Genetics and genomics of pulmonary arterial hypertension

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ABSTRACT Since 2000 there have been major advances in our understanding of the genetic and genomics of pulmonary arterial hypertension (PAH), although there remains much to discover. Based on existing knowledge, around 25–30% of patients diagnosed with idiopathic PAH have an underlying Mendelian genetic cause for their condition and should be classified as heritable PAH (HPAH). Here, we summarise the known genetic and genomic drivers of PAH, the insights these provide into pathobiology, and the opportunities afforded for development of novel therapeutic approaches. In addition, factors determining the incomplete penetrance observed in HPAH are discussed. The currently available approaches to genetic testing and counselling, and the impact of a genetic diagnosis on clinical management of the patient with PAH, are presented. Advances in DNA sequencing technology are rapidly expanding our ability to undertake genomic studies at scale in large cohorts. In the future, such studies will provide a more complete picture of the genetic contribution to PAH and, potentially, a molecular classification of this disease.

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Introduction

For half a century after the mid-1900s, when cardiac catheterisation first provided clinicians the ability to safely measure pulmonary haemodynamics, idiopathic pulmonary arterial hypertension (IPAH, then termed primary pulmonary hypertension (PPH)) was recognised as a lethal pulmonary vascular disease of enigmatic origin. Near the turn of this century, new understanding was developed of the specific genetic predisposition in families with PAH and that information was a driver to revise the classification of PAH by the 3rd World Symposium on Pulmonary Hypertension in 2003. Since 2000, investigators have contributed increasing knowledge of the genetics and genomics of PAH. Technological advances in genetic sequencing have enabled inexpensive and rapid sequencing of the coding regions of the genome (whole exome sequencing (WES)), or the whole genome (whole genome sequencing (WGS)), in families and large cohorts of patients (figure 1). Insights from human genetics are increasing our understanding of the pathobiology of PAH, identifying new potential drug targets, and informing the care of patients and their families.

State of the art

Mendelian inheritance

There is a family history of PAH in 6–10% of patients not associated with other underlying disorders [1]. In 2000, genetic analysis of such families identified heterozygous germline mutations in BMPR2, the gene encoding bone morphogenetic protein receptor type 2, a member of the transforming growth factor-β (TGF-β) superfamily [2, 3]. Subsequently, mutations were also identified in IPAH [4]. It is now well established that around 70–80% of families with PAH and 10–20% of IPAH cases are caused by mutations in BMPR2 [5].

Sequencing of genes encoding BMP receptor signalling intermediaries led to the identification of rare sequence variants in SMAD1, SMAD4 and SMAD9 [6, 7]. The identification of additional SMAD9 mutations in large cohorts has confirmed its role in PAH [8]. In addition, exome sequencing of BMPR2-negative individuals with more than one family member diagnosed with PAH revealed mutations in CAV1, which encodes caveolin-1 and functions to physically colocalise BMP receptors [9]. A rare mutation in CAV1 has been associated with both lipodystrophy and PAH in a young child. KCNK3 (potassium channel subfamily K member 3) mutations were also identified by exome sequencing and encode a potassium channel that contributes to the membrane potential to determine pulmonary vascular tone [10]. Array comparative hybridisation and sequencing in childhood and (rarely) adult-onset PAH identified deletions and loss-of-function mutations in TBX4 (T-box 4), a gene that is also associated with small patella syndrome [11]. Mutations in TBX4 are among the most common genetic causes of PAH in children and suggest that PAH is at least in part a developmental lung disease when it presents early in life [12, 13]. The recognition that severe PAH can also occur in families segregating hereditary haemorrhagic telangiectasia (HHT) implicated ACVRL1 (activin receptor-like kinase 1 (ALK1)) and ENG (endoglin) mutations in PAH [14-16].

Recently, a large survey was undertaken in a collaborative European cohort of adult-onset (>18 years) patients with IPAH, familial PAH (FPAH) and anorexigen-associated PAH [8]. This study of over 1000
that with right ventricular function [24]. Given the significant sex disparities in PAH and previous evidence warrant further study regarding a possible role in right ventricular remodelling in PAH.

Variants in novel genes may explain 19% of paediatric-onset IPAH cases [12]. The incomplete penetrance observed for PAH genes suggests that additional genetic, epigenetic and/or environmental factors contribute to disease risk/progression.

In 2014, biallelic mutations in EIF2AK4, a gene encoding eukaryotic translation initiation factor 2α kinase 4, were identified as a cause of heritable pulmonary capillary haemangiomatosis (PCH) [17] and pulmonary veno-occlusive disease (PVOD) [18]. PVOD and PCH are rare and pathologically distinct forms of PAH. Heritable PVOD and PCH are autosomal recessive and nearly completely penetrant, unlike other forms of PAH. Individuals diagnosed with apparently sporadic PVOD or PCH may also carry biallelic EIF2AK4 mutations in up to 25% of cases [17, 18]. Detection of biallelic pathogenic EIF2AK4 mutations establishes a precise and accurate molecular diagnosis of PVOD/PCH without requiring a lung biopsy [19].

A summary of the genes reported to date in patients with HPAH is shown in table 1. A high level of evidence needs to be established for the causal role of mutations in a particular gene before it is used in clinical screening and management.

### Common genetic variation

The role of common genetic variation contributing to the aetiology or clinical course of PAH is less well defined. To date only one genome-wide association study has been published to identify variants associated with PAH (n=625 cases). This study detected a significant association at the CBLN2 (cerebellin 2 precursor) locus mapping to 18q22.3, with the risk allele conferring an odds ratio for PAH of 1.97 (p=7.47 × 10^{-10}) [20]. Another large study evaluated associations between polymorphisms in genes comprising the endothelin signalling pathway and clinical outcomes in 715 PAH patients of European descent in the STRIDE study (sitaxsentan). An association was identified between a single nucleotide polymorphism (rs11157866) in the G-protein γ subunit gene GNG2 and a combined improvement in functional class and 6-min walk distance [21]. In a smaller study, levels of serum endostatin, a potent antiangiogenic protein with the capacity to induce endothelial cell apoptosis and inhibit endothelial cell proliferation, correlated with poor functional status and was a strong predictor of mortality in group 1 patients. In contrast, a missense variant (rs12483377) in COL18A1, which encodes endostatin, was associated with lower circulating protein and reduced mortality [22]. Abnormalities of mitochondrial metabolism are now well recognised in PAH. A recent study suggests that variants in the mitochondrial genome may influence the risk of developing PAH, with a lower rate of disease associated with haplogroup L, the oldest ancestral human haplogroup [23]. Lastly, in a study of 2761 healthy adults, variants in oestriadiol metabolism (CYP1B1 (cytochrome P450 1B1)) and androgen receptor genes were associated with right ventricular function [24]. Given the significant sex disparities in PAH and previous evidence that CYP1B1 variants may modulate disease penetrance in BMPR2 mutation carriers [25], these variants warrant further study regarding a possible role in right ventricular remodelling in PAH.

| Higher level of evidence | Lower level of evidence |
|--------------------------|-------------------------|
| BMPR2; EIF2AK4; TBX4; ATP13A3; GDF2; SOX17; AQP1; ACVRL1; SMAD9; ENG; KCNK3; CAV1 | SMAD4; SMAD1; KLF2; BMPR1B; KCNA5 |

Evidence includes de novo mutation, cosegregation studies, association with replication and functional studies.
Pathobiology links between mutations and disease

**PAH mutations and disease pathobiology**

BMPR2 is expressed on the surface of a wide number of cells, but it is particularly highly expressed on the pulmonary vascular endothelium, where it forms a complex with the type I receptors, ALK1 or ALK2. The ALK1/BMPR2 receptor complex signals specifically in response to the circulating BMP ligands, BMP9 and BMP10, utilising ENG as a coreceptor [26, 27]. ALK1 and ENG are also highly expressed in the pulmonary endothelium, and this requirement for high levels of BMPR2/ALK1 signalling in the pulmonary endothelium may contribute to the lung-specific effects of BMPR2 mutations. BMP9 (encoded by GDF2) functions as a circulating vascular quiescence factor, protects endothelial cells from apoptosis and excessive proliferation, and inhibits vascular permeability. Loss of BMPR2 also favours endothelial dysfunction and promotes endothelial-to-mesenchymal transition. Given that mutations in ALK1 and ENG (usually associated with HHT) can also cause PAH, the genetic evidence strongly suggests that the pulmonary endothelial cell is an important initiating cell type in PAH pathobiology. Nevertheless, loss of BMPR2 function in other cell types (e.g. smooth muscle cells, fibroblasts and immune cells) may also contribute to disease pathobiology. For example, pulmonary artery smooth muscle cells with BMPR2 mutations are hyperproliferative and resistant to the growth-suppressive effects of BMPs, through loss of antiproliferative Smad1/5 signalling [28].

Other less common PAH mutations are also related to the BMP pathway. Smad8 (encoded by the SMAD9 gene) is a downstream mediator of BMP signalling, together with Smad1 and 5. Heterozygous mutations of SMAD9 have relatively little effect on canonical BMP signalling through Smad4, presumably due to redundancy with Smad1/5 function [29]. However, in relation to a Smad4-independent pathway that promotes microRNA maturation [30], SMAD9 mutations lead to significant loss of function, suggesting that microRNAs regulated in this manner may play an important role in PAH pathogenesis [29]. Similar results were seen in a case with congenital heart disease (CHD)-associated PAH in which pulmonary artery endothelial cells carried a somatic heterozygous deletion of SMAD9 [31].

EIF2AK4, also known as general control non-derepressible 2 (GCN2), is a serine/threonine protein kinase that phosphorylates the α subunit of eukaryotic initiation factor 2, which plays a key role in modulating amino acid metabolism in response to nutrient deprivation. EIF2AK4 senses amino acid deficiency through binding to uncharged transfer RNA. The molecular and cellular mechanisms by which loss of function of this kinase promotes the development of PVOD/PCH is under active investigation. In the Eif2ak4−/− mouse, an increased inflammatory response to stress was observed in the intestine, secondary to decreased autophagy and increased oxidative stress and its effect on inflammasome activation and inflammation [32]. It remains to be demonstrated if similar mechanisms are involved in the intimal fibrosis and endothelial cell proliferation observed in the lung vessels of heritable PVOD. Alternatively, Eif2ak4 biallelic loss of function leads to decreased TRIB3 (Tribbles-like protein 3), whose downregulation has been shown to inhibit BMP-mediated cellular response [33]. Under this hypothesis, Eif2ak4 loss of function would have similar consequences to BMPR2 or SMAD9 mutations by decreasing BMP signalling.

CAV1 is a major constituent protein of caveolae (flask-shaped invaginations of the plasma membrane) and is highly expressed in endothelial cells. BMP receptors are localised in caveolae, as well as other regions of the plasma membrane, and studies suggest that caveolae are required for initiation of BMP signalling [34]. Loss of CAV1 decreases BMPR2 membrane localisation and signalling [35]. Conversely, BMPR2 mutations can lead to caveolar trafficking defects and intracellular localisation [36]. Lastly, it is notable that even in the absence of a heritable mutation, expression levels of BMPR2 and CAV1 are reduced in PAH lung tissues [37, 38].

**Factors affecting disease penetrance**

Despite the fact that pathogenic BMPR2 mutations clearly cause PAH, the penetrance of the disease phenotype is incomplete. The best estimate is that penetrance in male carriers is around 14%, whereas in females it is around 42% [39]. Thus, female sex is the single most important factor influencing the penetrance of BMPR2 mutations in PAH, possibly driven by oestrogen metabolism [25]. Additional factors influencing penetrance may be genetic, epigenetic and/or environmental. Genetic factors may include the expression level of wild-type BMPR2 from the unaffected allele [40], genetic variants affecting the expression levels of TGF-β [41] or alternative splicing of BMPR2 [42]. Studies in induced pluripotent stem cells derived from affected and unaffected BMPR2 mutation carriers have suggested that genetic background, specifically differences in expression of BMP pathway-modifying genes, might contribute to penetrance [43]. Additional large-scale longitudinal studies of affected and unaffected mutation carriers are required to more systematically search for genetic and environmental modifiers of penetrance.
Although no study has yet addressed potential environmental factors influencing the penetrance of BMPR2 mutations in human cohorts, studies suggest that inflammatory mediators, e.g. lipopolysaccharide [44], tumour necrosis factor-α (TNF-α) [45] and 5-lipoxygenase [46], can drive the development of PH in genetically modified mice and in patient-derived pluripotent stem cells [47]. TNF-α directly suppresses BMPR2 mRNA and protein expression. In addition, epigenetic mechanisms such as hypermethylation of the BMPR2 promoter may play a role [48]. Several microRNAs have also been shown to target BMPR2 mRNA to modify expression levels of the protein, including miR-21 [49] and miR-17-92 [50].

Another factor that may contribute to penetrance is somatic mutation within lung vascular cells [51]. Several groups have identified DNA damage in endothelial and smooth muscle cells from PAH lungs in comparison with controls [51–53]. Loss of BMPR2 was associated with a deficiency of DNA repair [54], but increased DNA damage was also evident in cells from IPAH and associated PAH cases, and may be induced by environmental exposures such as methamphetamine use [55]. Further studies are needed to understand the role of DNA damage within the lung and its contribution to disease pathogenesis.

Genetic counselling/testing and management of healthy mutation carriers

Genetic counselling

Mutations in PAH genes have been identified in IPAH and FPAH, anorexigen-associated PAH, PVOD/PCH, and in children with IPAH and PAH associated with CHD. There is a medicolegal duty to inform all patients in these groups about the possibility of a genetic condition and that family members could carry a mutation that increases the risk of PAH, allowing for screening and early diagnosis. Even if genetic testing is not performed, family members should be made aware of early signs and symptoms to ensure that a timely and appropriate diagnosis is made. If a mutation is identified in a patient, the symptomatic patient should be reclassified as HPAH.

Genetic education and counselling should be performed prior to genetic testing for PAH to address the complex issues of incomplete penetrance, questions of surveillance for genetically at-risk family members, reproductive questions, concerns about genetic discrimination, as well as psychosocial issues of guilt and blame that can accompany genetically based diseases. Pre-test genetic education of the affected individual can be performed by PAH providers and/or genetic professionals, and is facilitated by focused educational videos (e.g. www.youtube.com/watch?v=36rlvtj_Qrs). In-depth genetic counselling with genetic professionals including genetic counsellors or medical geneticists is critical prior to genetic testing for asymptomatic family members. Families should also be referred to a genetic counsellor and/or clinical geneticist if they wish to consider reproductive options. The genetic counselling experience of a national reference centre on PAH shows the potential interest in genetic testing in asymptomatic family members [56].

Genetic testing in the family should begin with an affected individual whenever possible to identify the relevant mutation in the family. Otherwise, a negative genetic test result in unaffected family members is not informative. If the familial mutation is known and an unaffected family member tests negative for that mutation, the risk of PAH for that person is the same as the general population (around 1 per 1 000 000 in North America). This can provide great psychological relief to that family member and they can forgo evaluations to screen for PAH.

Genetic testing

Genetic testing can help to explain the aetiology of the disease and stratify risk for other family members and for future children. Clinical genetic testing is available in North America and Europe, and material can be sent to American or European genetic laboratories from other parts of the world. The current cost of testing ranges from approximately USD1000 to USD1500 to analyse the first member of a family. Once the family-specific mutation is known, testing other family members costs USD300–500 in the USA. The exact number of genes included, cost of testing and insurance coverage for testing varies by country and insurer. The most commonly cited reason for genetic testing for PAH is to provide information to and about children, and interest in testing is especially high for paediatric-onset PAH [57].

Targeted sequencing

As the number of genes associated with PAH increases, it has become arduous to test for each of these genes individually. The advent of next-generation sequencing has enabled the development of gene panels to interrogate several genes simultaneously. Several different technologies and platforms are available, and many are now offered on a clinical basis (www.ncbi.nlm.nih.gov/gtr/conditions/CO152171/). In addition, several of these genes can be analysed for dosage changes to detect deletions or duplications of one or more exons. Such mutations are common in BMPR2 and TBX4. An appropriate methodology, such as multiplex ligation-dependent probe amplification, should be used since these types of mutations are not
readily detected by sequencing. It is important to check the genes included in the panel at the time of testing since the composition changes as genetic discoveries advance.

Whole exome sequencing and whole genome sequencing

If an up-to-date panel of PAH genes fails to identify a pathogenic variant in a patient with familial PAH or in a child, WES may be appropriate to identify potentially novel genes. In the case of paediatric-onset PAH, testing should ideally include the child and both parents to allow for facile identification of de novo variants. This trio approach was used to identify both the CAV1 and KCNK3 genes [9, 10]. The largest trio analyses suggest that 19% of childhood-onset IPAH is due to de novo mutations [12]. Exome sequencing is significantly more expensive than panel gene testing and should be ordered by a genetic professional who can discuss the option of learning about incidental or secondary findings, currently comprised of 59 genes recommended by the American College of Medical Genetics and Genomics that are medically actionable and potentially life saving, including genes for hereditary cancer and sudden cardiac death [58].

On the horizon is the use of WGS, which enables the identification of variants in the non-coding regions in a patient’s genome, but is not yet routinely used on a clinical basis. Exome and genome sequencing have the advantage of iterative reanalysis over time as novel genes are identified.

Interpretation of sequencing data

The main problem with WES and WGS is the large number of variants identified in any individual. Interpreting the functional consequences of missense and non-coding variants is particularly challenging. Therefore, identifying potentially pathogenic variants in novel genes requires filtering out common variants based upon large databases of reference allele frequencies and bioinformatic prediction of the likely functional effects. Segregation studies within families can sometimes clarify a variant of uncertain significance (VUS), especially if the variant is de novo or if it segregates with PAH in other affected family members [58], while appreciating that penetrance is usually incomplete and that carriers may be unaffected. Only genetic variants classified as pathogenic or likely pathogenic (not VUS) can be used to genetically risk stratify unaffected family members.

Psychosocial considerations and reproductive options

Genetic test results may cause more harm than good in some individuals because there is currently no effective way to prevent or differentially treat hereditary forms of PAH and because of the incomplete penetrance of mutations. Identifying a mutation in a family can be associated with feelings of guilt in the parent who has passed on a mutation to their child. There are also concerns about genetic discrimination in employment and insurance. Legal safeguards against genetic discrimination vary by country. In the USA, the Genetic Information Nondiscrimination Act protects insured members against discrimination in coverage or cost of health insurance and protects against discrimination in employment, but not against discrimination in life, long-term care or disability insurance based upon a genetic predisposition. In countries with universal healthcare, the concerns about genetic discrimination are not as great and genetic testing uptake rates have been higher.

Reproductive options for those who carry pathogenic mutations include adoption, use of donor gametes, pre-natal testing, in vitro fertilisation (IVF) with pre-implantation genetic diagnosis or not considering PAH genetic status. Accessibility and insurance coverage for IVF and pre-implantation genetic diagnosis vary by country, but coverage is often not readily available due to the cost to the patient.

Practical application of genetics and genomics

What is the utility of genetic diagnosis in PAH patients?

Pathogenic PAH mutations associate with disease characteristics that influence treatment and prognosis. For example, HPAH associated with BMPR2 or ACVRL1 mutations presents at a younger age, with more severe haemodynamic abnormalities, and a very low probability of acute vasoreactivity, as well as reduced survival in the current treatment era [59, 60]. Patients with a heritable form of PVOD are also younger at presentation than non-mutation carriers, but there is no significant difference in the event-free survival at 3 years [61].

Several studies have clearly shown that detection of pathogenic EIF2AK4 mutations is especially useful. The diagnosis of PVOD/PCH challenges PH clinicians, radiologists and pathologists. Identification of biallelic EIF2AK4 mutations allows confirmation of heritable PVOD/PCH without a lung biopsy and identifies a unique form of PAH that not only does not respond well to current PAH medication, but also predisposes to pulmonary oedema when these medications are administered [61]. A subgroup of individuals diagnosed with IPAH or HPAH may also carry biallelic pathogenic EIF2AK4 mutations. These individuals are characterised by presenting under the age of 50 years with a diffusing capacity of the lung
for carbon monoxide (DLCO) <50% of predicted [62–64]. Thus, we suggest that clinicians test younger PAH patients who have a low DLCO for EIF2AK4 mutations in order to guide treatment and for risk assessment in family members [63].

Findings from genomic studies are also starting to be used to better understand the disease process and therapeutic responses. Hennies et al. [65] identified a peripheral blood RNA expression signature in patients with a PAH subphenotype responsive to calcium channel blocker therapy, suggesting a non-invasive approach to predict drug responsiveness. Using WES, genetic variants that may underlie this subphenotype were also identified [66]. As discussed previously, common genetic variants have been associated with risk of PAH, survival and/or pharmacogenomic response to therapy. Most recently, it was shown that common functional variants in SIRT3 and UCP2 might predict the response to inhibition of pyruvate dehydrogenase kinase by dichloroacetate in a phase 2 clinical trial in PAH [67], paving the way for future precision medicine studies with this drug.

**From genes to therapies**

Molecular pathways highlighted by genetic studies are now the subject of several novel therapeutic approaches (figure 2). Long et al. [68] reported the beneficial effects of BMP9 administration in heterozygous BMPR2 knockout mice, suggesting that compensating for BMPR2 haploinsufficiency by increasing dose of the ligand might constitute a targeted therapy for human PAH. At the receptor level, approaches include translational readthrough of nonsense mutations using ataluren to restore full-length protein [69] or slowing down lysosomal degradation of BMPR2 with chloroquine to increase receptor density at the cell surface [70, 71]. Both approaches effectively restored BMP signalling in vitro and lysosomal blockade reversed PH in experimental models. Inhibition of TNF-α with etanercept not only

![Figure 2](https://doi.org/10.1183/13993003.01899-2018)
targets inflammation, but can reduce receptor shedding and proteosomal degradation of BMPR2 [45]. Elafin acts via CAV1 to promote receptor recruitment and enhance BMP signalling, reversing experimental PH [72]. Downstream of BMPR2, FK506 was found to increase BMP signalling and to reverse experimental PH, by binding FK-binding protein 12, an inhibitor of BMP signalling [73]. Phase 2a clinical trials have established safety and tolerability of low-dose FK506 in PAH [74]. Beyond the BMP pathway, KCNK3 mutations lead to reduced potassium channel conductance, which, at least for some mutations, can be recovered by the phospholipase A$_2$ inhibitor ONO-RS-082 [10]. These studies underline the considerable progress that has been made in translating basic genetic studies into potential therapeutic modalities. While direct correction of the underlying mutations remains challenging at present, rapid advances in gene-editing technologies may make targeted mutational correction within the lung vasculature a realistic prospect in the future.

**Proposed future directions**

These different genomic approaches highlight potential paths forward for using molecular medicine to improve PAH patient care. Several large-scale genetics/genomics studies are currently underway in the USA and Europe. Of note, the US National Biological Sample and Data Repository for PAH (the “PAH Biobank”; www.pahbiobank.org) is generating genetic data (targeted DNA sequencing, WES and genome-wide single nucleotide polymorphisms) for 3000 group 1 PAH patients. The BRIDGE Project in the UK includes PAH as one of the rare diseases for which WGS data are being generated (https://bridgestudy.medschl.cam.ac.uk/pah.shtml). Over 1250 IPAH/familial PAH patients from Europe are being analysed to investigate the underlying genetic variation contributing to the disease. In addition, the US National Institutes of Health PVDOMICS initiative aims to define new molecular classifications across the traditional World Health Organization groups by combining deep clinical phenotyping with multiple “omics” analyses [75]. It is only through such cohorts and initiatives that we will begin to have adequate power to discover the range of genetic factors underlying PAH to provide a more complete genomic understanding of the disease. Ideally, this will lead to the identification of additional novel targets for new drugs. It also may facilitate prevention strategies for PAH and the prediction of prognosis based on a genetic classification of PAH.

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