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Early View

Task force report

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Genetic counselling and testing in pulmonary arterial hypertension -A consensus statement on behalf of the International Consortium for Genetic Studies in PAH

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Take home message

Idiopathic, anorexigen-induced, congenital heart disease associated, heritable PAH and pulmonary veno-occlusive disease patients should be offered genetic counselling and testing with a gene panel including all disease genes for the condition.

Abstract

Pulmonary arterial hypertension (PAH) is a rare disease, which can be caused by (likely) pathogenic germline genomic variants. In addition to the most prevalent disease gene, termed *bone morphogenetic protein receptor 2* (*BMPR2*), several genes, some belonging to distinct functional classes, are also now known to predispose to the development of PAH. As a consequence, specialist and non-specialist clinicians and health care professionals, are increasingly faced with a range of questions regarding the need for, approaches to and the benefits and risks, of genetic testing for PAH patients and/or related family members. We provide a consensus-based approach to recommendations for genetic counselling and assessment of current best practice for disease gene testing. We provide a framework and the type of information to be provided to patients and relatives, through the process of genetic counselling, and describe the presently known disease causal genes, to be analysed. Benefits of including molecular genetic testing within the management protocol of patients with PAH, include identification of individuals misclassified by other diagnostic approaches, the optimisation of phenotypic characterisation for aggregation of outcome data, including in clinical trials and importantly through cascade screening, the detection of healthy causal variant carriers, to whom regular assessment should be offered.

Key words: pulmonary arterial hypertension, pulmonary veno-occlusive disease, genetic counselling, genetic testing

Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a rare and severe disorder characterised by the obliteration of remodelled pulmonary microvessels resulting in increased pulmonary vascular resistance (PVR), progressively elevated pulmonary artery pressure, right ventricle hypertrophy and failure as well as death if untreated [1]. PAH has been traditionally defined haemodynamically by a mean pulmonary arterial pressure (mPAP) greater or equal to 25 mmHg, a pulmonary arterial wedge pressure equal to or less than 15 mmHg and a PVR above 3 Wood Units [2]. The cut-off of 25 mmHg exceeds the upper limit of a normal mPAP, i.e. 20 mmHg, which was suggested as a new cut-off value for diagnosing pulmonary hypertension during the 6th World Symposium on Pulmonary Hypertension [3] and was recently accepted as a new haemodynamic cut off in addition to a PVR >2 WU [4]. Patients with PAH present with nonspecific symptoms (fatigue, dyspnoea on exertion, chest pain, and (pre)syncope that often leads to a delay for seeking medical advice by the patient and referral delay by the physician often resulting in the appropriate diagnosis at already advanced stages of the disease, leading to a worse prognosis. The mean delay between onset of symptoms and diagnosis of 2.8 years could be shortened by a greater awareness of this disease amongst clinicians [5]. Available vasodilator drugs targeting the nitric oxide, endothelin and prostacyclin pathways fail to cure the disease. New agents are being tested in clinical trials addressing underlying molecular mechanisms [6, 7] to meet the urgent need for new therapeutic targets. This set of new drugs underscores the importance of a thorough molecular characterisation of our patients to understand if subsets of patients respond or do not respond to particular treatments.

The first description of PAH as a distinct entity was made by Ernst von Romberg, a German physician who described the pulmonary vascular disease as 'pulmonary vascular sclerosis' on autopsy in 1891 [8]. Since the mid 1900's, when right heart catheterisation (RHC) became possible, idiopathic PAH (IPAH, called primary pulmonary hypertension at that time, PPH) was recognised. PAH has an incidence between 2.5 and 7.5 cases per million per year and a prevalence ranging from 15 to 50 per million, according to the French [5] and Scottish registries [9]. In the US REVEAL registry and the US PAH Biobank, patients with PAH associated with other conditions (APAH) comprised the largest subpopulation (50.7% and 48.2%, respectively), followed closely by IPAH [10]. Connective tissue disease accounted for half of the APAH cases [11].

A possible genetic origin was described in 1954 by Dresdale, who observed familial cases [12]. In the 1980's, an autosomal dominant mode of inheritance was described in 14 families with PPH/PAH [13]. In the late 90's and in the year 2000 the locus *PPH1* was mapped to chromosome 2q31-32 [14, 15], later in the year 2000 to 2q33 [16] and in 2000, heterozygous pathogenic germline variants in the

bone morphogenetic protein receptor type 2 (*BMPR2*) gene were found to be responsible for the majority of familial PAH (FPAH) cases [17, 18]. This finding opened a new era, highlighting the genetic causes of PAH, leading to the identification of several other genes associated with PAH. This process resulted in the refinement of the clinical classification of PAH, with heritable PAH (HPAH) categorised as a distinct subcategory of group 1 (PAH) during the 4th World Symposium on Pulmonary Hypertension in 2008 [19]. In the most recent classification of PH, this distinction remains including sub-types for IPAH (Group 1.1) and HPAH (Group 1.2) (Table 1). About 3 % of all PAH patients are characterised as HPAH and around 40 % as IPAH [5, 11].

Data from several PAH registries show a consistent female predominance; on average 70-80% of patients are female, with variation according to the subgroup, e.g. up to 90% are female in PAH associated with connective tissue disease [20, 21]. While, variable across registries, female predominance may be less apparent or even absent in elderly patients in particular with smoking history [22-24] and in prepubertal children [25]. In younger adult patients, the disease prevalence is also less biased with twice as many females compared to males [26].

The genetics of PAH

The term HPAH includes familial cases of PAH and sporadic cases when there is an underlying (likely) pathogenic variant in a predisposing gene. In about 70-87 % of familial PAH and 12-20 % of IPAH patients a genetic cause can be identified in the currently known PAH genes [10, 27, 28]. HPAH is most often caused by heterozygous pathogenic variants in the *BMPR2* gene, encoding a member of the bone morphogenetic protein receptor family of transmembrane serine/threonine kinases (BMPR-II). Pathogenic variants in this gene predispose to a narrowing of the small pulmonary arteries by driving cell proliferation and preventing apoptosis. Vascular remodelling results in increased proliferation of pulmonary arterial smooth muscle cells and fibroblasts. This can lead to the formation of neointimal lesions and complex plexiform lesions where nests of proliferation endothelial cells are also seen. More than 800 different, independent pathogenic variants in the *BMPR2* gene have been identified to date [29, 30]. In addition, by 2018 a total of 17 PAH genes had been acknowledged at the 6th World Symposium on Pulmonary Hypertension [31]. Many of these genes have been shown to belong to or to be associated with the *BMPR2* and transforming growth factor beta (TGF-β) pathway.

The main pattern of inheritance in PAH is autosomal dominant with incomplete penetrance. Thus, one inherited or newly arisen (*de novo*) variant can be sufficient to lead to disease development. However, not all heterozygous individuals develop PAH. For *BMPR2* variants the penetrance has been estimated to be around 30 % with 42 % of heterozygous women 14 % of heterozygous men

developing PAH [32]. Hence, for *BMPR2* the penetrance is also sexually dimorphic with the penetrance for PAH in female *BMPR2* variant carriers being at least twice that of male variant carriers [30]. Thus, the disease may "skip" a generation and manifest again in the following generation [33].

Of note, a few PAH genes can also be autosomal recessively inherited or act semi-dominantly. Patients with biallelic variants have been shown to present with more severe and earlier onset clinical phenotypes [27, 34, 35].

In contrast to classical PAH, the heritable form of pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis (PVOD/PCH) is characterised by autosomal recessive inheritance due to biallelic pathogenic variants in the *eukaryotic translation initiation factor 2 alpha kinase 4* (*EIF2AK4*) gene [36-39]. While PVOD patients usually present with reduced diffusion capacity for carbon monoxide (DLCO) and abnormalities in the computed tomography (CT), heritable PVOD patients are in addition characterised by an earlier age of onset than PVOD patients without biallelic *EIF2AK4* variants [39, 40].

Genetic testing in PAH with and without associated conditions

Genetic testing may help clinicians to characterise better the phenotype of PAH patients and identify potentially misclassified patients, facilitating appropriate management. Considering that individuals with *BMPR2* pathogenic variants, on average, develop PAH at a younger age, present with a more compromised haemodynamic profile and carry a higher risk of death or lung transplantation [41], a genetic diagnosis may have significant consequences for clinical management and therapeutic strategies. For example, more frequent monitoring may be recommended, to allow for early therapy escalation and/or starting with combination therapy. Furthermore, genetic testing can be helpful for risk stratification of family members and it may allow for reproductive options such as pre-implantation genetic testing.

PVOD/PCH can be difficult to diagnose and is characterised by radiological abnormalities, low DLCO and poor response to PAH therapies [42]. PVOD/PCH has likely been underdiagnosed as distinguishing features may not be apparent in early stages of disease. Biallelic *EIF2AK4* pathogenic variants represent at least 25% of all PVOD/PCH cases [43]. Genetic testing may identify biallelic *EIF2AK4* variants in patients misclassified as having IPAH [39, 44]. As PVOD/PCH patients have a poor prognosis and can develop pulmonary oedema with PAH therapies, genetic testing could identify these misclassified patients, allowing appropriate management and early referral for lung transplantation as rapid disease progression in *EIF2AK4* patients has been reported [39, 45]. PVOD/PCH have also been described more frequently in consanguineous families [36, 38].

The development of PAH in patients with hereditary haemorrhagic telangiectasia (HHT) (or Osler-Weber-Rendu disease) led to the identification of other PAH-predisposing genes: *activin A receptor type II-like kinase 1* (*ACVRL1*), *endoglin* (*ENG*), and *mothers against decapentaplegic homolog 4* (*SMAD4*). These genes also belong to the BMP-TGF-β family. The cardinal features of HHT are mucocutaneous telangiectasias, recurrent epistaxis, macroscopic arteriovenous malformations and, in rare cases, PAH. HHT is autosomal dominantly transmitted with onset around puberty and essentially complete penetrance of HHT by the age of 60 years, although there is a much lower penetrance for PAH. Only very few HHT predisposing variant carriers with PAH older than 60 years have been described without any signs for HHT [10]. In contrast, PAH associated with an *ACVRL1* mutation is characterised by a young median age of onset of 20 years-old and thus, PAH may be the first obvious sign of subsequently developing HHT in these young patients [46]. Indeed, genetic testing can identify these patients and facilitate the recognition of HHT complications (arteriovenous malformations) in the patients and their relatives.

Several predisposing PAH genes are also associated with lung development abnormalities in children and adults, such as *T-box protein 4* (*TBX4*). This gene has been previously associated with small patella syndrome (OMIM# 601719) with or without PAH and is inherited in an autosomal dominant fashion with incomplete penetrance and variable expressivity [47, 48]. An enrichment of pathogenic *TBX4* variants has been observed in paediatric PAH [49, 50]. Genetic diagnosis can assist in assessment of associated hip and knee issues. Similarly, protein truncating variants in the gene *KDR* encoding the vascular endothelial growth factor receptor 2 were identified in patients with interstitial lung disease in addition to PAH [48, 50-52]. In these conditions, PAH may be associated with low DLCO (*KDR*) or bronchial abnormalities (*TBX4*). Genetic testing may help to differentiate these patients from Group 3 PH due to chronic lung diseases in particular if low DLCO is present together with a past smoking history [24, 53].

In patients with congenital heart disease APAH, particularly, in children with this condition, pathogenic variants in the transcription factor *SOX17* have been identified in 3 %-7 % of the patients [54-56]. The majority of these patients presented with simple heart defects such as arterial septal defect, patent ductus arteriosus, patent foramen ovale and ventricular septal defect [54, 56]. In addition, chest CT abnormalities such as dilated, tortuous pulmonary vessels and ground-glass opacities and haemoptysis were described in a subset of patients [56]. While pathogenic *SOX17* variants are more common in congenital heart disease APAH they have also been described in familial and IPAH [10, 27, 28, 56], see Table 2. The same *SOX17* variant was even identified in PAH patients from the same family with and without associated congenital heart disease [57]. Similarly,

pathogenic variants in CHD-APAH patients were not only identified in *SOX17* but also in other PAH genes such as *TBX4* and *BMPR2* [10].

Little evidence is available for patients with drugs and toxin induced PAH. Current evidence shows that only patients with previous anorexigen intake may harbour pathogenic variants and should, therefore, undergo genetic counselling and testing [59, 60]. For other specific drug or toxin exposures only few cases with pathogenic variants were reported [61, 62]. In most other patients no predisposing genetic variant was identified in addition to the drug or toxin exposure. For example, in a subset of more than 20 patients who ingested colza oil and subsequently developed PAH no pathogenic variant could be identified (J.A. Tenorio-Castano, personal communication). Similarly, in a subset of 13 PVOD patients with previous intermediate or high trichlorethylene exposure no pathogenic variant could be identified while five out of twelve PVOD patients with absent or trivial exposure to the same substance were carriers of biallelic *EIF2AK4* variants [63].

Our recommendation is that genetic testing should be performed at least in patients with a family history of PAH, patients with IPAH, patients with anorexigen-induced PAH and patients with congenital heart disease APAH. Patients with suspected or confirmed PVOD/PCH and other developmental lung disorders should also be offered genetic counselling and testing. Cascade genetic testing should be offered to relatives of index cases with PAH with identified pathogenic/likely pathogenic variants according to the guidelines by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG) [64].

Currently there is insufficient evidence to recommend genetic testing for pulmonary hypertension patients in Group 2-5 (Table 2). Only if there is familial aggregation of pulmonary hypertension or any of the above-mentioned differential diagnosis is being considered, should genetic be offered.

Genetic testing and clinical screening for relatives of PAH patients

Regular screening and follow-up of at-risk family members of HPAH patients is particularly important to diagnose HPAH at an early stage, initiate early treatment and hopefully improve prognosis. Asymptomatic individuals at genetic risk should have regular clinical assessments. Screening assessments intervals may vary between 6 months to 3 years, based on gene, assessment results, trajectory, gender, and family history. As soon as any symptoms such as breathlessness appear, a full clinical work up should be immediately conducted. Therefore, family members should be informed to be alert for these symptoms.

Screening should ideally be performed annually and include medical history, New York Heart Association (NYHA) / World Health Organization (WHO) dyspnoea class, physical examination, electrocardiogram, pulmonary function tests, NT-proBNP or BNP, and echocardiography [2]. In addition, echocardiography during exercise and cardiopulmonary exercise testing can provide valuable additional information on pulmonary artery pressure increase during exercise [15]. Subtle clinical changes may only be obvious during exercise in an otherwise healthy person [4]. An increase of systolic pulmonary arterial pressure >40 mmHg during mild exercise has been demonstrated in 30 % of family members of HPAH patients and in only 10 % of controls [65]. This hypertensive pulmonary response could indicate early vascular changes as only family members with this response and a pathogenic *BMPR2* variant subsequently developed manifest PAH [66]. Screening intervals of asymptomatic variant carriers with a hypertensive exercise response may therefore be even shorter than of individuals without an hypertensive exercise response.

An absolute minimum screening should include NT-proBNP/BNP levels, which may be checked by the local general practitioner and anamnesis via phone by the treating PH expert albeit at shorter intervals such as every 6 months. While this more frequent minimum screening is less burdensome and costly for the family member, it cannot substitute for a full clinical screening with the full set of the above-mentioned assessments which should then be conducted at less frequent time intervals.

Regular screening can help to identify family members at risk who can be offered diagnostic right heart catheterisation [67]. Therefore, informing the patient of the familial implications and providing genetic counselling to interested family members is a medicolegal responsibility once a (likely) pathogenic variant has been identified in a PAH proband. A starting point is the family history and pedigree. With consent of the PAH patient first-degree family members should be offered genetic counselling and testing. During genetic counselling disadvantages of knowing about a potential genetic predisposition also have to be addressed (see section on genetic counselling). By identifying those variant carriers with PAH in a given family further insight may be gained regarding possible second hits of genetic, epigenetic or environmental nature, providing clues about the disease penetrance [68].

A recent study (DELPHI-2 study, NCT01600898) [69] performed annual screening in asymptomatic *BMPR2* mutation carriers including clinical assessment, electrocardiogram, pulmonary function tests, DLCO measurement, 6-minute walk test, cardiopulmonary exercise test, chest X-ray, echocardiography, and NT-proBNP level. In addition, an optional RHC at rest and exercise was performed at baseline. Fifty-five subjects (median age 37 years) were included. At baseline, no PAH was suspected based on echocardiography and NT-proBNP levels. All subjects accepted the optional RHC at inclusion, which identified two mild PAH cases (3.6%), and 12 subjects with exercise PH

(21.8%). At long term follow-up (>5 years), three additional cases were diagnosed, who were still in functional class I-II. In these patients, echocardiography and NT-proBNP were useful in detecting mildly symptomatic PAH with more severe haemodynamic impairment. In clinical practice, screening is often extended to include mildly symptomatic patients, and these data suggest that echocardiography remains a useful screening tool in this setting. Also, in this study patients with exercise PH at baseline seemed to be at higher risk for developing PAH than patients with normal haemodynamics at rest and exercise [69]. Finally, a PAH incidence in *BMPR2* mutation carriers of 2.3 %/year (0.99 %/year in men and 3.5 %/year in women) was calculated for the first time. All PAH cases have remained at low-risk status on oral therapy at last follow-up illustrating the benefit of an early detection. International multicentre studies are needed to define the best multimodal screening programmes and follow-up intervals to allow early detection and effective treatment of PAH and to accurately estimate the numbers of at-risk family members who develop disease across the lifespan and by sex.

Which PAH genes should be included in genetic testing?

In addition to *BMPR2* as the gene responsible for the largest proportion of HPAH, pathogenic variants in its co-receptors ALK1 (*ACVRL1* gene) and endoglin (*ENG*) are found predominantly in patients with HHT, of whom around 2 % also develop PAH [70, 71]. Moreover, heterozygous or biallelic pathogenic variants in a ligand of BMPR-II, the bone morphogenetic protein 9 (*GDF2* gene) [35] and the BMPR-II downstream pathway gene *SMAD9* [72] are less common genetic causes of PAH.

Apart from *BMPR2* pathway genes, disease causing variants in genes strongly associated with PAH have also been identified in genes that encode a plasma membrane protein (*CAV1*), a potassium channel protein (*KCNK3*), and an ion channel protein (*ATP13A3*)[28, 73, 74]. For the genes *KCNK3, ATP13A3* and *GDF2* not only monoallelic but also biallelic variants have been described with a more severe presentation of the respective patients [27, 34, 35, 75].

The transcription factors *SOX17* and *TBX4* have been implicated in PAH often manifesting already in childhood [28, 47, 49]. As detailed above also *EIF2AK4* should be analysed to help to clarify a diagnosis of PVOD/PCH particularly in patients with CT abnormalities and low DLCO. Another subset of PAH patients with low DLCO and parenchymal lung abnormalities may carry a pathogenic variant of the *KDR* gene, a result consistent with those obtained by Bayesian inference on the DLCO phenotype [51, 52].

The ClinGen gene curation expert panel for pulmonary hypertension has identified the above listed genes (*ACVRL1, ATP13A3, BMPR2, CAV1, EIF2AK4, ENG, GDF2, KCNK3, KDR, SMAD9, SOX17, TBX4*) as

having a “strong” or “definitive” gene disease relationship (personal communication). Three genes were classified as having a “moderate” gene disease relationship including the only recently identified potassium channel *ABCC8* gene [76], the vitamin K pathway gene *GGCX* [10] and a gene involved in DNA-methylation regulation by catalysing the conversion of methylcytosine to 5-hydroxymethylcytosine *TET2* [77]. Finally, six additional genes involved in PAH have been recently reported and so far, identified in only a few PAH patients. Therefore, they have limited evidence for a gene disease relationship. These genes include the water channel gene *AQP1* [28], the transcription factor *KLF2* [78],the BMPR2 ligand gene *BMP10* [79], kallikrein 1 gene (*KLK1*) [10], the extracellular matrix gene fibulin 2 (*FBLN2*) and the platelet derived growth factor D gene (*PDGFD*) [80]. Thus, since the 6th World Symposium on Pulmonary Hypertension, the list of PAH genes has grown steadily. The genes with a strong recommendation for inclusion on the PAH panel are underlined in Figure 1.

How should testing be performed?

The clinical features of the patient and family history may guide the genetic testing chosen. Depending on the resources available, genetic testing should start with an affected individual and include *BMPR2* at a minimum. A PAH gene panel sequencing approach should be employed that includes *BMPR2* as well as at least all strong evidence PAH genes as defined by the ClinGen gene curation expert panel for pulmonary hypertension genes using next generation sequencing as a gold standard. This approach replaces the previous step-wise method of using sequential Sanger sequencing of selected genes due to lower costs and greater efficiency of panel testing with next generation sequencing [81]. The gene list should be revised as additional genes are identified. PAH patients who had received testing of only a limited number of genes in the past should be offered a new round of sequencing [82]. In addition, testing should include methods for large genomic rearrangements screening including copy number variations. Methods such as multiplex ligation-dependent probe amplification, quantitative polymerase chain reaction (PCR), digital droplet PCR, and microarrays (mainly cGH-arrays and single nucleotide polymorphism-arrays) are the gold standard techniques. Alternatively, sequence-based methods such as whole exome sequencing (WES) and gene panel sequencing can also be used to quantify read count to assess intragenic or whole gene deletions or duplications in particular regarding the genes *BMPR2, ACVRL1, ENG* and *TBX4*, since these occur frequently.

For children, familial cases or PAH patients with a congenital anomaly regardless of age without an identified pathogenic variant after panel gene testing including all disease genes for the condition, WES or whole genome sequencing (WGS) can be useful and should include parents to assess better *de novo* variants in offspring employing a trio-WES or trio-WGS strategy. Once a (likely) pathogenic

variant is identified in the family, targeted testing can be performed for the familial variant in symptomatic or asymptomatic family members. Variants should be classified according to ACMG guidelines [64] and submitted to the ClinVar database by the diagnostic laboratory.

How are the genetic test results derived and how is the report interpreted?

After the blood/saliva/buccal swab sample of the proband together with all required signed consent forms for genetic testing arrive in the genetic diagnostic laboratory, genomic DNA is extracted. Sequence data are generated for the genes requested. The sequences are aligned and compared to databases of known benign variants. Rare variants and copy number variations are classified by using defined criteria, such as those outlined by the ACMG guidelines [64]. The criteria assess different aspects of the variant and consider: 1) the variant frequency in the general population (i.e. genome aggregation database) and in the affected patient population; 2) the predicted impact of a nucleotide change leading to an amino acid substitution, premature stop codon, alternate splicing leading to skipping of an exon or inclusion of an intron, deletion or insertions of amino acids; 3) the location of the variant within important protein domains; 4) published functional studies for the given variant; 5) co-segregation of the variant with the disease in the family; 6) *de novo* appearance of the variant in the proband; 7) pathogenicity prediction according to *in-silico* bioinformatic tools; 8) pattern of inheritance of the variants and disease mode of inheritance.

According to these criteria each variant is classified as benign (class I), likely benign (class II), variant of uncertain significance (class III), likely pathogenic (class IV) or pathogenic (class V) [83]. Class IV and V variants are considered to be likely causative for the disease, while class I and II variants are considered not to be disease-causing. Variants with either insufficient or conflicting evidence are classified as variants of uncertain significance.

For example, variants present in more than 1% of the general population are considered benign for PAH (class I), while a rare variant (<0.001%), which has never been described in a healthy individual but has been observed in other PAH patients and was functionally shown to reduce or abolish protein function would be classified as a pathogenic variant (class V). In between (likely) benign and (likely) pathogenic variants are those variants of uncertain significance (class III). For these variants not enough evidence is available to up- or downgrade them to give them a clear benign or pathogenic character. Such variants should be re-evaluated in regular intervals, such as every three years to consider newly published data like functional evidence or additional cases in patients or controls. In addition, co-segregation with the disease in further affected family members can add helpful information. Association of a class III variant with a pathogenic variant on the other allele of a gene whose biallelic loss of function is lethal is of great help. Also, a segregation of the variant in the

parents can inform about its inherited or *de novo* nature. PH experts may monitor the public database ClinVar for further information of a specific variant or contact laboratories to request a re-evaluation if it is not done automatically. The large majority of these variants will eventually be downgraded to likely benign variants as they are discovered in further healthy control probands. Variants of uncertain significance are not to be acted on clinically and pre-implantation diagnostics for these variants should not be performed.

To correctly classify such variants and interpret the results appropriately, laboratories performing genetic testing should ideally be certified by an official agency and participate in a regular quality control for the genomic studies by external agencies (such as the European Molecular Quality Network, EMQN) to adhere to all regulations and use established and accepted methods for sample handling, sequencing, data analyses and reporting. Depending on the country and the regulatory agency, the final genetic diagnostic report is approved and signed by at least one professionally trained human geneticist.

Genetic counselling

Genetic testing for symptomatic individuals should be performed in PAH centres of excellence. The multidisciplinary team should include clinicians with expertise in genetics including cardiologists, pulmonologists, geneticists and genetic counsellors. Policies and regulations about who can order genetic testing differ by country. Pre-symptomatic genetic testing of asymptomatic individuals should include a genetic professional consultation to explore understanding of the genetic test, implications of a positive or negative result, risk of disease based upon age/gender/genetic status, and actions to be taken if genetic results are positive (surveillance and possible reproductive decisions). Pre-symptomatic genetic testing in minors/adolescents should be carefully considered to preserve the right to an open future for the child since there is not a proven method of disease prevention and because penetrance is incomplete. A healthy child with a pathogenic variant may unconsciously be treated differently by his / her parents compared to siblings without such pre-disposition. This should be carefully discussed during genetic counselling in particular considering the incomplete penetrance of PAH associated variants. Thus, the most immediate benefits for the child at hereditary risk would be to obviate the need for clinical assessments and the relief of anxiety in case of a negative test result. Not all countries allow genetic testing of minors for genetic conditions associated with PAH. When appropriate based upon the maturity of the adolescent, the adolescent and the parents should all be included in the discussion to ensure the opinions of the adolescent are considered. If a genetic test identified a familial variant, pre-implantation diagnostics may be discussed. While pregnancy itself is to be avoided in female PAH patients [2] it is being debated as a trigger for disease onset in

asymptomatic variant carriers **[84, 85]**. Because there is no proven way to prevent or cure PAH, genetic counselling is important to explore whether the individual is psychologically prepared to determine his/her genetic status and availability of emotional support. Although many countries have laws protecting against genetic discrimination, these laws do not necessarily cover life insurance, private health insurances, disability insurance or long-term care, and exploring policies prior to genetic testing is a consideration. Any positive result revealing a genetic predisposition could possibly lead to a rejection of a new insurance policy or a higher premium.

Upon return of results, many individuals who test positive are concerned because of the poorer prognosis associated with some PAH genes (i.e. *BMPR2*) and/or worries/guilt about having passed it onto their children. Even those who test negative may need time to adjust to "survivor's guilt". Those who test negative for a pathogenic variant in the family do not have to be concerned about genetic testing for any of their descendants since the pathogenic variant cannot be transmitted from someone who does not carry the variant. In contrast, variant carriers have a 50% chance of passing the variant to their offspring. Specific genetic counselling should be given for recessive autosomal transmission of *EIF2AK4* linked PVOD/PCH. Twenty five percent of the offspring will be carrier of biallelic pathogenic variants when both parents carry a single heterozygous pathogenic variant. Moreover, the risk of consanguineous unions should be explained to families with pathogenic *EIF2AK4* variants. Carrying a pathogenic variant of *EIF2AK4* on a single allele is not considered sufficient to lead to disease onset. Disseminating information about genetic test results to family members can be challenging due to the complex nature of the information, guilt, and family dynamics [86]. Genetic professionals can act as a critical liaison for families to educate and counsel family members once a (likely) pathogenic variant has been identified. The path of genetic counselling and testing is illustrated in Figure 2.

For some patients and relatives, access to testing can be difficult. In some countries, insurance may not cover genetic counselling and testing despite the benefits for the proband. This situation can be even worse in minority or underprivileged communities. Thus, a registration of the patient in a PAH expert centre may help to overcome these obstacles.

Areas of uncertainty

While PAH is a disease with a mainly autosomal dominant inheritance pattern, a few patients have been described in whom more than one pathogenic variant in different genes was present and might contribute to disease manifestation. For example, these so-called oligogenic cases have been described in a family with a *BMPR2* frameshift variant and an *EIF2AK4* exon skipping variant [87]. An

earlier study had reported a severe case of PAH in a child with a *BMPR2* and a *KCNA5* variant [88]. Thus, in rare cases two variants may increase the severity of the disease or lead to an earlier age of onset of PAH. However, the contribution of this potential oligogenic inheritance remains to be established in PAH.

It is unknown whether the substantial variation usually observed among PAH patients in the response to available treatments might be determined, at least in part, by genetic predisposition. Already more than a decade ago it was shown that *BMPR2* variant carriers rarely respond acutely to vasodilators and are hence less likely to benefit from calcium channel blocker therapy [89, 90]. Relatively few studies focused on gene variants that may interact with drug responses to PAH therapies. Benza et al. demonstrated that variants in endothelin metabolism may predict outcomes in PAH patients treated with endothelin receptor antagonists [91]. Two recent case series explored the hypothesis that pathogenic variants in the *BMPR2* gene may be associated with a reduced haemodynamic response to inhaled or parenteral prostanoids [92, 93]. The conclusions were different, but it is interesting to notice that the different results might be related to the aggressiveness of the therapeutic approach. These efforts might ultimately provide a step towards the application of precision medicine in PAH. In this regard, it will be interesting to determine whether patients with a genetic risk show a differential response to novel emerging drugs which aim to reduce cellular proliferation and vascular remodelling through the BMP pathway such as sotatercept [6] or the platelet derived growth factor receptor pathway such as the inhaled tyrosine kinase inhibitor seralutinib [7].

Patients with persistent pulmonary hypertension of the newborn (group 1.7 in Table 1) often present with complex comorbidities or disorders and may harbour genetic variants in developmental genes [55]. The exact influence of the genes on the phenotype of the patients apart from the acknowledged PAH genes such as *TBX4* and *BMPR2* remains to be established [94, 95].

Patients with congenital heart disease PAH may present with a diverse range of cardiac phenotypes. How much the specific cardiac defects contribute to PAH or whether there are pleiotropic effects of some genes on cardiac and pulmonary vascular development remains to be determined. Among patients with congenital heart defects, those repaired early may be more likely to harbour a pathogenic variant.

PVOD/PCH is defined by clear clinical characteristics in the CT, reduced DLCO and in hereditary forms by biallelic *EIF2AK4* pathogenic variants. *EIF2AK4* variants have also been reported in a limited number of PAH patients, which may have been unrecognised PVOD patients [39]. Interestingly, the protein expression of *EIF2AK4* was similarly reduced in *BMPR2* heterozygotes and sporadic PVOD

patients without variants in *EIF2AK4* [40]. The presence of an additional *EIF2AK4* variant in *BMPR2* heterozygotes explaining the disease penetrance in a HPAH family could also point towards an autosomal dominant contribution of *EIF2AK4* mutations in some cases [87]. Thus, despite being characterised as two different forms of PAH, one might speculate that PVOD and PAH are a spectrum of the same disease. An eight-year follow-up of a sibling with two sisters with PVOD illustrated pulmonary arterial remodelling as a first sign of a lung disease with mild dyspnoea without elevated pulmonary artery pressures while after eight years lung transplantation revealed pronounced venous remodelling and capillary proliferation [96]. This hypothesis is also supported by a PVOD-like phenotype, which has been described in *BMPR2* mutation carriers [97, 98] and which has even received an OMIM entry (# 265450) prior to the detection of *EIF2AK4* as a genetic cause for PVOD. Accordingly, it was proposed that we should use a new terminology for the classification of PVOD/PCH patients at the 6th World Symposium on Pulmonary Hypertension: PAH with overt signs of venous or capillary involvement (PVOD/PCH) for this subgroup of PAH.

The strength of the international consortium for genetic studies in PAH (PAH-ICON) is the connection of worldwide experts, large and small centres and together achieving larger numbers of patients to address questions like oligogenic inheritance, genetic contributions to persistent pulmonary hypertension of the newborn and characterise patients' phenotypes and rare genotypes in detail. Data collected and analysed by PAH-ICON will also provide clues to therapeutic responses of novel molecular agents addressing the underlying pathophysiologic imbalances.

Summary of recommendations

At least probands diagnosed with IPAH, FPAH, PVOD/PCH, and anorexigen-induced and congenital heart disease associated PAH should be offered genetic counselling and genetic testing.

A PAH gene panel sequencing approach should be employed that includes at least all strong evidence PAH genes *ACVLR1, ATP13A3, BMPR2, CAV1, EIF2AK4, ENG, GDF2, KCNK3, KDR, SMAD9, SOX17, TBX4* using next generation sequencing. The gene list should be revised as additional genes are identified and new supporting evidence for previously described possible disease genes becomes available.

The testing method should be complemented by methods to quantify read count to assess intragenic or whole gene deletions or duplications particularly for the genes *BMPR2, ACVRL1, ENG* and *TBX4*.

Symptomatic individuals with FPAH, children with normal genetic results on a PAH gene panel and patients with a congenital anomaly regardless of age should consider exome/genome sequencing

including multiple affected family members in FPAH and both parents in children to increase the chance of identifying the disease-causing variant.

Cascade genetic testing should be offered in consultation with genetic professionals in families with an identified PAH associated pathogenic/likely pathogenic variant.

Asymptomatic genetically at-risk individuals should be followed regularly and should be aware of PAH associated symptoms to enable early diagnosis of PAH and early initiation of treatment if evidence of PAH emerges.

Variants of uncertain significance should be re-evaluated in regular intervals to take into account newly published data. PH experts may monitor ClinVar for further information of a specific variant or contact laboratories to request a re-evaluation.

Table 1. Classification of pulmonary hypertension [4]

1 Pulmonary arterial hypertension (PAH)

1.1 Idiopathic PAH

1.1.1 Non-responders at vasoreactivity testing

1.1.2 Acute responders at vasoreactivity testing

1.2 Heritable

1.3 Associated with drugs- and toxins

1.4 Associated with:

1.4.1 Connective tissue disease

1.4.2 HIV infection

1.4.3 Portal hypertension

1.4.4 Congenital heart disease

1.4.5 Schistosomiasis

1.5 PAH with overt features of venous/capillaries (PVOD/PCH) involvement

1.6 Persistent pulmonary hypertension of the newborn syndrome

2 Pulmonary hypertension associated with left heart disease

2.1 PH due to heart failure with preserved LVEF

2.1.1 with preserved ejection fraction

2.1.2 with reduced or mildly reduced ejection fraction

2.2 Valvular heart disease

2.3 Congenital/acquired cardiovascular conditions leading to post-capillary PH

3 Pulmonary hypertension associated with lung diseases and/or hypoxia

3.1 Obstructive lung disease or emphysema

3.2 Restrictive lung disease

3.3 Lung disease with mixed restrictive/obstructive pattern

3.4 Hypoventilation syndromes

3.5 Hypoxia without lung disease (e.g. high altitude)

3.6 Developmental lung disorders

4 Pulmonary hypertension associated with pulmonary artery obstructions

4.1 Chronic thromboembolic PH

4.2 Other pulmonary artery obstructions

5 Pulmonary hypertension with unclear and/or multifactorial mechanisms

5.1 Haematological disorders

5.2 Systemic disorders

5.3 Metabolic disorders

5.4 Chronic renal failure with or without haemodialysis

5.5. Pulmonary tumour thrombotic microangiopathy

5.6 Fibrosing mediastinitis

PAH: pulmonary arterial hypertension; PCH: pulmonary capillary haemangiomatosis; PVOD: pulmonary veno-occlusive disease.

Table 2. Reported disease causing variants across genes and PH groups

	Group 1 PAH										Group 2	Group 3	Group 4		Group 5
	IPAH	**HPAH**	**DPAH**	**CTD-APAH**	**HIV-APAH**	**PoPH**	**CHD-APAH**	**Sch-APAH**	**PVOD/PCH**	**PPHN**	**PH LHD**	**PH lung**	**CTEPH**	**Other obstructions**	**Multifactorial mechanisms**
ACVRL1	20-25	5-10	<5	<5	-	-	<5	-	-	-	-	-	-	-	-
ATP13A3	20-25	<5	<5	<5	-	-	<5	-	-	-	-	-	-	-	-
BMPR2	>650	>350	<5	<5	<5	-	10-15	-	<5	-	-	-	<5	-	<5
CAV1	5-10	5-10	-	<5	-	<5	<5	-	-	-	-	-	-	-	-
EIF2AK4	10-15	5-10	-	<5	-	-	-	-	40-50	-	-	-	-	-	-
ENG	5-10	<5	-	<5	<5	-	<5	-	-	-	-	-	-	-	-
GDF2	50-60	<5	-	<5	<5	-	-	-	-	-	-	-	-	-	-
KCNK3	15-20	5-10	-	<5	-	-	-	-	-	-	-	-	-	-	-
KDR	5-10	<5	-	-	-	-	<5	-	-	-	-	-	-	-	-
SMAD9	15-20	<5	-	<5	<5	-	5-10	-	-	-	-	-	-	-	-
SOX17	30-40	<5	<5	-	-	<5	10-15	-	-	-	-	-	-	-	-
TBX4	70-80	5-10	<5	<5	-	-	10-15	-	-	10-15	-	-	-	-	-

I/H/D/APAH: idiopathic / heritable / associated with drugs and toxins / associated pulmonary arterial hypertension; CHD: congenital heart disease; CTD: connective tissue disease; CTEPH: chronic thromboembolic pulmonary hypertension, HIV: human immune deficiency virus; PCH: pulmonary capillary haemangiomatosis; PVOD: pulmonary veno-occlusive disease; PPHN: persistent pulmonary hypertension of the newborn; Sch: Schistosomiasis; Data compiled from: [10, 50, 54, 56, 57, 82, 99-102]

Figure Legends

Figure 1. Main genes recommended to be included in genetic testing of PAH patients and their relatives (underlined). The underlined genes are the genes which should definitely be included in the PAH gene panel. The other genes may be included but results should be interpreted with caution as they await further supporting evidence. Genes associated with *hereditary haemorrhagic telangiectasia (HHT)/Osler-Rendu disease; $pulmonary veno-occlusive disease (PVOD)/pulmonary capillary haemangiomatosis (PCH); #lung development abnormalities

Figure 2. Genetic counselling path for PAH patients and their relatives.

Patients should receive genetic counselling before and after genetic testing. If a (likely) pathogenic variant is identified, family testing should be encouraged. If asymptomatic carriers are identified they should undergo regular clinical follow-up.

IPAH, idiopathic pulmonary arterial hypertension; FPAH, familial pulmonary arterial hypertension; PVOD, pulmonary veno-occlusive disease; CPET, cardio-pulmonary exercise test; TTE, transthoracic echocardiography.

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Conflict of Interest: Christina A. Eichstaedt reports lecture fees from MSD, outside the submitted work; and has patent “Gene panel specific for pulmonary hypertension and its uses” European Patent ID: EP3507380 issued.

Catharina Belge reports consulting fees, participation on advisory board and lecture honoraria from Janssen, MSD/Bayer; travel support from MSD/Bayer; outside the submitted work.

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References

1. Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. *N Engl J Med* 2004; 351: 1425-1436.
2. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M, Aboyans V, Vaz Carneiro A, Achenbach S, Agewall S, Allanore Y, Asteggiano R, Paolo Badano L, Albert Barbera J, Bouvaist H, Bueno H, Byrne RA, Carerj S, Castro G, Erol C, Falk V, Funck-Brentano C, Gorenflo M, Granton J, Iung B, Kiely DG, Kirchhof P, Kjellstrom B, Landmesser U, Lekakis J, Lionis C, Lip GY, Orfanos SE, Park MH, Piepoli MF, Ponikowski P, Revel MP, Rigau D, Rosenkranz S, Voller H, Luis Zamorano J. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J* 2016; 37: 67-119.
3. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, Williams PG, Souza R. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2019; 53: 1801913.
4. Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, Carlsen J, Coats AJS, Escribano-Subias P, Ferrari P, Ferreira DS, Ghofrani HA, Giannakoulas G, Kiely DG, Mayer E, Meszaros G, Nagavci B, Olsson KM, Pepke-Zaba J, Quint JK, Radegran G, Simonneau G, Sitbon O, Tonia T, Toshner M, Vachiery JL, Vonk Noordegraaf A, Delcroix M, Rosenkranz S, Group EESD. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Respir J* 2022.
5. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Simonneau G. Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med* 2006; 173: 1023-1030.
6. Humbert M, McLaughlin V, Gibbs JSR, Gomberg-Maitland M, Hoeper MM, Preston IR, Souza R, Waxman A, Escribano Subias P, Feldman J, Meyer G, Montani D, Olsson KM, Manimaran S, Barnes J, Linde PG, de Oliveira Pena J, Badesch DB, Investigators PT. Sotatercept for the Treatment of Pulmonary Arterial Hypertension. *N Engl J Med* 2021; 384: 1204-1215.
7. Frantz RP, Benza RL, Channick RN, Chin K, Howard LS, McLaughlin VV, Sitbon O, Zamanian RT, Hemnes AR, Cravets M, Bruey JM, Roscigno R, Mottola D, Elman E, Zisman LS, Ghofrani HA. TORREY, a Phase 2 study to evaluate the efficacy and safety of inhaled seralutinib for the treatment of pulmonary arterial hypertension. *Pulm Circ* 2021; 11: 20458940211057071.
8. von Romberg E. Über Sklerose der Lungenarterie [On sclerosis of pulmonary arteries]. *Dtsch Archiv Klin Med* 1891; 48: 197-206.
9. Hurdman J, Condliffe R, Elliot CA, Davies C, Hill C, Wild JM, Capener D, Sephton P, Hamilton N, Armstrong IJ, Billings C, Lawrie A, Sabroe I, Akil M, O'Toole L, Kiely DG. ASPIRE registry: assessing the Spectrum of Pulmonary hypertension Identified at a REferral centre. *Eur Respir J* 2012; 39: 945-955.
10. Zhu N, Pauciulo MW, Welch CL, Lutz KA, Coleman AW, Gonzaga-Jauregui C, Wang J, Grimes JM, Martin LJ, He H, Investigators PAHBEC, Shen Y, Chung WK, Nichols WC. Novel risk genes and mechanisms implicated by exome sequencing of 2572 individuals with pulmonary arterial hypertension. *Genome Med* 2019; 11: 69.
11. McGoon MD, Miller DP. REVEAL: a contemporary US pulmonary arterial hypertension registry. *Eur Respir Rev* 2012; 21: 8-18.
12. Dresdale DT, Michtom RJ, Schultz M. Recent studies in primary pulmonary hypertension, including pharmacodynamic observations on pulmonary vascular resistance. *Bull N Y Acad Med* 1954; 30: 195-207.

13. Loyd JE, Primm RK, Newman JH. Familial primary pulmonary hypertension: clinical patterns. *Am Rev Respir Dis* 1984; 129: 194-197.
14. Nichols WC, Koller DL, Slovis B, Foroud T, Terry VH, Arnold ND, Siemieniak DR, Wheeler L, Phillips JA, 3rd, Newman JH, Conneally PM, Ginsburg D, Loyd JE. Localization of the gene for familial primary pulmonary hypertension to chromosome 2q31-32. *Nat Genet* 1997; 15: 277-280.
15. Grünig E, Janssen B, Mereles D, Barth U, Borst MM, Vogt IR, Fischer C, Olschewski H, Kuecherer HF, Kübler W. Abnormal pulmonary artery pressure response in asymptomatic carriers of primary pulmonary hypertension gene. *Circulation* 2000; 102: 1145-1150.
16. Deng Z, Haghighi F, Helleby L, Vanterpool K, Horn EM, Barst RJ, Hodge SE, Morse JH, Knowles JA. Fine mapping of PPH1, a gene for familial primary pulmonary hypertension, to a 3-cM region on chromosome 2q33. *Am J Respir Crit Care Med* 2000; 161: 1055-1059.
17. Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA, Loyd JE, Nichols WC, Trembath RC. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet* 2000; 26: 81-84.
18. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, Kalachikov S, Cayanis E, Fischer SG, Barst RJ, Hodge SE, Knowles JA. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 2000; 67: 737-744.
19. Galiè N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G, Guidelines ESCCfP. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J* 2009; 30: 2493-2537.
20. McGoon MD, Benza RL, Escribano-Subias P, Jiang X, Miller DP, Peacock AJ, Pepke-Zaba J, Pulido T, Rich S, Rosenkranz S, Suissa S, Humbert M. Pulmonary arterial hypertension: epidemiology and registries. *J Am Coll Cardiol* 2013; 62: D51-59.
21. Badesch DB, Raskob GE, Elliott CG, Krichman AM, Farber HW, Frost AE, Barst RJ, Benza RL, Liou TG, Turner M, Giles S, Feldkircher K, Miller DP, McGoon MD. Pulmonary arterial hypertension: baseline characteristics from the REVEAL Registry. *Chest* 2010; 137: 376-387.
22. Galiè N, Simonneau G. The Fifth World Symposium on Pulmonary Hypertension. *J Am Coll Cardiol* 2013; 62: D1-3.
23. Hoeper MM, Gibbs JSR. The changing landscape of pulmonary arterial hypertension and implications for patient care. *Eur Respir Rev* 2014; 23: 450-457.
24. Hoeper MM, Pausch C, Grünig E, Klose H, Staehler G, Huscher D, Pittrow D, Olsson KM, Vizza CD, Gall H, Benjamin N, Distler O, Opitz C, Gibbs JSR, Delcroix M, Ghofrani HA, Rosenkranz S, Ewert R, Kaemmerer H, Lange TJ, Kabitz HJ, Skowasch D, Skride A, Jureviciene E, Paleviciute E, Miliauskas S, Claussen M, Behr J, Milger K, Halank M, Wilkens H, Wirtz H, Pfeuffer-Jovic E, Harbaum L, Scholtz W, Dumitrescu D, Bruch L, Coghlan G, Neurohr C, Tsangaris I, Gorenflo M, Scelsi L, Vonk-Noordegraaf A, Ulrich S, Held M. Idiopathic pulmonary arterial hypertension phenotypes determined by cluster analysis from the COMPERA registry. *J Heart Lung Transplant* 2020; 39: 1435-1444.
25. Berger RM, Beghetti M, Humpl T, Raskob GE, Ivy DD, Jing ZC, Bonnet D, Schulze-Neick I, Barst RJ. Clinical features of paediatric pulmonary hypertension: a registry study. *Lancet* 2012; 379: 537-546.
26. Hoeper MM, Huscher D, Ghofrani HA, Delcroix M, Distler O, Schweiger C, Grünig E, Staehler G, Rosenkranz S, Halank M, Held M, Grohe C, Lange TJ, Behr J, Klose H, Wilkens H, Filusch A, Germann M, Ewert R, Seyfarth HJ, Olsson KM, Opitz CF, Gaine SP, Vizza CD, Vonk-Noordegraaf A, Kaemmerer H, Gibbs JS, Pittrow D. Elderly patients diagnosed with idiopathic pulmonary arterial hypertension: results from the COMPERA registry. *Int J Cardiol* 2013; 168: 871-880.

27. Eichstaedt CA, Sassmannshausen Z, Shaukat M, Cao D, Xanthouli P, Gall H, Sommer N, Ghofrani HA, Seyfarth HJ, Lerche M, Halank M, Kleymann J, Benjamin N, Harutyunova S, Egenlauf B, Milger K, Rosenkranz S, Ewert R, Klose H, Hoeper MM, Olsson KM, Lankeit M, Lange TJ, Hinderhofer K, Grünig E. Gene panel diagnostics reveals new pathogenic variants in pulmonary arterial hypertension. *Respir Res* 2022; 23: 74.
28. Gräf S, Haimel M, Bleda M, Hadinnapola C, Southgate L, Li W, Hodgson J, Liu B, Salmon RM, Southwood M, Machado RD, Martin JM, Treacy CM, Yates K, Daugherty LC, Shamardina O, Whitehorn D, Holden S, Aldred M, Bogaard HJ, Church C, Coghlan G, Condliffe R, Corris PA, Danesino C, Eyries M, Gall H, Ghio S, Ghofrani HA, Gibbs JSR, Girerd B, Houweling AC, Howard L, Humbert M, Kiely DG, Kovacs G, MacKenzie Ross RV, Moledina S, Montani D, Newnham M, Olschewski A, Olschewski H, Peacock AJ, Pepke-Zaba J, Prokopenko I, Rhodes CJ, Scelsi L, Seeger W, Soubrier F, Stein DF, Suntharalingam J, Swietlik EM, Toshner MR, van Heel DA, Vonk Noordegraaf A, Waisfisz Q, Wharton J, Wort SJ, Ouwehand WH, Soranzo N, Lawrie A, Upton PD, Wilkins MR, Trembath RC, Morrell NW. Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. *Nat Commun* 2018; 9: 1416.
29. Machado RD, Southgate L, Eichstaedt CA, Aldred MA, Austin ED, Best DH, Chung WK, Benjamin N, Elliott CG, Eyries M, Fischer C, Graf S, Hinderhofer K, Humbert M, Keiles SB, Loyd JE, Morrell NW, Newman JH, Soubrier F, Trembath RC, Viales RR, Grünig E. Pulmonary arterial hypertension: A current perspective on established and emerging molecular genetic defects. *Hum Mutat* 2015; 36: 1113-1127.
30. Southgate L, Machado RD, Gräf S, Morrell NW. Molecular genetic framework underlying pulmonary arterial hypertension. *Nat Rev Cardiol* 2020; 17: 85-95.
31. Morrell NW, Aldred MA, Chung WK, Elliott CG, Nichols WC, Soubrier F, Trembath RC, Loyd JE. Genetics and genomics of pulmonary arterial hypertension. *Eur Respir J* 2019; 53: 1801899.
32. Larkin EK, Newman JH, Austin ED, Hemnes AR, Wheeler L, Robbins IM, West JD, Phillips JA, 3rd, Hamid R, Loyd JE. Longitudinal analysis casts doubt on the presence of genetic anticipation in heritable pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2012; 186: 892-896.
33. Pfarr N, Fischer C, Ehlken N, Becker-Grunig T, Lopez-Gonzalez V, Gorenflo M, Hager A, Hinderhofer K, Miera O, Nagel C, Schranz D, Grünig E. Hemodynamic and genetic analysis in children with idiopathic, heritable, and congenital heart disease associated pulmonary arterial hypertension. *Respir Res* 2013; 14: 3.
34. Navas Tejedor P, Tenorio Castano J, Palomino Doza J, Arias P, Gordo G, Lopez Meseguer M, Roman Broto A, Lapunzina P, Escribano Subias P. An Homozygous Mutation in *KCNK3* is Associated with an Aggressive Form of Hereditary Pulmonary Arterial Hypertension. *Clin Genet* 2017; 91: 453-457.
35. Wang G, Fan R, Ji R, Zou W, Penny DJ, Varghese NP, Fan Y. Novel homozygous BMP9 nonsense mutation causes pulmonary arterial hypertension: a case report. *BMC Pulm Med* 2016; 16: 17.
36. Eyries M, Montani D, Girerd B, Perret C, Leroy A, Lonjou C, Chelghoum N, Coulet F, Bonnet D, Dorfmüller P, Fadel E, Sitbon O, Simonneau G, Tregouet DA, Humbert M, Soubrier F. *EIF2AK4* mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension. *Nat Genet* 2014; 46: 65-69.
37. Best DH, Sumner KL, Austin ED, Chung WK, Brown LM, Borczuk AC, Rosenzweig EB, Bayrak-Toydemir P, Mao R, Cahill BC, Tazelaar HD, Leslie KO, Hemnes AR, Robbins IM, Elliott CG. *EIF2AK4* mutations in pulmonary capillary hemangiomatosis. *Chest* 2014; 145: 231-236.
38. Tenorio J, Navas P, Barrios E, Fernandez L, Nevado J, Quezada CA, Lopez-Meseguer M, Arias P, Mena R, Lobo JL, Alvarez C, Heath K, Escribano-Subias P, Lapunzina P. A founder *EIF2AK4* mutation causes an aggressive form of pulmonary arterial hypertension in Iberian Gypsies. *Clin Genet* 2014; 88: 579-583.
39. Hadinnapola C, Bleda M, Haimel M, Screaton N, Swift AJ, Dorfmüller P, Preston SD, Southwood M, Hernandez-Sanchez J, Martin J, Treacy C, Yates K, Bogaard H, Church C, Coghlan G,

Condliffe R, Corris PA, Gibbs SR, Girerd B, Holden S, Humbert M, Kiely DG, Lawrie A, Machado RD, MacKenzie Ross R, Moledina S, Montani D, Newnham M, Peacock AJ, Pepke-Zaba J, Rayner-Matthews PJ, Shamardina O, Soubrier F, Southgate L, Suntharalingam J, Toshner MR, Trembath RC, Vonk Noordegraaf A, Wilkins MR, Wort SJ, Wharton J, Graf S, Morrell NW, Consortium NB-RD, Idiopathic UKNCSo, Heritable PAH. Phenotypic Characterisation of *EIF2AK4* Mutation Carriers in a Large Cohort of Patients Diagnosed Clinically with Pulmonary Arterial Hypertension. *Circulation* 2017; 136: 2022-2033.

40. Nossent EJ, Antigny F, Montani D, Bogaard HJ, Ghigna MR, Lambert M, Thomas de Montpreville V, Girerd B, Jais X, Savale L, Mercier O, Fadel E, Soubrier F, Sitbon O, Simonneau G, Vonk Noordegraaf A, Humbert M, Perros F, Dorfmüller P. Pulmonary vascular remodeling patterns and expression of general control nonderepressible 2 (GCN2) in pulmonary veno-occlusive disease. *J Heart Lung Transplant* 2018; 37: 647-655.

41. Evans JD, Girerd B, Montani D, Wang XJ, Galie N, Austin ED, Elliott G, Asano K, Grünig E, Yan Y, Jing ZC, Manes A, Palazzini M, Wheeler LA, Nakayama I, Satoh T, Eichstaedt C, Hinderhofer K, Wolf M, Rosenzweig EB, Chung WK, Soubrier F, Simonneau G, Sitbon O, Graf S, Kaptoge S, Di Angelantonio E, Humbert M, Morrell NW. BMPR2 mutations and survival in pulmonary arterial hypertension: an individual participant data meta-analysis. *Lancet Respir Med* 2016; 4: 129-137.

42. Montani D, Lau EM, Dorfmüller P, Girerd B, Jais X, Savale L, Perros F, Nossent E, Garcia G, Parent F, Fadel E, Soubrier F, Sitbon O, Simonneau G, Humbert M. Pulmonary veno-occlusive disease. *Eur Respir J* 2016; 47: 1518-1534.

43. Montani D, Girerd B, Jais X, Levy M, Amar D, Savale L, Dorfmüller P, Seferian A, Lau EM, Eyries M, Le Pavec J, Parent F, Bonnet D, Soubrier F, Fadel E, Sitbon O, Simonneau G, Humbert M. Clinical phenotypes and outcomes of heritable and sporadic pulmonary veno-occlusive disease: a population-based study. *Lancet Respir Med* 2017.

44. Best DH, Sumner KL, Smith BP, Damjanovich-Colmenares K, Nakayama I, Brown LM, Ha Y, Paul E, Morris A, Jama MA, Dodson MW, Bayrak-Toydemir P, Elliott CG. *EIF2AK4* Mutations in Patients Diagnosed with Pulmonary Arterial Hypertension. *Chest* 2017; 151: 821-828.

45. Zeng X, Chen F, Rathinasabapathy A, Li T, Adnan Ali Mohammed Mohammed A, Yu Z. Rapid disease progress in a PVOD patient carrying a novel EIF2AK4 mutation: a case report. *BMC Pulm Med* 2020; 20: 186.

46. Girerd B, Montani D, Coulet F, Sztrymf B, Yaici A, Jais X, Tregouet D, Reis A, Drouin-Garraud V, Fraisse A, Sitbon O, O'Callaghan DS, Simonneau G, Soubrier F, Humbert M. Clinical outcomes of pulmonary arterial hypertension in patients carrying an ACVRL1 (ALK1) mutation. *Am J Respir Crit Care Med* 2010; 181: 851-861.

47. Kerstjens-Frederikse WS, Bongers EM, Roofthooft MT, Leter EM, Douwes JM, Van Dijk A, Vonk-Noordegraaf A, Dijk-Bos KK, Hoefsloot LH, Hoendermis ES, Gille JJ, Sikkema-Raddatz B, Hofstra RM, Berger RM. TBX4 mutations (small patella syndrome) are associated with childhood-onset pulmonary arterial hypertension. *J Med Genet* 2013; 50: 500-506.

48. Thore P, Girerd B, Jais X, Savale L, Ghigna MR, Eyries M, Levy M, Ovaert C, Servettaz A, Guillaumot A, Dauphin C, Chabanne C, Boiffard E, Cottin V, Perros F, Simonneau G, Sitbon O, Soubrier F, Bonnet D, Remy-Jardin M, Chaouat A, Humbert M, Montani D. Phenotype and outcome of pulmonary arterial hypertension patients carrying a TBX4 mutation. *Eur Respir J* 2020; 55.

49. Zhu N, Gonzaga-Jauregui C, Welch CL, Ma L, Qi H, King AK, Krishnan U, Rosenzweig EB, Ivy DD, Austin ED, Hamid R, Nichols WC, Pauciulo MW, Lutz KA, Sawle A, Reid JG, Overton JD, Baras A, Dewey F, Shen Y, Chung WK. Exome Sequencing in Children With Pulmonary Arterial Hypertension Demonstrates Differences Compared With Adults. *Circ Genom Precis Med* 2018; 11: e001887.

50. Galambos C, Mullen MP, Shieh JT, Schwerk N, Kielt MJ, Ullmann N, Boldrini R, Stucin-Gantar I, Haass C, Bansal M, Agrawal PB, Johnson J, Peca D, Surace C, Cutrera R, Pauciulo MW, Nichols WC, Griese M, Ivy D, Abman SH, Austin ED, Danhaive O. Phenotype characterisation of TBX4 mutation and deletion carriers with neonatal and paediatric pulmonary hypertension. *Eur Respir J* 2019; 54.

51. Eyries M, Montani D, Girerd B, Favrolt N, Riou M, Faivre L, Manaud G, Perros F, Graf S, Morrell NW, Humbert M, Soubrier F. Familial pulmonary arterial hypertension by KDR heterozygous loss of function. *Eur Respir J* 2020; 55: 1902165.
52. Swietlik EM, Greene D, Zhu N, Megy K, Cogliano M, Rajaram S, Pandya D, Tilly T, Lutz KA, Welch CCL, Pauciulo MW, Southgate L, Martin JM, Treacy CM, Penkett CJ, Stephens JC, Bogaard HJ, Church C, Coghlan G, Coleman AW, Condliffe R, Eichstaedt CA, Eyries M, Gall H, Ghio S, Girerd B, Grünig E, Holden S, Howard L, Humbert M, Kiely DG, Kovacs G, Lordan J, Machado RD, Mackenzie Ross RV, McCabe C, Moledina S, Montani D, Olschewski H, Pepke-Zaba J, Price L, Rhodes CJ, Seeger W, Soubrier F, Suntharalingam J, Toshner MR, Vonk Noordegraaf A, Wharton J, Wild JM, Wort SJ, Lawrie A, Wilkins MR, Trembath RC, Shen Y, Chung WK, Swift AJ, Nichols WC, Morrell NW, Gräf S. Bayesian Inference Associates Rare KDR Variants with Specific Phenotypes in Pulmonary Arterial Hypertension. *Circ Genom Precis Med* 2021; 14: e003155.
53. Hoeper MM, Dwivedi K, Pausch C, Lewis RA, Olsson KM, Huscher D, Pittrow D, Grunig E, Staehler G, Vizza CD, Gall H, Distler O, Opitz C, Gibbs JSR, Delcroix M, Park DH, Ghofrani HA, Ewert R, Kaemmerer H, Kabitz HJ, Skowasch D, Behr J, Milger K, Lange TJ, Wilkens H, Seyfarth HJ, Held M, Dumitrescu D, Tsangaris I, Vonk-Noordegraaf A, Ulrich S, Klose H, Claussen M, Eisenmann S, Schmidt KH, Swift AJ, Thompson AAR, Elliot CA, Rosenkranz S, Condliffe R, Kiely DG, Halank M. Phenotyping of idiopathic pulmonary arterial hypertension: a registry analysis. *Lancet Respir Med* 2022.
54. Zhu N, Welch CL, Wang J, Allen PM, Gonzaga-Jauregui C, Ma L, King AK, Krishnan U, Rosenzweig EB, Ivy DD, Austin ED, Hamid R, Pauciulo MW, Lutz KA, Nichols WC, Reid JG, Overton JD, Baras A, Dewey FE, Shen Y, Chung WK. Rare variants in SOX17 are associated with pulmonary arterial hypertension with congenital heart disease. *Genome Med* 2018; 10: 56.
55. Welch CL, Chung WK. Genetics and Genomics of Pediatric Pulmonary Arterial Hypertension. *Genes* 2020; 11.
56. Montani D, Lechartier B, Girerd B, Eyries M, Ghigna MR, Savale L, Jais X, Seferian A, Jevnikar M, Boucly A, Riou M, Traclet J, Chaouat A, Levy M, Le Pavec J, Fadel E, Perros F, Soubrier F, Remy-Jardin M, Sitbon O, Bonnet D, Humbert M. An emerging phenotype of pulmonary arterial hypertension patients carrying SOX17 variants. *Eur Respir J* 2022.
57. Wang TM, Wang SS, Xu YJ, Zhao CM, Qiao XH, Yang CX, Liu XY, Yang YQ. SOX17 Loss-of-Function Mutation Underlying Familial Pulmonary Arterial Hypertension. *Int Heart J* 2021.
58. !!! INVALID CITATION !!! [56].
59. Humbert M, Deng Z, Simonneau G, Barst RJ, Sitbon O, Wolf M, Cuervo N, Moore KJ, Hodge SE, Knowles JA, Morse JH. BMPR2 germline mutations in pulmonary hypertension associated with fenfluramine derivatives. *Eur Respir J* 2002; 20: 518-523.
60. Souza R, Humbert M, Sztrymf B, Jais X, Yaici A, Le Pavec J, Parent F, Herve P, Soubrier F, Sitbon O, Simonneau G. Pulmonary arterial hypertension associated with fenfluramine exposure: report of 109 cases. *Eur Respir J* 2008; 31: 343-348.
61. Lerche M, Eichstaedt CA, Hinderhofer K, Grünig E, Tausche K, Ziemssen T, Halank M, Wirtz H, Seyfarth HJ. Mutually reinforcing effects of genetic variants and interferon-beta 1a therapy for pulmonary arterial hypertension development in multiple sclerosis patients. *Pulm Circ* 2019; 9: 2045894019872192.
62. Perrin S, Montani D, O'Connell C, Gunther S, Girerd B, Savale L, Guignabert C, Sitbon O, Simonneau G, Humbert M, Chaumais MC. Nasal decongestant exposure in patients with pulmonary arterial hypertension: a pilot study. *Eur Respir J* 2015; 46: 1211-1214.
63. Montani D, Lau EM, Descatha A, Jais X, Savale L, Andujar P, Bensefa-Colas L, Girerd B, Zendah I, Le Pavec J, Seferian A, Perros F, Dorfmuller P, Fadel E, Soubrier F, Sitbon O, Simonneau G, Humbert M. Occupational exposure to organic solvents: a risk factor for pulmonary veno-occlusive disease. *Eur Respir J* 2015; 46: 1721-1731.
64. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of

Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405-424.
65. Grünig E, Weissmann S, Ehlken N, Fijalkowska A, Fischer C, Fourme T, Galie N, Ghofrani A, Harrison RE, Huez S, Humbert M, Janssen B, Kober J, Koehler R, Machado RD, Mereles D, Naeije R, Olschewski H, Provencher S, Reichenberger F, Retailleau K, Rocchi G, Simonneau G, Torbicki A, Trembath R, Seeger W. Stress Doppler echocardiography in relatives of patients with idiopathic and familial pulmonary arterial hypertension: results of a multicenter European analysis of pulmonary artery pressure response to exercise and hypoxia. *Circulation* 2009; 119: 1747-1757.
66. Hinderhofer K, Fischer C, Pfarr N, Szamalek-Hoegel J, Lichtblau M, Nagel C, Egenlauf B, Ehlken N, Grünig E. Identification of a new intronic *BMPR2*-mutation and early diagnosis of heritable pulmonary arterial hypertension in a large family with mean clinical follow-up of 12 years. *PLoS One* 2014; 9: e91374.
67. Coghlan JG, Denton CP, Grünig E, Bonderman D, Distler O, Khanna D, Müller-Ladner U, Pope JE, Vonk MC, Doelberg M, Chadha-Boreham H, Heinzl H, Rosenberg DM, McLaughlin VV, Seibold JR. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann Rheum Dis* 2014; 73: 1340-1349.
68. Yang P, Yu PB. In Search of the Second Hit in Pulmonary Arterial Hypertension. *Circ Res* 2019; 124: 6-8.
69. Montani D, Girerd B, Jais X, Laveneziana P, Lau EMT, Bouchachi A, Hascoet S, Gunther S, Godinas L, Parent F, Guignabert C, Beurnier A, Chemla D, Herve P, Eyries M, Soubrier F, Simonneau G, Sitbon O, Savale L, Humbert M. Screening for pulmonary arterial hypertension in adults carrying a *BMPR2* mutation. *Eur Respir J* 2021; 58: 2004229.
70. Lyle MA, Fenstad ER, McGoon MD, Frantz RP, Krowka MJ, Kane GC, Swanson KL. Pulmonary Hypertension in Hereditary Hemorrhagic Telangiectasia. *Chest* 2016; 149: 362-371.
71. Margelidon-Cozzolino V, Cottin V, Dupuis-Girod S, Traclet J, Ahmad K, Mornex JF, Turquier S. Pulmonary hypertension in hereditary haemorrhagic telangiectasia is associated with multiple clinical conditions. *ERJ Open Res* 2021; 7.
72. Nasim MT, Ogo T, Ahmed M, Randall R, Chowdhury HM, Snape KM, Bradshaw TY, Southgate L, Lee GJ, Jackson I, Lord GM, Gibbs JS, Wilkins MR, Ohta-Ogo K, Nakamura K, Girerd B, Coulet F, Soubrier F, Humbert M, Morrell NW, Trembath RC, Machado RD. Molecular genetic characterization of SMAD signaling molecules in pulmonary arterial hypertension. *Hum Mutat* 2011; 32: 1385-1389.
73. Ma L, Roman-Campos D, Austin ED, Eyries M, Sampson KS, Soubrier F, Germain M, Tregouet DA, Borczuk A, Rosenzweig EB, Girerd B, Montani D, Humbert M, Loyd JE, Kass RS, Chung WK. A novel channelopathy in pulmonary arterial hypertension. *N Engl J Med* 2013; 369: 351-361.
74. Austin ED, Ma L, LeDuc C, Berman Rosenzweig E, Borczuk A, Phillips JA, 3rd, Palomero T, Sumazin P, Kim HR, Talati MH, West J, Loyd JE, Chung WK. Whole exome sequencing to identify a novel gene (caveolin-1) associated with human pulmonary arterial hypertension. *Circ Cardiovasc Genet* 2012; 5: 336-343.
75. Machado R, Welch CL, Haimel M, Bleda M, Colglazier E, Coulson JD, Debeljak M, Ekstein J, Fineman JR, Golden WC, Griffin EL, Hadinnapola C, Harris MA, Hirsch Y, Hoover-Fong J, Nogee L, Romer LH, Vesel S, Graf S, Morrell N, Southgate L, Chung W. Biallelic variants of ATP13A3 cause dose-dependent childhood-onset pulmonary arterial hypertension characterised by extreme morbidity and mortality. *J Med Genet* 2021.
76. Bohnen MS, Ma L, Zhu N, Qi H, McClenaghan C, Gonzaga-Jauregui C, Dewey FE, Overton JD, Reid JG, Shuldiner AR, Baras A, Sampson KJ, Bleda M, Hadinnapola C, Haimel M, Bogaard HJ, Church C, Coghlan G, Corris PA, Eyries M, Gibbs JSR, Girerd B, Houweling AC, Humbert M, Guignabert C, Kiely DG, Lawrie A, MacKenzie Ross RV, Martin JM, Montani D, Peacock AJ, Pepke-Zaba J, Soubrier F, Suntharalingam J, Toshner M, Treacy CM, Trembath RC, Vonk Noordegraaf A, Wharton J, Wilkins MR, Wort SJ, Yates K, Graf S, Morrell NW, Krishnan U, Rosenzweig EB, Shen Y, Nichols CG, Kass RS, Chung WK. Loss-of-Function *ABCC8* Mutations in Pulmonary Arterial Hypertension. *Circ Genom Precis Med* 2018; 11: e002087.

77. Potus F, Pauciulo MW, Cook EK, Zhu N, Hsieh A, Welch CL, Shen Y, Tian L, Lima P, Mewburn J, D'Arsigny CL, Lutz KA, Coleman AW, Damico R, Snetsinger B, Martin AY, Hassoun PM, Nichols WC, Chung WK, Rauh MJ, Archer SL. Novel Mutations and Decreased Expression of the Epigenetic Regulator TET2 in Pulmonary Arterial Hypertension. *Circulation* 2020.
78. Eichstaedt CA, Song J, Rodríguez Viales R, Pan Z, Benjamin N, Fischer C, Hoeper M, Ulrich S, Hinderhofer K, Grünig E. First identification of Krüppel-like factor 2 mutation in heritable pulmonary arterial hypertension. *Clinical science (London, England : 1979)* 2017; 131: 689-698.
79. Eyries M, Montani D, Nadaud S, Girerd B, Levy M, Bourdin A, Tresorier R, Chaouat A, Cottin V, Sanfiorenzo C, Prevot G, Reynaud-Gaubert M, Dromer C, Houeijeh A, Nguyen K, Coulet F, Bonnet D, Humbert M, Soubrier F. Widening the landscape of heritable pulmonary hypertension mutations in paediatric and adult cases. *Eur Respir J* 2019; 53: 1801371.
80. Zhu N, Swietlik EM, Welch CL, Pauciulo MW, Hagen JJ, Zhou X, Guo Y, Karten J, Pandya D, Tilly T, Lutz KA, Martin JM, Treacy CM, Rosenzweig EB, Krishnan U, Coleman AW, Gonzaga-Jauregui C, Lawrie A, Trembath RC, Wilkins MR, Regeneron Genetics C, Investigators PAHBEC, Diseases NBfTR-R, National Cohort Study of I, Heritable PAH, Morrell NW, Shen Y, Graf S, Nichols WC, Chung WK. Rare variant analysis of 4241 pulmonary arterial hypertension cases from an international consortium implicates FBLN2, PDGFD, and rare de novo variants in PAH. *Genome Med* 2021; 13: 80.
81. Song J, Eichstaedt CA, Rodríguez Viales R, Benjamin N, Harutyunova S, Fischer C, Grünig E, Hinderhofer K. Identification of genetic defects in pulmonary arterial hypertension by a new gene panel diagnostic tool. *Clinical science (London, England : 1979)* 2016; 130: 2043-2052.
82. van den Heuvel LM, Jansen SMA, Alsters SIM, Post MC, van der Smagt JJ, Handoko-De Man FS, van Tintelen JP, Gille H, Christiaans I, Vonk Noordegraaf A, Bogaard H, Houweling AC. Genetic Evaluation in a Cohort of 126 Dutch Pulmonary Arterial Hypertension Patients. *Genes* 2020; 11.
83. Plon SE, Eccles DM, Easton D, Foulkes WD, Genuardi M, Greenblatt MS, Hogervorst FB, Hoogerbrugge N, Spurdle AB, Tavtigian SV, Group IUGVW. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* 2008; 29: 1282-1291.
84. Limoges M, Langleben D, Fox BD, Shear R, Wieczorek P, Rudski LG, Hirsch AM, Schlesinger RD, Lesenko L. Pregnancy as a possible trigger for heritable pulmonary arterial hypertension. *Pulm Circ* 2016; 6: 381-383.
85. Jaliawala HA, Parmar M, Summers K, Bernardo RJ. A second hit? Pulmonary arterial hypertension, *BMPR2* mutation, and exposure to prescription amphetamines. *Pulm Circ* 2022; 12: e12053.
86. Girerd B, Montani D, Jais X, Eyries M, Yaici A, Sztrymf B, Savale L, Parent F, Coulet F, Godinas L, Lau EM, Tamura Y, Sitbon O, Soubrier F, Simonneau G, Humbert M. Genetic counselling in a national referral centre for pulmonary hypertension. *Eur Respir J* 2016; 47: 541-552.
87. Eichstaedt CA, Song J, Benjamin N, Harutyunova S, Fischer C, Grünig E, Hinderhofer K. *EIF2AK4* mutation as "second hit" in hereditary pulmonary arterial hypertension. *Respir Res* 2016; 17: 141.
88. Wang G, Knight L, Ji R, Lawrence P, Kanaan U, Li L, Das A, Cui B, Zou W, Penny DJ, Fan Y. Early onset severe pulmonary arterial hypertension with 'two-hit' digenic mutations in both *BMPR2* and *KCNA5* genes. *Int J Cardiol* 2014; 177: e167-169.
89. Rosenzweig EB, Morse JH, Knowles JA, Chada KK, Khan AM, Roberts KE, McElroy JJ, Juskiw NK, Mallory NC, Rich S, Diamond B, Barst RJ. Clinical implications of determining BMPR2 mutation status in a large cohort of children and adults with pulmonary arterial hypertension. *J Heart Lung Transplant* 2008; 27: 668-674.
90. Elliott CG, Glissmeyer EW, Havlena GT, Carlquist J, McKinney JT, Rich S, McGoon MD, Scholand MB, Kim M, Jensen RL, Schmidt JW, Ward K. Relationship of BMPR2 mutations to vasoreactivity in pulmonary arterial hypertension. *Circulation* 2006; 113: 2509-2515.
91. Benza RL, Gomberg-Maitland M, Demarco T, Frost AE, Torbicki A, Langleben D, Pulido T, Correa-Jaque P, Passineau MJ, Wiener HW, Tamari M, Hirota T, Kubo M, Tiwari HK. Endothelin-1

Pathway Polymorphisms Affect Outcome in Pulmonary Arterial Hypertension. *Am J Respir Crit Care Med* 2015; 192: 1345-1354.
92. Scelsi L, Greco A, Acquaro M, Olivieri C, Sobrero M, Turco A, Cappelletti D, Visconti LO, Ghio S. *BMPR2* mutations and response to inhaled or parenteral prostanoids: a case series. *Pulm Circ* 2021; 11: 20458940211037275.
93. Boucly A, Savale L, Jaïs X, Humbert M, Sitbon O, Montani D. Sequential combination therapy with parenteral prostacyclin in BMPR2 mutations carriers. *Pulm Circ* 2022: e12023.
94. Taha F, Southgate L. Molecular genetics of pulmonary hypertension in children. *Curr Opin Genet Dev* 2022; 75: 101936.
95. Haarman MG, Kerstjens-Frederikse WS, Vissia-Kazemier TR, Breeman KTN, Timens W, Vos YJ, Roofthooft MTR, Hillege HL, Berger RMF. The Genetic Epidemiology of Pediatric Pulmonary Arterial Hypertension. *J Pediatr* 2020.
96. Montani D, Dorfmüller P, Girerd B, Le Pavec J, Fadel E, Simonneau G, Sitbon O, Humbert M. Natural History Over 8 Years of Pulmonary Vascular Disease in a Patient Carrying Bi-allelic EIF2AK4 Mutations. *Am J Respir Crit Care Med* 2018.
97. Oriaku I, LeSieur MN, Nichols WC, Barrios R, Elliott CG, Frost A. A novel BMPR2 mutation with widely disparate heritable pulmonary arterial hypertension clinical phenotype. *Pulm Circ* 2020; 10: 2045894020931315.
98. Montani D, Achouh L, Dorfmüller P, Le Pavec J, Sztrymf B, Tcherakian C, Rabiller A, Haque R, Sitbon O, Jais X, Dartevelle P, Maitre S, Capron F, Musset D, Simonneau G, Humbert M. Pulmonary veno-occlusive disease: clinical, functional, radiologic, and hemodynamic characteristics and outcome of 24 cases confirmed by histology. *Medicine (Baltimore)* 2008; 87: 220-233.
99. !!! INVALID CITATION !!! [27, 33, 49, 50, 95-101].
100. Tenorio Castaño JA, Hernandez-Gonzalez I, Gallego N, Perez-Olivares C, Ochoa Parra N, Arias P, Granda E, Acebo GG, Lago-Docampo M, Palomino-Doza J, Lopez Meseguer M, Del Cerro MJ, Pah Consortium S, Valverde D, Lapunzina P, Escribano-Subias P. Customized Massive Parallel Sequencing Panel for Diagnosis of Pulmonary Arterial Hypertension. *Genes* 2020; 11.
101. Tsoi SM, Jones K, Colglazier E, Parker C, Nawaytou H, Teitel D, Fineman JR, Keller RL. Persistence of persistent pulmonary hypertension of the newborn: A case of de novo TBX4 variant. *Pulm Circ* 2022; 12: e12108.
102. Wang XJ, Lian TY, Jiang X, Liu SF, Li SQ, Jiang R, Wu WH, Ye J, Cheng CY, Du Y, Xu XQ, Wu Y, Peng FH, Sun K, Mao YM, Yu H, Liang C, Shyy JY, Zhang SY, Zhang X, Jing ZC. Germline BMP9 mutation causes idiopathic pulmonary arterial hypertension. *Eur Respir J* 2019; 53: 1801609.

Appendix

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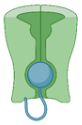
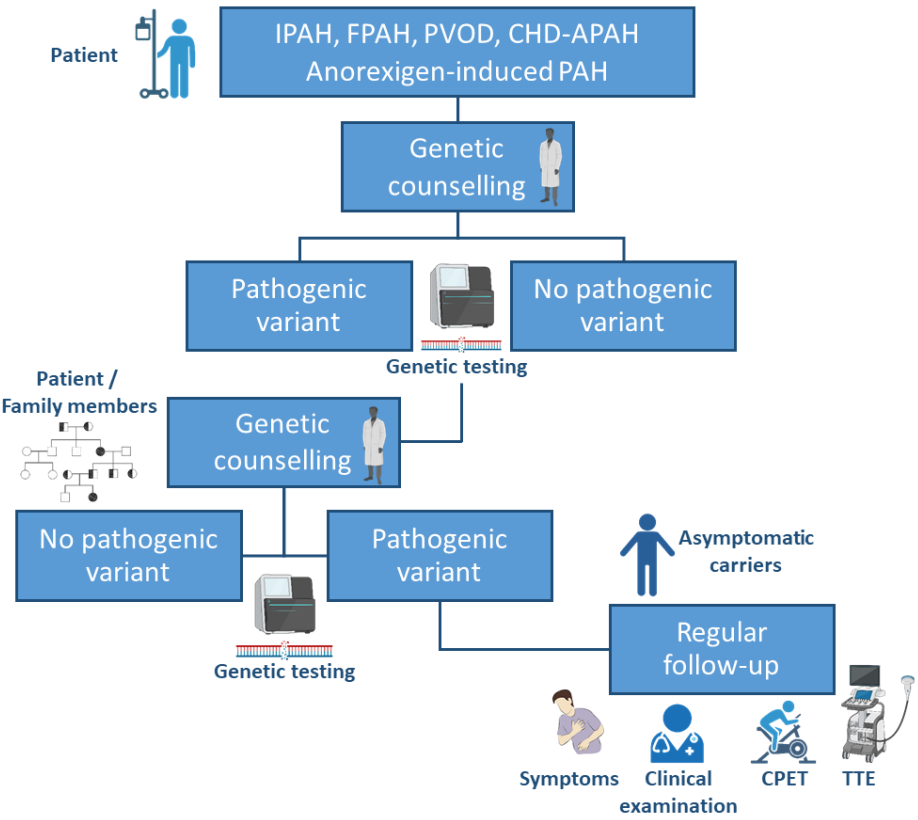



BMP/TGFβ family	Channels	Transcription factors	Other
- *ACVRL1* (ALK1)*	- *ATP13A3* (ATPase 13A3)	- *EIF2AK4* (GCN2)[$]	- *KDR* (VEGFR-2)
- *BMPR2* (BMPRII)	- *KCNK3* (TASK-1)	- *SOX17* (SOX-17)[#]	- *TET2* (TET2)
- *ENG* (endoglin)*	- *ABCC8* (MRP8)	- *TBX4* (TBX4)[#]	- *GGCX* (GGCX)
- *GDF2* (BMP9)			
- *SMAD9* (SMAD8)			
- *CAV1* (Caveolin-1)			


Patient
IPAH, FPAH, PVOD, CHD-APAH
Anorexigen-induced PAH
Genetic counselling
Pathogenic variant
No pathogenic variant
Genetic testing
Patient / Family members
Genetic counselling
No pathogenic variant
Pathogenic variant
Genetic testing
Asymptomatic carriers
Regular follow-up
Symptoms
Clinical examination
CPET
TTE