Implementing pulmonary arterial hypertension screening among *TBX4* mutation carriers: a timely endeavour

Received: 31 July 2024
Accepted: 23 Sept 2024

To the Editor:

Pulmonary arterial hypertension (PAH) is a rare disease characterised by progressive remodelling of the small pulmonary arteries, leading to right heart failure and death if left untreated. It is a form of precapillary pulmonary hypertension (PH) defined on right heart catheterisation (RHC) as having a mean pulmonary arterial pressure (mPAP) >20 mmHg, pulmonary vascular resistance (PVR) >2 Wood units (WU) and a pulmonary arterial wedge pressure (PAWP) ⩽15 mmHg [1, 2]. The identification of pathogenic variants in the *T-box transcription factor 4* (*TBX4*) gene was associated with small patella syndrome (SPS) [3, 4]. The *TBX4* gene is also implicated in both pulmonary and vascular development, and has recently been identified as a predisposing gene for PAH [5–7]. PH may occur in various forms among *TBX4* carriers: as cases of persistent pulmonary hypertension of the newborn with or without congenital heart disease, more or less associated with major pulmonary developmental anomalies, including pulmonary vein misalignment, and as PAH with mild or no pulmonary development; the latter two may be seen in both the adult and paediatric populations [6, 7]. In heritable PAH, *BMPR2* is the most frequently identified gene, and screening asymptomatic relatives carrying *BMPR2* mutations, as recommended, facilitates early disease diagnosis [1, 8, 9]. However, there are currently neither data nor recommendations regarding screening in *TBX4* carriers. Here, we illustrate the impact of genetic counselling and early diagnosis through familial cases of PAH among *TBX4* carriers.

The index case was referred for suspected PH at the age of 14 years (figure 1a and b). She was in New York Heart Association functional class (NYHA-FC) III, with a 6-min walk distance (6MWD) of 128 m, and a normal brain natriuretic peptide (BNP) value of 58 ng·L^{-1}. RHC confirmed severe precapillary PH with an mPAP of 73 mmHg, a PAWP of 4 mmHg, a cardiac index (CI) of 2.9 L·min^{-1}·m^{-2}, PVR of 19.2 WU and indexed PVR of 23.8 WU·m^{-2}. Computed tomography (CT) was normal. During follow-up, she started complaining of knee pains, and after investigation, a diagnosis of SPS was made. Genetic testing, performed as previously described [10], identified a nonsense heterozygous variant (c.231G>A, p.(Trp77*)) in the *TBX4* gene. This null variant, located in exon 3, results in a non-functional truncated protein in a gene where loss-of-function is a known mechanism of disease. This variant, absent from controls, was previously described and classified as pathogenic according to the American College of Medical Genetics and Genomics classification [11]. Despite receiving oral sequential therapy including an endothelin receptor antagonist, a phosphodiesterase type 5 inhibitor (PDE5i) and intravenous epoprostenol, her condition worsened; she received a transplant 8 years later, and died 6 months after the transplant.

Throughout her daughter's follow-up, the mother of the patient (figure 1b, II-2) reported a progressive dyspnoea evolving for at least 6 years. A complete work-up was performed: 6MWD was 378 m, CT showed abnormal bronchial and parenchymal abnormalities compatible with *TBX4* lung involvement [7], pulmonary function tests were normal except for low diffusing capacity of the lung for carbon monoxide (D_{LCO}) (figure 1a), and echocardiography showed a tricuspid regurgitation velocity of 3.5 m·s^{-1}. RHC confirmed precapillary PH with an mPAP value of 42 mmHg, a CI of 3.0 L·min^{-1}·m^{-2}, and a PVR value of 6.9 WU. The familial *TBX4* variant was identified. She was subsequently treated with a combination oral therapy of PDE5i and endothelin receptor antagonist. She is currently stable in NYHA-FC I after 3 years. Afterwards, genetic counselling was proposed in the other first-degree relatives, and the *TBX4* variant was identified in both sisters of the index patient (figure 1b). Based on the experience of the

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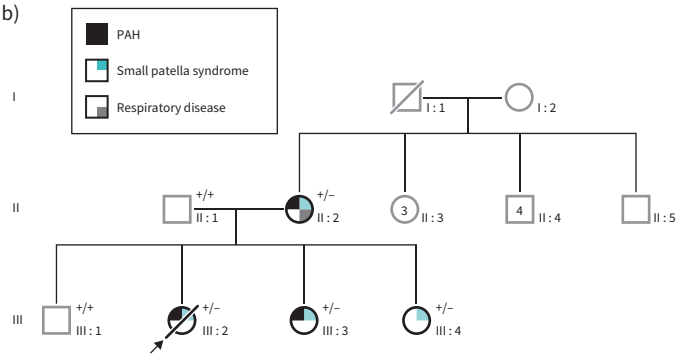
Systematic genetic counselling for *TBX4* carriers and their relatives enables screening for small patella syndrome and early diagnosis of pulmonary arterial hypertension https://bit.ly/4eKaPzY

Cite this article as: Montani D, Grynblat J, Coulet F, *et al*. Implementing pulmonary arterial hypertension screening among *TBX4* mutation carriers: a timely endeavour. *ERJ Open Res* 2025; 11: 00760-2024 [DOI: 10.1183/23120541.00760-2024].

a)

	III-2	II-2	III-3	III-4
Age at diagnosis, years	14	48	23	14
Gender, M/F	F	F	F	F
Signs of small patella syndrome, y/n	Yes	Yes	Yes	Yes
PAH, y/n	Yes	Yes	Yes	No
Chronic respiratory disease, y/n	No	Yes	No	No
Baseline characteristics				
NYHA-FC, I–IV	III	III	I	I
TRV, m·s^{-1}	3.4	3.5	No TRV	2.48
TAPSE, mm	14	19	23	24
6MWD, m	128	378	500	566
Increased BNP/ NT-proBNP, y/n	No	No	No	No
Haemodynamics at diagnosis				
mPAP, mmHg	73	42	22	-
PAWP, mmHg	4	3	8	-
Cardiac output, L·min^{-1}	3.48	5.77	6.17	-
Cardiac index, L·min^{-1}·m^{-2}	2.9	3	3.4	-
PVR , WU	19.8	6.9	2.3	-
Pulmonary function tests				
FEV_1, % pred	84	82	92	84
FVC, % pred	67	83	88	98
FEV_1/FVC	0.84	0.85	0.92	0.85
TLC, % pred	72	71	84	76
D_{LCO}, % pred	72	48	65	-
High resolution computed tomography of the chest				
Signs of TBX4 lung involvement	Mild septal lines	Multiple cysts, irregular bronchial walls, subpleural septal lines	No	No
PAH-approved drugs	ERA + PDE5i	ERA + PDE5i	PDE5i	None
Outcomes	LTx after 8 years Death 6 months after LTx	Stable at 3 years	Stable at 2 years	No PH

b)



c)

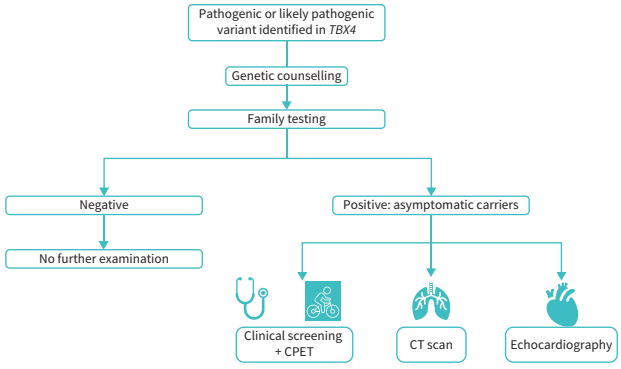


FIGURE 1 a) Characteristics of *TBX4* carriers. b) Family tree of all the PH cases and *TBX4* carriers and their different mode of presentation. The arrow points at the index case. The green squares represent a patient with small patella syndrome. The black square represents a pulmonary arterial hypertension case. The grey square represents the mother of the index case who developed a respiratory disease associated with PH. c) Assessment among patients with *TBX4* pathogenic or likely pathogenic variant. PAH: pulmonary arterial hypertension; NYHA-FC: New York Heart Association functional class; TRV: tricuspid regurgitation velocity; TAPSE: tricuspid annular plane systolic excursion; 6MWD: 6-min walk distance; BNP: brain natriuretic peptide; NT-proBNP: N-terminal pro-brain natriuretic peptide; mPAP: mean pulmonary arterial pressure; PAWP: pulmonary arterial wedge pressure; PVR: pulmonary vascular resistance; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; ERA: endothelin receptor antagonist; PDE5i: phosphodiesterase type 5 inhibitor; LTx: lung transplant; PH: pulmonary hypertension; CPET: cardiopulmonary exercise testing; CT: computed tomography.

DELPHI-2 screening programme [7], a yearly systematic screening programme was proposed to the two younger sisters (III-3 and III-4) carrying the *TBX4* variants. The patient III-3 (24-years-old) was in NYHA FC I but reported malaise at exercise. ECG showed an incomplete right bundle branch, her echocardiography and N-terminal pro-brain natriuretic peptide (NT-proBNP) were normal, D_{LCO} was mildly decreased, contrasting with normal spirometry, lung volumes and CT. Cardiopulmonary exercise testing (CPET) revealed a decreased peak oxygen consumption (70% pred) with static dead space to tidal volume fraction during exercise. The probability score for PAH previously used in the DELPHI-2 screening programme [7] was calculated at 3/10, indicating "possible" PAH. Based on unexplained malaise, a mild decrease in D_{LCO} and mild abnormalities on CPET, RHC at rest and exercise was performed. It showed mild precapillary PH at rest with mPAP 22 mmHg, PAWP 8 mmHg, CI 3.4 L·min^{-1}·m^{-2}, PVR 2.3 WU and exercise PH at 80 W with mPAP 47 mmHg, PAWP 14 mmHg, CI 7.87 L·min^{-1}·m^{-2}, PVR 2.3 WU and a mPAP/cardiac output slope of 3.1 mmHg·min·L^{-1}. An initial specific PAH oral monotherapy with PDE5i was initiated and 2 years later the patient was stable in NYHA-FC I and PVR at 2.3 WU. The other younger sister (III-4) is annually screened and did not develop any patent signs of PAH. Notably, among all family members in whom the c.231G>A, p.(Trp77*) variant was identified an SPS diagnosis was made.

The history of this family illustrates three situations: 1) diagnosis based on symptoms in a patient (III-2) with no personal or familial history, leading to a severe diagnosis and progression to lung transplantation; 2) diagnosis based on unexplained dyspnoea (II-2) in a context of familial PAH history, which allowed for an early PH screening but with already significant PH; and 3) early diagnosed PAH in a patient without respiratory limitation diagnosed in screening PAH programme. This observation in three members of one family supports the importance of genetic counselling for relatives of PAH patients in whom a predisposing

gene has been identified, as is currently recommended [1, 8, 9]. Notably, those recommendations are based on relatives of *BMPR2* carriers, in whom the penetrance is low. While in *TBX4* carriers the PAH penetrance is also incomplete, penetrance regarding the complete clinical manifestation, including lower limb and other skeletal abnormalities, is nearly 100% [12]. To date, the prevalence of PAH among *TBX4* carriers is unknown, thus, the effectiveness of systematic screening remains the subject of discussion. Indeed, the factors leading to PAH are unknown, and there are no possible preventive actions. Furthermore, it is important to keep in mind that genetic testing and screening of relatives who have not developed PAH may generate additional psychological burden, including fear, anxiety and guilt [13]; even more so in a population where the prevalence of PAH is unknown. However, the interest in identifying *TBX4* carriers among relatives is not limited to screening for PAH but also allows for the search and prevention of orthopaedic complications of SPS, the search and management of associated chronic respiratory diseases, and ultimately provides information on the risk of transmission in case of pregnancy. The latter point is a major issue as there is wide phenotypic variability, as displayed in our study where family members presenting the same variant display various phenotypes. Furthermore, developmental abnormalities in bone and respiratory systems may be severe in children, thus suggesting a specialised screening for antenatal complications among those patients [14, 15].

We previously demonstrated that a non-invasive, multi-faceted, annual follow-up approach including echocardiography, exercise testing, pulmonary function tests, and NT-proBNP is feasible in the follow-up of healthy *BMPR2* carriers [7]. Tóth *et al.* [16] recently confirmed that the majority of *BMPR2* carriers will not develop PAH and the screening's efficiency for early PAH identification among those patients. However, they also showed that *BMPR2* unaffected carriers presented with smaller right and left cavities compared to control subjects on cardiac magnetic resonance imaging. Our study suggests that ECG, D_{LCO} and CPET may detect early PH in relatives carrying a *TBX4* pathogenic variant as well, although further studies need to better delineate the most relevant strategy regarding the screening among those patients. Within the French PH network, we decided to implement and adapt this screening for relatives carrying *TBX4* pathogenic or likely pathogenic variants, adding an orthopaedic assessment and an initial thoracic CT scan screening for pulmonary abnormalities (figure 1c). Hopefully, this approach will improve our understanding of the prevalence of PAH in this context, the risk factors associated with the development of PAH, and allow for early screening of PAH. The relevance of the screening for orthopaedic and respiratory complications remains to be discussed, and patients should be well informed of the absence of proof regarding the efficiency of the screening in those clinical manifestations.

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Provenance: Submitted article, peer reviewed.

Conflict of interest: D. Montani reports grants from Acceleron, Janssen and Merck MSD; consulting fees from Acceleron, Merck MSD, Janssen and Ferrer; and payment or honoraria for lectures, presentations, manuscript writing or educational events from Bayer, Janssen, Boerhinger, Chiesi, GSK, Ferrer and Merck MSD. D. Montani is an associate editor of this journal. D. Bonnet reports consulting fees from Jansen and Merck; and participation on a data safety monitoring board or advisory board with Lupin. M. Humbert reports grants from Acceleron, AOP Orphan, Janssen, Merck and Shou Ti; consulting fees from Acceleron, Aerovate, Altavant, AOP Orphan, Bayer, Chiesi, Ferrer, Janssen, Merck, MorphogenIX, Shou Ti and United Therapeutics; payment or honoraria for lectures, presentations, manuscript writing or educational events from Janssen and Merck; and participation on a data

safety monitoring board or advisory board with Acceleron, Altavant, Janssen, Merck and United Therapeutics. The remaining authors have nothing to disclose.

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