

Genetics and genetic testing in pulmonary arterial hypertension (RCD code: II-1A.1)

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Abstract

Pulmonary arterial hypertension (PAH) is a rare disease with a high mortality and complex pathomechanism. Recent studies suggest an important role of genetic factors in the development of PAH. It was shown that patients with *BMPR2* mutations present with disease at an earlier age and have more severe haemodynamic disturbances at the time of diagnosis. Interestingly, in this disorder, lifelong penetrance is estimated to be only about 20% and the mechanism of this phenomenon remains unknown. More recent studies have focused on mutational analyses of genes involved in the transforming growth factor-beta signalling pathway in this group of patients. Results of these studies are very promising, however, they still need to be confirmed. Moreover, data on the impact of identified mutations on the clinical course, PAH-specific treatment, and prognosis is required. A multi-center study is planned in Poland to include patients from referral centers for pulmonary hypertension with diagnosed idiopathic, hereditary and drug-induced PAH, PAH developing after surgical correction of a congenital heart defect, or of pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis. The aim of the study is to perform an extended molecular analysis to better understand the molecular basis of PAH pathogenesis, incomplete penetrance, and to create an algorithm for the molecular diagnosis of PAH patients. In addition, the correlation of molecular and clinical data with the effects of specific treatment and prognosis in PAH will be assessed. JRC D 2018; 3 (7): 226–229

Key words: rare disease, genetics, pulmonary arterial hypertension, pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis

Pulmonary arterial hypertension (PAH) is a rare disease characterised by progressive remodelling within the small pulmonary arteries resulting in increased pulmonary vascular resistance, pulmonary arterial pressure, and ultimately right ventricular failure and death. A recent definition from March 2018, proposed during the 6th World Symposium on Pulmonary Hypertension in Nice, France, states that PAH is characterised by a mean pulmonary artery pressure (mPAP) >20 mm Hg with a normal pulmonary artery wedge pressure (≤15 mm Hg) and pulmonary vascular resistance > 3 Wood units measured via right heart catheterisation, in the absence of other causes of pre-capillary pulmonary hypertension. Disorders classified as PAH Group 1 include idiopathic, heritable, drug- and toxin-induced, and associated with congenital heart disease, connective tissue disease, portal hypertension, and human immunodeficiency virus infection [1].

Current therapy, including PAH-specific pharmacological treatment, is insufficient, and the disease is characterised by high mortality [2]. Early diagnosis is crucial to improve long-term survival. In this regard, an effective strategy is genetic testing, since identi-

fying asymptomatic carriers of PAH-associated alterations enables early management if PAH symptoms occur. Molecular testing allows the implementation of genetic counselling for severe disease.

Mutational analysis helped to determine the genetic background in less than 5% patients enrolled in the most important PAH registries. In the REVEAL registry, 2.7% of patients had heritable PAH [3], while 3.9% of patients in the French registry had heritable PAH [4]. To date, comprehensive molecular testing has not been performed in a group of Polish patients with PAH. Therefore, an extended molecular analysis in PAH patients from referral PAH centers in Poland and treated according to the Polish National Health Fund program has been planned.

In 1954, Dresdale et al. [5] described familial PAH affecting a patient, her sister, and son, suggesting a heritable form of the disease. In 1984, Loyd et al. [6] reported 14 families with two or more members affected by PAH. In 2000, mutations of the *BMPR2* gene (bone morphogenetic protein receptor type II) were identified as the main cause of heritable PAH [7, 8].

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Table. Genes in which pathogenic variants have been detected in patients with pulmonary arterial hypertension (PAH) or pulmonary veno-occlusive disease (PVOD)/pulmonary capillary hemangiomatosis (PCH) [13, 28]

Symbol	Name
ACVRL1	Activin A receptor like type 1
AGTR1	Angiotensin II receptor type 1
AQP1	Aquaporin 1
ATP13A3	P-type ATPase
BMPR1B	Bone morphogenetic protein receptor type 1B
BMPR2	Bone morphogenetic protein receptor type 2
CAV1	Caveolin 1
EDN1	Endothelin 1
EDNRA	Endothelin receptor type A
EIF2AK4	Eukaryotic translation initiation factor 2 alpha kinase 4
ENG	Endoglin
GDF2 (BMP9)	Growth differentiation factor 2 (bone morphogenetic protein 9)
KCNA5	Potassium voltage-gated channel subfamily A member 5
KCNK3	Potassium two pore domain channel subfamily K member 3
KLF2	Kruppel like factor 2
KLF4	Kruppel like factor 4
KLF5	Kruppel like factor 5
NOS2	Nitric oxide synthase 2
NOTCH1	Notch 1
NOTCH3	Notch 3
SERPINE1	Serpin family E member 1
SIRT3	Sirtuin 3
SMAD1	Smad family member 1
SMAD4	Smad family member 4
SMAD5	Smad family member 5
SMAD9	Smad family member 9
SOX17	SRY-box 17
TBX4	T-box 4
THBS1	Thrombospondin 1
TOPBP1	Topoisomerase (DNA) II binding protein 1
TRPC6	Transient receptor potential cation channel subfamily C member 6

Hereditary haemorrhagic telangiectasia (HHT) is characterised by telangiectasias and arteriovenous malformations, including the pulmonary circulation. Furthermore, patients with HHT may develop PAH. Molecular diagnostics performed in PAH patients with a personal or familial history of HHT revealed a mutation of two genes: *ACVRL1* (activin A receptor type II-like kinase) and *ENG* (endoglin) [9, 10]. Additionally, in individuals with coexisting HHT and PAH, mutations in *BMP9* (*GDF2*) were detected.

In 2012, mutations in *CAV1* (caveolin1) were found in PAH patients [11], and in 2013, channelopathy of *KCNK3* was described [12]. *KCNK3* belongs to a family of mammalian potassium channels and is localised on the cell membrane of many cells including pulmonary arterial smooth muscle cells. Potassium channels play an important role in the regulation of resting membrane potential and pulmonary vascular tone. It has been postulated that *KCNK3* is involved in the regulation of vascular remodelling and abnormal vascular proliferation in PAH patients via the prevention of apoptosis. *CAV1* is highly expressed in adipocytes, endothelial cells, and fibroblasts. In patients with lipodystrophy or partial lipodystrophy, homozygous or heterozygous *CAV1* mutations have been identified. More recently, in patients with isolated PAH and PAH associated with lipodystrophy, heterozygous mutations of this gene were found. *BMPR2* localises to caveolae and also directly interacts with *CAV1* in vascular smooth muscle cells.

All PAH-causing mutations have been discovered in the transforming growth factor-beta (*TGFβ*) pathway, including mutations in *BMPR2*, *ACVRL1*, *ENG*, *CAV1*, and *SMAD* family genes [13]. Pulmonary arterial hypertension caused by mutations in these genes is an autosomal dominant disease with incomplete penetrance. Nearly 30 proteins have been identified as members of the *TGFβ* superfamily, playing important roles in the regulation of embryonic development, multiple cellular functions, and homeostasis, including proliferation, differentiation, and apoptosis. *TGFβ* has an important role in the respiratory system. In humans, five type II and seven type I receptors have been identified, which together comprise the family of serine/threonine kinase receptors. The activated receptor complex propagates the signal into the pathway through phosphorylation of proteins from the family of small mothers against decapentaplegic (*SMAD*) – *SMAD* 1/5/9 and *SMAD* 2/3, subsequently forming complexes with common-mediator *SMAD4* and translocating to the nucleus to regulate gene expression.

In 2013, biallelic recessive mutations in the *EIF2AK4* (eukaryotic translation initiation factor 2 alpha kinase 4) gene have been detected in patients with pulmonary veno-occlusive disease (PVOD) [14] and pulmonary capillary hemangiomatosis (PCH) [15], a rare disease which can be misdiagnosed as PAH. However, PVOD/PCH vascular remodelling predominates in pulmonary venules and small veins, while in PAH this applies to small arterioles. *EIF2AK4* mutations can be detected in both PVOD and PCH patients, which suggests that there are two pathologic manifestations of the same disorder. PVOD/PCH due to *EIF2AK4* mutations are autosomal recessive disorders with unknown penetrance.

A recent meta-analysis has revealed genetic evidence for at least 30 genes, of which 21 have been shown to possess specific mutations related to PAH [13], however, the *BMPR2* mutations are un-

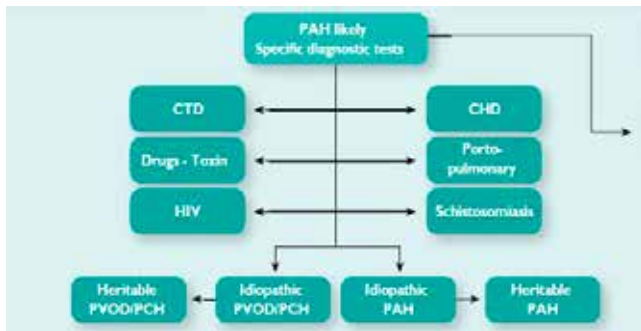


Figure 1. Place of genetic testing to establish the cause of pulmonary arterial hypertension (PAH) or if the pulmonary veno-occlusive disease (PVOD)/pulmonary capillary hemangiomatosis (PCH) is suspected with respect to the ESC recommendations [1]. CHD – congenital heart disease; CTD – connective tissue disease; HIV – Human immunodeficiency virus

doubtedly the most common. Initially, the BMP type II receptor was described to be involved in the regulation of differentiation and growth of bone and cartilage, but in further studies, it was found to play an important role in the regulation of differentiation, growth, and apoptosis of other cell types, including pulmonary artery endothelial cells and smooth muscle cells [16]. Alterations of the *BMPR2* gene are loss-of-function mutations resulting in reduced signalling downstream of the receptor. To date, more than 300 different *BMPR2* mutations have been identified, which were detected in approximately 75% and 11-40% of heritable and sporadic PAH cases, respectively [17, 18, 19, 20]. The penetrance of *BMPR2* mutations is estimated to be 27% in the overall population, 14% for males and 42% for females [21]. Low penetrance observed for *BMPR2* mutations is complex and multifactorial. Variation in sex hormone metabolism, *TGF β 1* polymorphism, concurrent inflammation, or multiple second hit hypotheses have been considered [17].

Several studies have shown that PAH patients with *BMPR2* mutations are diagnosed at an earlier age, have more severe haemodynamic disturbances at diagnosis with higher mean pulmonary artery pressure, pulmonary vascular resistance, and lower cardiac index. Only a minority of *BMPR2* mutation carriers have a positive response to acute vasodilator testing and these individuals are more likely to undergo lung transplantation [22–25]. It should be emphasised that vasodilator non-responsiveness is associated with a worse prognosis, as it indicates the predominance of extensive vascular remodelling rather than vasoconstriction. Moreover, it has been suggested that impaired right ventricular adaptation to increased afterload is an important factor in these patients, which may account for a reduced cardiac index at diagnosis [25, 26].

Recent guidelines of the European Cardiology Society [1] indicate that mutational analysis of the *BMPR2* gene should be offered by referral centers to patients with sporadic idiopathic or anorexigen-induced PAH and to individuals with a family history of PAH. In patients with familial PAH or HHT, or individuals < 40 years old with idiopathic or anorexigen-induced PAH negative for *BMPR2* mutations, molecular diagnostics of *ACVRL1* and *ENG* genes may be performed. In individuals with no pathogenic variant

detected within the *BMPR2*, *ACVRL1* and *ENG* genes, mutational analyses of other genes should be considered (*KCNK3*, *CAV1*, etc.).

Patients with both sporadic and familial PVOD/PCH should be tested for *EIF2AK4* mutations, since the presence of biallelic recessive pathogenic variants in this gene confirms the PVOD/PCH diagnosis, eliminating the need for lung biopsy and histological examination.

Recently, different diagnostic tests have been developed to perform mutational analyses in patients with PAH [13]. Most of them allow the simultaneous analyses of approximately eight genes. All kits allow testing of *BMPR2*, *ENG*, *ACVRL1*, *CAV1*, and *SMAD4* genes. In contrast, none of the methods included mutational analysis of *AGTR1*, *TBX4*, *EDNN1*, *EDNRA*, *NOS2*, *SERPINE*, *SIRT3*, *THBS1*, *TOPBP1*, or *TRCP6* genes. Current data show that PAH-specific mutations are recurrent in *BMPR2*, *ACVRL1*, *ENG*, *EIF2AK4*, *KCNA3* and *SMAD4* genes. In contrast, in patients with familial PAH, no pathogenic variants were detected in the tested genes. Girerd et al. [27] were unable to detect a mutation in 11% of patients with a family history of PAH. Recently, a study performed in 1038 PAH individuals [28] revealed novel pathogenic variants in *ATP13A3*, *AQP1*, *SOX17*, and *GDF2* (*BMP9*) genes. In this study, mutations in genes accepted as a molecular basis of PAH were found in 19.9% of individuals (15.3% with *BMPR2* pathogenic variants). However, including alterations detected in new genes, pathogenic variants were detected in only 23.5% of PAH patients. Interestingly, in patients with the *SOX17* mutation, the coexistence of PAH with congenital heart disease was observed (patent ductus arteriosus or atrial septal defect). Of interest is the genetic background of PAH which develops after correction of congenital heart defect in some patients. A list of genes in which mutations are accepted to be a molecular basis of PAH or PVOD/PCH is presented in Table, while the location of genetic testing to establish the cause of PAH or if PVOD/PCH is suspected is shown in Figure.

Genetic counselling is important for family members of individuals with PAH. Molecular testing allows us to identify asymptomatic individuals with pathogenic variants. This is crucial, as early diagnosis and management of PAH are associated with better long-term outcomes. All asymptomatic carriers of a PAH-related mutation should undergo clinical, electrocardiographic, and echocardiographic assessment yearly or when symptoms occur. The ongoing DELPHI-2 study, which included 57 asymptomatic *BMPR2* carriers over 18 years old, is aimed at determining predictors of progression to PAH during the 3-year observation period [29]. However, genetic testing for PAH and PVOD/PCH is not recommended for asymptomatic relatives under 18 years old, as there are currently no effective interventions or medications to prevent disease in mutation carriers [27].

Moreover, genetic testing offers a unique opportunity to discover novel genetic causes of PAH, as well as to provide novel therapies for PAH. It was found that application of a phospholipase A2 inhibitor was capable of rescuing potassium channel activity in some *KCNK3* mutants [12,30]. Genetic discoveries are promising for gene correction therapy in PAH.

To date, there are no detailed data regarding the molecular basis of Polish patients with PAH and PVOD/PCH. Only one study has reported results of the *BMPR2* mutation analysis in 50 patients

with PAH of various aetiologies, in which 20 patients with idiopathic PAH were also screened for mutations in *ACVRL1* and *ENG* [31]. Currently, a multi-center study is planned to include patients from referral centers for a comprehensive analysis of the Polish population regarding genetic factors in the development of PAH. The inclusion criteria include patients with diagnosed idiopathic, hereditary and drug-induced PAH or PVOD/PCH, and PAH developing after surgical correction of a congenital heart defect. Patients with PAH of a different aetiology will be excluded from the study. The aim of the study is to perform a comprehensive molecular analysis to better understand the molecular basis of PAH pathogenesis, incomplete penetrance, and to create an algorithm of molecular diagnosis of PAH patients. In addition, this study will allow the assessment of molecular and clinical correlations, effects of PAH-specific treatment, and the prognosis of individuals with PAH.

Based on the clinical data, 300 patients will be enrolled in the study at the Department of Cardiology and Electrotherapy, Medical University of Gdańsk. Genetic examinations will be performed at the Department of Biology and Medical Genetics, Medical University of Gdańsk. The mutational analysis of selected genes, mainly encoding proteins associated with the TGF β signaling pathway will be performed using new generation sequencing (NGS). Identification of novel genetic variants can give new insights into the pathogenesis of PAH. Finally, when combined with clinical data, the study will enable us to assess the impact of identified mutations on the clinical course, PAH-specific treatment, and prognosis in PAH.

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