Landscape of mutational profiles in a Polish cohort of patients diagnosed with idiopathic pulmonary arterial hypertension

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare vascular disorder, characterized by narrowing of the pulmonary arteries due to vasoconstriction and vascular remodeling [1–3]. The national registry reports a PAH incidence of 5.2 cases per million per year and a prevalence of 30.8 cases per million in Poland [4, 5]. While its etiology remains partly unclear, genetic factors have been implicated in certain patient groups [6–8]. However, the molecular background of this population has not been systematically studied. Therefore, we aimed to comprehensively characterize the genetic landscape of PAH in Polish patients.

METHODS

Between June 2009 and June 2020, we enrolled consecutive patients with idiopathic PAH (IPAH), defined by a mean pulmonary artery pressure >25 mm Hg and pulmonary vascular resistance >3 WU. All participants gave written informed consent, and the study was approved by the Jagiellonian University Bioethics Committee (No. 122.6120.125.2016).

We analyzed a targeted panel of 46 genes potentially implicated in PAH pathogenesis, including bone morphogenetic proteins (BMP)/ /small mothers against decapentaplegic (SMAD) signaling (*BMPR2*, *BMPR1B*, *ACVRL1*, *EIF2AK4*, *ENG*, *KCNK3*, *KLF2*, *NOTCH3*, *SMAD1–7*, *SMAD9*, *TBX4*, *TOPBP1*, *GDF5*, *CAV1*, *INHBB*, *BMP2/4/6*, *ACVR2A*, *NOTCH1*, *YAP1*, *ID1*, *ID2*), the *TGF-β* cascade (*TGFBR1*, *TGFBR2*, *TGFB1–3*, *PDPK1*), the peroxisome proliferator-activated receptor (PPAR) network (*PPARγ*, *PPARα*, *PPARδ*, *FBP1*, *RXRA*, *PCK*, *GK*, *AQP7*), the potassium channel *KCNA5*, hypoxia regulator *ILK*, and inflammatory mediators interleukin 6 and tumor necrosis factor α.

The detailed methodology for the genetic analyses has been previously described [9]. In short, genetic libraries were prepared with SureSelect XT Low Input Target Enrichment (Agilent) and sequenced on a NextSeq 500 (Illumina) using a v2.5 kit (2 × 150 bp). For large rearrangement detection in *ENG*, *ACVRL1*, and *BMPR2*, 100 ng of DNA was analyzed using the SALSA MLPA P093-C2 HHT/HPAH Probemix and MLPA reagents (MRC-Holland), following the manufacturer's protocol; DNA from a healthy donor served as the reference.

Variants predicted as D (disease-causing) or A (disease-causing automatic) by the MutationTaster (http://www.mutationtaster.org/) were classified according to American College of Medical Genetics and Genomics guidelines [10] (Franklin platform, Genoox) as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign.

Variants were deemed novel if they lacked an rs number in NCBI SNP and were absent from ClinVar. Due to descriptive nature of the current study, we did not use statistical calculations.

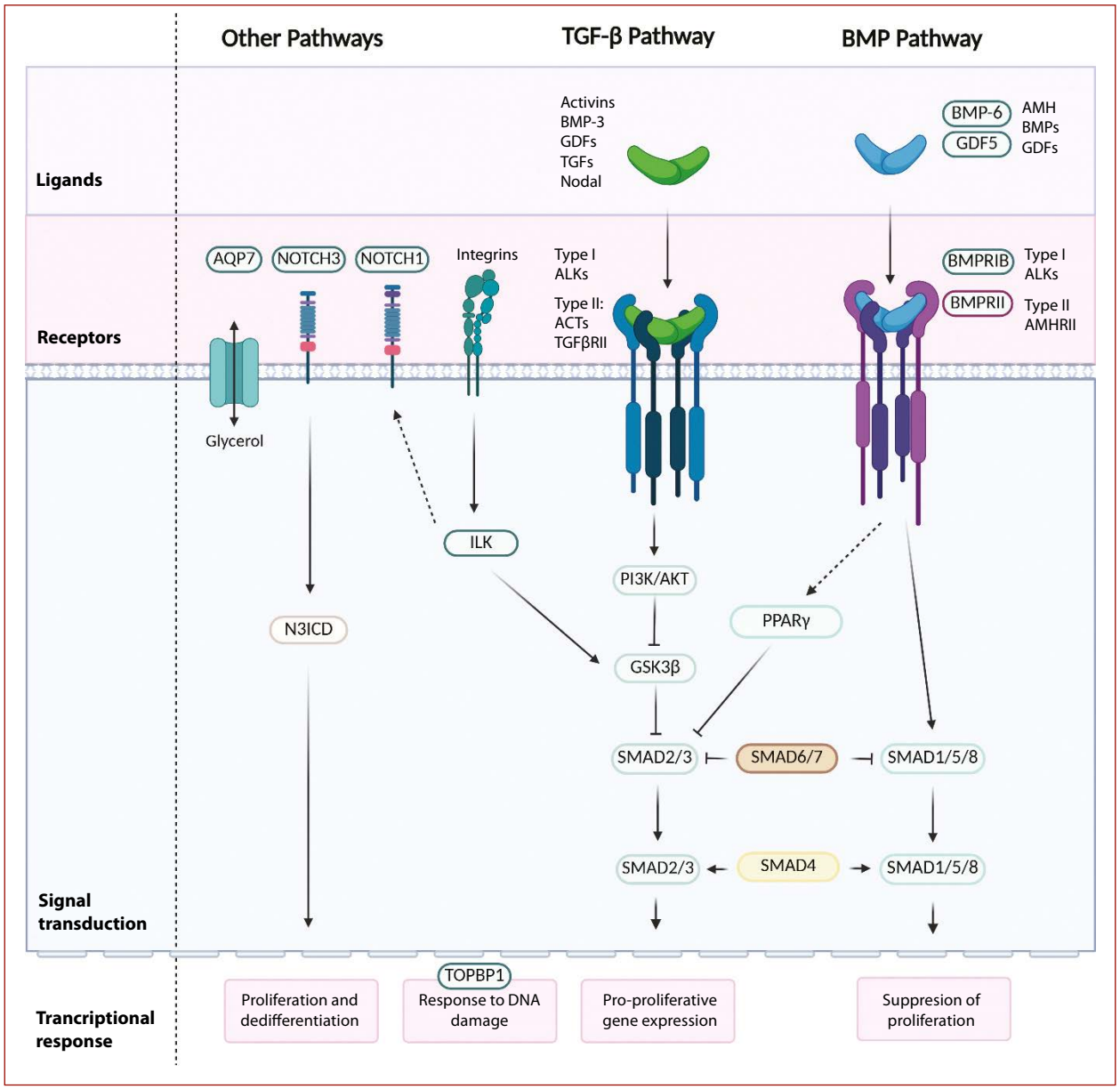

Figure 1. Pathways associated with pulmonary arterial hypertension (adapted from Supplementary references). Genes with pathogenic or likely pathogenic alterations in our cohort are marked with a dark pink outline, while variants of uncertain significance are marked with a cyan outline. Dashed arrows indicate unclear or multi-level interactions. Created with BioRender.com

Abbreviations: ACT, activin receptor; AKT, AKT serine/threonine kinase; ALK, activin receptor-like kinase; AMH, anti-Müllerian hormone; AMHRII, AMH receptor type II; BMP, bone morphogenetic proteins; BMPRIB, BMP receptor type I B; BMPRII, BMP receptor type II; GDF, growth differentiation factor; GSK3β, glycogen synthase kinase 3β; ILK, integrin-linked kinase; N3ICD, NOTCH3 intracellular domain; NOTCH, neurogenic locus notch homolog; PI3K, phosphoinositide 3-kinase; PPARγ, peroxisome proliferator-activated receptor γ; SMAD, small mothers against decapentaplegic; TGF-β, transforming growth factor β; TGFβRII, TGF-β receptor type II

RESULTS AND DISCUSSION

We enrolled 93 Caucasian IPAH patients (65 females; aged 53 years [interquartile range 41–69]). Most of them were in an advanced World Health Organization functional class at first presentation, as described in detail previously [9]. We identified 49 distinct variants in 21 genes among 51 (54.8%) patients (Supplementary material, *Table S1*). This included pathogenic or likely pathogenic variants in *BMPR2* (9 variants in 11 patients, as previously reported by our group [9]), *EIF2AK4* (1 pathogenic novel frameshift variant, consistent with pulmonary veno-occlusive disease [11]), *NOTCH3* (1 likely pathogenic variant, known but not associated with any specific condition), and *SMAD4* (1 likely pathogenic novel variant). A total of 20 variants (40.8%) were classified as VUS, and 17 variants (34.7%) as benign or likely benign. No large genomic rearrangements were detected by MLPA.

Overall, 14 (15.1%) patients carried pathogenic or likely pathogenic variants (*BMPR2*, n = 11, *EIF2AK4*, n = 1, *NOTCH3*, n = 1, *SMAD4*, n = 1), while 21 patients (22.6%) carried

VUS including the following: *NOTCH3* (n = 7 patients), *NOTCH1* (n = 3), *BMPR2* (n = 2), *BMP6* (n = 2), *BMPR1B* (n = 2), *GDF5* (n = 1), *ILK* (n = 3), *AQP7* (n = 2), and *TOPBP* (n = 1).

A new likely pathogenic splice acceptor variant in the *SMAD4* gene was identified in a woman diagnosed with IPAH at the age of 64 years, who also had atrial fibrillation and liver cirrhosis of unknown etiology. Another patient carrying a likely pathogenic *NOTCH3* variant was a woman diagnosed with IPAH at age 42 years, who subsequently died of ovarian cancer at age 51 years. The clinical phenotype of a patient with *EIF2AK4* mutation was previously described by our group [11].

Alterations in the BMP pathway in PAH typically affect upstream components — receptors and ligands [12–14] — a pattern mirrored in our cohort (Figure 1). Outside pathogenic or likely pathogenic variants, we found several variants of unknown significance in this pathway exemplified by patient 837, who harbored a VUS (rs142167481) in *BMP6*, a ligand known to promote PASMC proliferation in the absence of *BMPR2*, or patient 23, who carried a VUS in the propeptide domain of *GDF5*, a BMP receptor ligand related to *GDF2*, which has been previously linked to PAH. Additional studies are needed to clarify the significance of these VUS and uncover novel pathogenic mechanisms.

CONCLUSIONS

We identified a genetic background in 15.1% of Polish patients with IPAH. The majority of pathogenic or likely pathogenic variants were found in *BMPR2*, which was altered in the largest number of patients, while only a single patient carried pathogenic or likely pathogenic variants in *EIF2AK4*, *NOTCH3*, and *SMAD4*.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/polish_heart_journal.

Article information

Conflict of interest: None.

Declaration of artificial intelligence use: Nothing to disclose.

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