced by hypermethylation of a CpG island in an enhancer region within intron 2 and the promoter of *SOD2*.<sup>3,103</sup> In addition, there is growing interest in the contribution of non-coding RNA such as microRNA (miRs) to the pathobiology of PAH, and tremendous progress has been made to mature our understanding of the integrative functions of these crucial molecular regulators in this disease.<sup>3,104–106</sup>

## From Genetics to Pharmacological Treatment

Recent evidence suggests that targeting molecular pathways highlighted by genetic studies may provide promising new approaches for the treatment of PAH (Figure 2). Long et al demonstrated that BMP9 administration may enhance



**Figure 2** From genetics to pharmacological treatment.

**Notes:** Bone morphogenetic protein receptor II, BMPR-II; BMP-responsive element, BRE; Caveolin-1, CAV1; 12-kDa FK506-binding protein, FKBP12. BMP-II signaling in pulmonary vascular endothelial cells may be mediated by the ligands BMP9 and BMP10 through the ALK1/BMPR2 receptor complex. Endoglin may serve as an accessory receptor. Pathway may be mediated via phosphorylation of the receptor Smads (Smad1, 5 and 8), which in turn may interact with Smad4 and translocate to the nucleus, modulating genes that contain BREs. CAV1 may promote receptor colocalization, while KCNK3 encodes a potassium channel that may enhance pulmonary vascular tone. Genes that are mutated in HPAH are in bold. Potential therapeutic strategies targeted to these signaling pathways may include: administration of BMP9 ligand, enhancing availability of functional BMPR2 receptors (hydroxychloroquine, etanercept), enhancing readthrough of nonsense mutations to restore functional BMPR2 or Smad8 protein (ataluren), promoting downstream signaling by relieving FKBP12 inhibition of BMP type I receptors (FK506), enhancing CAV1-mediated receptor recruitment (elafin), or recovering KCNK3 potassium-channel current (ONO-RS-082). Adapted with permission from Morrell NW, Aldred MA, Chung WK, et al. Genetics and genomics of pulmonary arterial hypertension. *Eur Respir J*. 2019;53(1):1801899.<sup>107</sup>

endothelial BMPR-II-mediated signaling and reverse established PAH in experimental models bearing a heterozygous knock-in of a human BMPR-II mutation as well as in other experimental PAH models.<sup>107</sup> The authors demonstrated that BMP9 not only enhances vascular stability and prevents apoptosis of the pulmonary arterial endothelial cells, but also promotes *BMPR2* gene expression, which may result in further enhancement of BMPR-II-mediated signaling models.<sup>107</sup> Therapeutic strategies may, therefore, include translational readthrough of premature stop codons or inhibition of lysosomal degradation of BMPR2.<sup>108</sup> Drake et al showed that the investigational drug, ataluren, not only may suppress a high proportion of BMPR2 and SMAD9 nonsense mutations in patients with HPAH, but also may correct BMP signaling in vitro.<sup>109</sup> Treatment with chloroquine and hydroxychloroquine may also enhance the

expression of BMPR-II protein and thus restore cell surface BMPR-II via lysosomal degradative pathway.<sup>110,111</sup> Other potential therapeutic approaches have been suggested in the literature.<sup>108</sup> The anti-tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) agent etanercept may reverse PAH progression by targeting not only the inflammation, but also by reducing BMPR-II cleavage in pulmonary artery smooth muscle cells.<sup>112</sup> The endogenous elastase inhibitor elafin may reverse the obliterative changes in pulmonary arteries via elastase inhibition and through caveolin-1-dependent amplification of BMPR2 signaling.<sup>113</sup> Downstream of BMPR2, the calcineurin inhibitor FK506 (tacrolimus) was found to potentiate BMP signaling and to reverse severe experimental PAH, by binding FK-binding protein 12, a repressor of BMP signaling.<sup>114</sup> Beyond the BMP signaling, Ma et al demonstrated that loss-of-function *KCNK3* mutations lead to reduced potassium-

channel current, which, at least for some mutations, may be remedied by the phospholipase A<sub>2</sub> inhibitor ONO-RS-082.<sup>32</sup> Although targeted mutational corrections may be challenging, the above studies make these potential therapeutic strategies a realistic future prospect.

## Genetic Testing for PAH

Clinical genetic testing for HPAH and IPAH is now available to assess risk for family members ie their hereditary predisposition; and in some cases, it is done as part of a broader evaluation as to the etiology of the PAH.<sup>3</sup> Clinical genetic testing can be offered to any subject with a family history of PAH or IPAH and pediatric PAH. Although still controversial due to the potential psychosocial burden both for the indexed subject as well as for the informed family members, the identification of an heritable disease may provide an opportunity for family screening and closer monitoring in the hope of earlier disease detection and earlier institution of therapy.<sup>3</sup> After weighing potential risks of genetic screening (psychosocial implications of the probability of developing a deadly disease for which there is no prevention and no specific cure) against potential benefits (including early detection and thus earlier initiation of treatment when indicated), the 6th World Symposium on Pulmonary Hypertension (WSPH) task force recommended that genetic screening be performed under the guidance of a clinical geneticist or genetic counsellor.<sup>108</sup> There are currently different ways of assessing the genetics of PAH-affected patients.<sup>115</sup> In addition to commercially available diagnostic PAH/PVOD gene panels, WES or WGS may also be considered for subjects with a negative PAH-causing gene panel.<sup>115</sup>

Although there are no studies to determine the optimal strategy for screening at-risk family members, patient education to ensure awareness of PAH-related symptoms, annual clinical examination and disease surveillance by echocardiogram at least every 3–5 years should be considered.<sup>116</sup> While there are currently no approved PH-specific therapies for asymptomatic subjects who have tested positive for a PAH-causing mutation, there is no evidence to suggest that early diagnosis will even improve the outcome of these individuals. Primary prevention trials are thus needed to determine what PH-specific therapies may be helpful, and the optimal time to initiate them.

## Conclusion

Since its initial description, limited progress has been made to mature our in-depth understanding of the complex biologic

basis of PAH, and this is, at least in part, largely due to its genetic heterogeneity, incomplete penetrance and sexual dimorphism.<sup>4</sup> As genetic and other types of inherent biologic variations rarely occur in isolation, major advancements can be expected in the next few years in the identification of additional genes as well as genetic and environmental modifiers for PAH. Larger genetic and biomarkers studies, with a close interplay of animal and human approaches will be necessary to better understand the complex genetic networks and events that promote PAH in genetically at-risk subjects.

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