

# Mutually reinforcing effects of genetic variants and interferon- $\beta$ 1a therapy for pulmonary arterial hypertension development in multiple sclerosis patients

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## Abstract

Based on a small number of cases, interferon beta (IFN- $\beta$ ) has been added to the list of drugs that might induce pulmonary arterial hypertension (PAH) in the current European guidelines for the diagnosis and treatment of pulmonary hypertension. Here, we propose that multiple sclerosis patients who are genetically predisposed to PAH may be at higher risk to develop disease when treated with IFN- $\beta$ . We included two patients with multiple sclerosis who developed a manifest PAH after five and eight years on IFN- $\beta$  1a therapy, respectively (without confirmed right heart catheterization). In both patients, PAH markedly improved after discontinuation of IFN- $\beta$  1a and initiation of targeted PAH therapy. For genetic analysis, we used a PAH-gene panel based on next-generation sequencing of 16 PAH and 38 candidate genes. In one of the two patients, we could identify a nonsense variant in the PAH gene *ATP13A3*. The second patient showed a missense variant of the *CYP11B1* gene, which might be linked to PAH predisposition. The results of this study support the hypothesis that multiple sclerosis patients who receive IFN- $\beta$  1a therapy might be at higher risk for the development of manifest PAH, if they carry a pathogenic variant or sequence variant genetically predisposing to the disease. However, further studies are necessary to systematically investigate the presence of predisposing PAH gene variants in these patients.

## Keywords

pulmonary arterial hypertension, interferon beta, multiple sclerosis, *ATP13A3*, next-generation sequencing

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Pulmonary arterial hypertension (PAH) is a rare disease characterized by increased mean pulmonary artery pressure (mPAP) measured by right heart catheterization (RHC), which leads to right heart failure. Apart from associated diseases and hereditary causes, it may also be induced by drugs and toxins such as the appetite suppressants fenfluramine and aminorex.<sup>1,2</sup> Interferon beta (IFN- $\beta$ ) and interferon alpha (IFN- $\alpha$ ) were listed among the substances with a possible risk of PAH induction.<sup>1</sup> IFN- $\beta$  has been the first available disease-modifying medical treatment for multiple sclerosis (MS) patients.<sup>3</sup> It still ranges among the first-line

therapies in relapse remitting multiple sclerosis (RRMS) to slow down disease progression.<sup>4</sup> While PAH is a rare side effect of IFN- $\beta$  in RRMS patients,<sup>5</sup> the PAH incidence in this cohort was much higher than expected compared to the general population.<sup>6</sup> In some of these patients, a withdrawal

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of IFN- $\beta$  with or without provision of PAH-targeted therapy led to a normalization of hemodynamics.<sup>3</sup> In other patients, right heart failure progressed even after withdrawal of IFN- $\beta$  and led to death.<sup>3</sup> Hence, it would be beneficial to identify those RRMS patients who are at risk of developing PAH upon IFN- $\beta$  treatment.

In this regard, additional risk factors such as pathogenic variants in the bone morphogenetic protein receptor 2 (*BMPR2*) gene and pathway genes could also play a role in RMSS patients who develop PAH. Recently, up to 16 PAH genes have been described causing hereditary PAH.<sup>7-9</sup> The investigation of genetic predisposition to PAH in RMSS patients has been suggested<sup>3</sup> but not carried out so far. Therefore, we screened for PAH gene variants in two RMSS patients who developed PAH after IFN- $\beta$  treatment.

## Methods

Patients were diagnosed with RRMS, treated with IFN- $\beta$  1a, and referred to a pulmonary hypertension (PH) expert center once suspicion of PAH was raised. Clinical work-up for PAH followed current European Society of Cardiology (ESC) and European Respiratory Society (ERS) guidelines.<sup>1</sup> Briefly, electrocardiogram and transthoracic echocardiography (TTE) were performed. Laboratory parameters including N-terminal pro brain natriuretic peptide (NT-proBNP), thyroid function, and markers for connective tissue disorders were measured. Routine lung function measurements, blood gas analysis and six-minute walking distance (6MWD), high-resolution computed tomography, abdominal ultrasound, hematological and immunology assessment, human immune deficiency virus and hepatitis tests were performed to examine other causes of PH. Ventilation and perfusion (V/Q) lung scan was used to exclude chronic thromboembolic PH. RHC was conducted to characterize hemodynamics including Iloprost inhalation to test for vasoreactivity. Lastly, World Health Organization (WHO) functional class was determined to assess the risk profile.

Genetic testing for PAH variants was carried out using a customized PAH specific gene diagnostics panel (SureSelect, Agilent) based on next-generation sequencing (NGS) as described previously.<sup>10</sup> Panel design, validation, quality control, and data analysis were performed at the University Hospital Heidelberg.

In total 16 PAH genes (*ACVRL1*, *AQP1*, *ATP13A3*, *BMPR1B*, *BMPR2*, *CAV1*, *EIF2AK4*, *ENG*, *GDF2*, *KCNA5*, *KCNK3*, *KLF2*, *SMAD4*, *SMAD9*, *SOX17*, and *TBX4*) and 38 further candidate genes were sequenced. Variants were classified according to current American College of Medical Genetics and Genomics (ACMG) guidelines and termed either likely pathogenic or pathogenic (classes IV or V), respectively.<sup>11</sup> Missense variants were only considered if the combined annotation depletion depended (CADD) score was above 20.<sup>12</sup>

## Results

### Clinical presentation

The first patient who developed PAH under IFN- $\beta$  therapy was a 46-year-old woman diagnosed with RRMS in 2005. She was treated with IFN- $\beta$  1a for five years from time of diagnosis and remained stable with an expanded disability status scale (EDSS) of 1.0 (maximum 10 points, equals death). In 2010, the patient developed progressive dyspnea on exertion over a period of 10 months before hospitalization. At hospital admission in 2010 she suffered from dyspnea corresponding to WHO functional class III, decreased exercise capacity, dizziness, and angina pectoris. She was on no other medication than IFN- $\beta$  1a. 6MWD was reduced to 280 m. TTE showed right ventricular hypertrophy and a dilated right atrium and ventricle, as well as a D-shape formation and paradox septal deviation. In contrast, a previous TTE in 2009 had shown normal heart function and size indicating a rapid progression. The left ventricular (LV) systolic and diastolic function was already reduced to 45%. A coronary angiography disclosed a one-vessel coronary artery disease at initial diagnosis of PH of unknown origin. Placing a drug eluting stent in the left main coronary artery treated this. LV-end-diastolic pressure of 12 mmHg excluded left heart failure as origin of the severe PH.

RHC revealed an mPAP of 66 mmHg, cardiac index (CI) of 1.51/min/m<sup>2</sup>, and a pulmonary vascular resistance (PVR) of 14.1 Wood Units (WU) (Table 1). After exclusion of known causes of PH, the diagnosis of drug induced PAH was made. Up-front dual-targeted PAH-combination treatment was started using the endothelin receptor antagonist (ERA) ambrisentan and the phosphodiesterase-5 inhibitor (PDE-5i) tadalafil according to the current treatment recommendation.<sup>1,13</sup> The patient was classified as intermediate risk. Due to the presence of clinical disease activity, IFN- $\beta$  1a was escalated to fingolimod therapy, as described by Thomas et al.<sup>14</sup> The follow-up assessments after 3 and 6 months including RHC after 12 months showed a rapid improvement of PAH. The patients' dyspnea enhanced from WHO functional class III to II, NT-proBNP levels dropped from 1754 to 144 ng/l. The walking distance in 6MWD increased to 400 m and hemodynamics improved to mPAP of 29 mmHg, PAWP of 9 mmHg, PVR of 3.8 WU, and cardiac output (CO) of 2.91/min. In 2018, the RVSP was 55 mmHg and 6MWD increased further to 480 m.

The second RRMS patient who developed PAH after IFN- $\beta$  1a treatment was a 53-year-old woman. She was diagnosed with RRMS in 2008, treated with subcutaneous IFN- $\beta$  1a for a total of eight years. Her EDSS was 1.0 with her medical history being unremarkable at the same time. In 2016, she presented with edema of both legs, shortness of breath, and chest pain equivalent to a WHO functional class of III. Clinical signs indicated PH, which was confirmed by RHC. IFN- $\beta$  1a medication was withdrawn and three months later an invasive measurement of hemodynamics

**Table 1.** Parameters of biochemistry, transthoracic echocardiography, blood gas analysis, and right heart catheterization in the two patients with multiple sclerosis.

Parameters	Patient 1 at diagnosis	Patient 1 after 7 years of PAH treatment	Patient 2 at diagnosis	Patient 2 after 6 month of PAH treatment
NT-proBNP (pmol/l)	207	10	333	76
LVEF (%)	65	70	74	87
6 MWD (m)	100	412	400	510
pO <sub>2</sub> at rest (mmHg)	85	76	87	92
pO <sub>2</sub> after 6MWT (mmHg)	n.d.	n.d.	83	72
pCO <sub>2</sub> at rest (mmHg)	29	38	31	30
pCO <sub>2</sub> after 6MWT (mmHg)	n.d.	n.d.	23	32
DLCO (%)	49	53	53	57
DLCO/VA (%)	55	60	92	66
FVC (%)	81	81	43	84
FEV1 (l/min)	2.82	2.66	1.72	1.99
PAP mean (sys/dia) (mmHg)	66 (101/51)	29 (48/19)	52 (84/ 36)	38 (65/ 24)
CI (l/min/m <sup>2</sup> )	1.5	2.7	1.56	2.71
CO (l/min)	2.90	5.2	2.57	4.78
PAWP (mmHg)	12	9	6	7
CVP (mmHg)	14	8	9	6
PVR (WU)	18.6	3.8	18	6.4

PAH: pulmonary arterial hypertension; NT-proBNP: N-terminal pro brain natriuretic peptide; LVEF: left ventricular ejection fraction; 6 MWD: 6 minute walking distance; pO<sub>2</sub>: oxygen partial pressure; pCO<sub>2</sub>: carbon dioxide partial pressure; DLCO: transfer factor of the lung for carbon monoxide; DLCO/VA: Krogh factor; FVC: functional vital capacity; FEV1: forced expiratory volume in 1 s; PAP: mean (sys/dia) pulmonary arterial pressure mean (systolic/diastolic); CI: cardiac index; CO: cardiac output; PAWP: pulmonary arterial wedge pressure; CVP: central venous pressure; PVR: pulmonary vascular resistance; n.d.: not determined.

demonstrated consistent impairment of right heart function. Targeted medication for PAH was initiated, according to the recommendations of the ESC/ERS guidelines for WHO functional class III/intermediate risk using up-front combination therapy with the ERA macitentan and the PDE-5i sildenafil. Nine months later, the patient clinically presented without any signs of right heart failure, WHO functional class improved from III to I. Serum NT-proBNP levels dropped from 3329 ng/ml to 550 ng/ml and the 6MWD improved from 400 m to 510 m compared to time of PAH diagnosis. RHC showed a marked improvement: mPAP decreased to 38 mmHg from 56 mmHg at diagnosis and PVR dropped by 11.6 WU to 6.4 WU. CO and CI improved to 4.81/min and 2.71/min/m<sup>2</sup>, respectively (Table 1). Since the cessation of IFN-β 1a, the patient remained without any medication for RRMS. Latest cerebral magnet resonance imaging displayed no active lesions (new or contrast-enhancing). No relapses of RRMS occurred and the EDSS was unchanged at 1.0 until latest follow-up.

### Genetic testing

Genetic screening in the first patient with a PAH gene panel identified a nonsense variant (class V variant according to ACMG criteria) in the recently described PAH

gene *ATP13A3*.<sup>7</sup> The heterozygous variant c.1540C>T p.(Glu514\*) introduced a premature stop codon in exon 3 out of 32 exons most likely leading to nonsense mediated decay of the messenger RNA and was classified as disease causing. No family history of PAH was known indicating that the variant in concert with IFN-β could have triggered disease manifestation.

In the second patient, a missense variant c.1417G>A p.(Glu473Lys) was found in exon 3 of the gene *Cytochrome p450 Family B1 (CYP1B1)*. The variant led to an amino acid change from glutamic acid to lysine and was considered as deleterious by four in silico prediction programs (SIFT: 0, PolyPhen2: 1, Align GVGD: C55, MutationTaster: damaging; CADD: 24.8). However, it has also been found in seven as healthy classified subjects (Genome Aggregation Data Base) and *CYP1B1* has been so far not described as PAH gene. Nevertheless, we assume this variant may affect PAH signaling. Two further variants of unknown significance (class III) were identified in the genes *KLF2* and *ID1*. In contrast to the variant in *CYP1B1*, these two variants most likely had no impact on PAH development. The *KLF2* variant (c.201\_203delGCC p.(Pro71del)) was a deletion among a stretch of eight other prolines and the *ID1* variant led to no alteration of the amino acid (c.147C>T p.(Ala49Ala)).

## Discussion

In this study, we describe for the first time a genetic predisposition in two patients who developed PAH, IFN- $\beta$  1a therapy. In one case, a pathogenic variant in a PAH gene, and in a second case, a missense variant possibly affecting PAH signaling support the hypothesis that patients carrying pathogenic or likely pathogenic variants are more prone to developing PAH during IFN- $\beta$  1a therapy.

The induction of PAH by certain drugs and toxins firstly became evident after wide-spread appetite suppressant use in the late 1960s.<sup>15</sup> The tyrosine kinase inhibitor dasatinib prescribed to chronic myelogenous leukemia patients represents another type of drug which likely induces PAH in a subset of patients.<sup>16</sup> The mechanisms by which drugs lead to PAH be often insufficiently understood. This also applies to IFN- $\beta$ , for which 26 cases of associated PAH have been published to date,<sup>3,5,17</sup> including 11 PAH patients in a cohort of 7190 MS patients treated with IFN- $\beta$  in the USA between 2001 and 2012.<sup>6</sup> Despite the absence of data demonstrating increased PAH cases due to IFN- $\beta$  development in randomized controlled trials in MS patients, the published cases, the histopathological findings of typical proliferative lesions in an explanted lung MS lung after treatment with IFN- $\beta$ <sup>18</sup> and data from interferon receptor knockout (IFNAR<sup>-/-</sup>) mice,<sup>19</sup> underline the assumed causal link of IFN- $\beta$  and PAH.<sup>18</sup> However, considering that over 2.3 million people suffer from MS worldwide<sup>20</sup> and IFN- $\beta$  is still ranging among first-line therapies, the number of reported cases of PAH is relatively low. Thus, it is highly suggestive that some MS patients harbor an unknown predisposition developing PAH in response to IFN- $\beta$ .

In order to clarify this predisposition, we performed genetic testing in our study. As a result, we described for the first time a genetic predisposition in two patients who developed PAH after five and eight years of IFN- $\beta$  1a therapy, respectively.

In one of the RRMS patients in this study, a variant in the gene *ATP13A3* was identified. We are the third group to report *ATP13A3* variants in PAH patients worldwide.<sup>7,21</sup> *ATP13A3* has been identified as a candidate gene for PAH, although its function is poorly understood.<sup>21</sup> So far, it is known that it is a membrane ATPase with high expression in pulmonary endothelium of the lung, smooth muscle cells of pulmonary arteries (PASCs), and cardiomyocytes.<sup>21</sup> Furthermore, it was also found to be expressed within plexiform lesions of endothelial cells of patients with idiopathic PAH.<sup>7</sup> *ATP13A3* encodes a cation channel for potassium transport and is essential for stabilization of the membrane potential in PASCs and thus for vascular tone regulation.<sup>21</sup> As hypothesized by Barozzi et al., pathogenic variants in *ATP13A3* may lead to initiation of PAH similar to those in *KCNK3* and *KCNA5*, in which disturbance of channel function activity lead to vasoconstriction and vascular remodeling by cell proliferation and reduced apoptosis.<sup>7,21,22</sup> No data on how drugs like IFN- $\beta$  influence *ATP13A3* function is yet available.

Although not to be excluded, coincidental co-occurrence of both disease-mediating factors in the same patient appears very unlikely, given the rareness of both *ATP13A3* variants and IFN- $\beta$  induced PAH. With regard to incomplete penetrance of variants within *ATP13A3*,<sup>21</sup> additional triggers, such as IFN- $\beta$ , may be needed to lead to a manifest disease. Hence, this description supports systematic screening of all IFN- $\beta$  induced PAH patients for predisposing genetic variants.

In the second patient, we could identify a missense variant in the *CYP1B1* gene, which is closely connected to the *BMPR2* gene and its pathway.<sup>23</sup> Variants in *CYP1B1* have been shown to act as modifiers for PAH disease penetrance in *BMPR2* mutation carriers, leading to disease manifestation in comparison to healthy carriers. The messenger RNA of *CYP1B1* was highly decreased in female PAH patients with a *BMPR2* mutation in comparison to carriers without PAH.<sup>23</sup> *CYP1B1* encodes an estrogen-metabolizing enzyme and is moderately expressed in endothelial cells and highly expressed in the lung and pulmonary arterial lesions of patients with PAH.<sup>23,24</sup> Specific gene polymorphisms can result in reduced enzymatic activity, subsequent increase of estrogen levels, and proliferation of PASCs.<sup>24</sup> Interestingly, Dempsie et al. showed that development of experimentally induced PAH by the appetite suppressant dexfenfluramine can be attenuated by *CYP1B1* inhibition.<sup>25</sup> An impact of *CYP1B1* function on IFN induced PAH, similar to dexfenfluramine, could be hypothesized. This assumption is reinforced by the suggested gene-environmental interaction of *CYP1B1* metabolizing environmental toxins.<sup>23</sup> Thus, one could speculate these variants may facilitate disease manifestation in concert with IFN treatment.

Both patients of our study showed an impressive response to targeted therapy with ERA and PDE-5i. One explanation may be the inhibition of the endothelin-1 (ET-1) response by the ERA. Since ET-1 can be induced by IFN expression,<sup>26</sup> ET-1 could have been increased in the two IFN- $\beta$  1a-treated RRMS patients and contributed to PAH development. This is in line with a study showing increased ET-1 in a dose-dependent manner in two hepatitis C virus patients treated with IFN $\alpha$ .<sup>27</sup> Moreover, ET-1 was elevated in PAH patients compared to controls<sup>28</sup> and it was increased in systemic sclerosis (SSc) patients with associated PAH compared to SSc patients without PAH.<sup>19</sup> In SSc patients, the augmented autoimmune response led to the production of endogenous IFN, inducing the expression of IFN-stimulated gene products in vascular smooth muscle cells such as ET-1.<sup>19</sup> Moreover, the ET-1 level correlated positively with biomarkers for PAH, such as NT-proBNP.<sup>19</sup> In both of our patients NT-proBNP was increased before targeted PAH therapy, portraying similarities to described pathogenesis of PAH in SSc. On the other hand, only 12% to 15% of patients with SSc suffer from PAH.<sup>29</sup> This indicates possible additional underlying mechanisms similar to the even lower proportion of RRMS patients who develop PAH. While this study describes only two patients, it may still add to the



body of evidence in particular considering the rareness of PAH in IFN-treated RRMS patients.

## Conclusion

It is of particular importance to raise awareness of PAH as a rare but serious side effect of IFN- $\beta$  treatment in MS patients. The findings of this study suggest a possible genetic predisposition in some MS patients to develop PAH during IFN- $\beta$  1a therapy. This requires further investigation into the prevalence of pathogenic variants and the biological mechanism as well as further factors triggering the development of PAH in MS patients. To this end, the diagnosis of drug induced PAH has to be clearly established by the systematic exclusion of other factors causing PH. A genetic screening of identified cases facilitates diagnosis of PAH and corroborates the assumption that pathogenic or likely pathogenic variants in concert with PAH-inducing drugs could lead to PAH development in a subset of MS patients. Therefore, it would be highly informative to sequence all patients who developed PAH after IFN- $\beta$  treatment as well as a matched control group without PAH after IFN- $\beta$  treatment. Such study could determine the impact of pathogenic variants and their potential as additional PAH triggers during IFN- $\beta$  therapy in MS patients.

## Conflict of interest

The author(s) declare that there is no conflict of interest.

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