



HAL
open science

Screening for pulmonary arterial hypertension in adults carrying a **BMPR2** mutation

David Montani, Barbara Girerd, Xavier Jaïs, Pierantonio Laveneziana, Edmund M T Lau, Amir Bouchachi, Sebastien Hascoet, Sven Günther, Laurent Godinas, Florence Parent, et al.

► To cite this version:

David Montani, Barbara Girerd, Xavier Jaïs, Pierantonio Laveneziana, Edmund M T Lau, et al.. Screening for pulmonary arterial hypertension in adults carrying a BMPR2 mutation. *European Respiratory Journal*, inPress, 10.1183/13993003.04229-2020 . hal-03104056

HAL Id: hal-03104056

<https://hal.sorbonne-universite.fr/hal-03104056>

Submitted on 13 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Screening for pulmonary arterial hypertension in adults carrying a *BMPR2* mutation

David Montani^{1,2,3}, MD*, Barbara Girerd^{1,2,3}, PhD*, Xavier Jais^{1,2,3}, MD, Pierantonio Laveneziana^{4,5}, MD, Edmund MT Lau⁶, MD, Amir Bouchachi^{3,7}, MD, Sébastien Hascoët^{3,8}, MD, Sven Günther⁹, MD, Laurent Godinas^{1,2,3}, MD, Florence Parent^{1,2,3}, MD, Christophe Guignabert^{1,3}, PhD, Antoine Beurnier^{3,10}, MD, Denis Chemla^{3,10}, MD, Philippe Hervé^{3,11}, MD, Mélanie Eyries^{12,13}, PhD, Florent Soubrier^{12,13}, MD, Gérald Simonneau^{1,2,3}, MD, Olivier Sitbon^{1,2,3}, MD, Laurent Savale^{1,2,3}, MD, and Marc Humbert^{1,2,3}, MD.

* DM and BG contributed equally

¹ Université Paris-Saclay, School of Medicine, Le Kremlin-Bicêtre, France

² Assistance Publique - Hôpitaux de Paris (AP-HP), Service de Pneumologie et Soins Intensifs Respiratoires, Hôpital Bicêtre, Le Kremlin-Bicêtre, France

³ INSERM UMR_S 999, Hôpital Marie Lannelongue, Le Plessis-Robinson, France

⁴ Sorbonne Université, INSERM, UMRS1158 Neurophysiologie respiratoire expérimentale et clinique, Paris, France.

⁵ AP-HP Sorbonne Université, Service des Explorations Fonctionnelles de la Respiration, de l'Exercice et de la Dyspnée, Hôpitaux Universitaires Pitié-Salpêtrière, Tenon et Saint-Antoine, Département Médico-Universitaire « APPROCHES », Paris, France

⁶ Department of Respiratory Medicine, Royal Prince Alfred Hospital, Missenden Road, Camperdown, Australia

⁷ AP-HP, Service de Cardiologie, Hôpital Bicêtre, Le Kremlin Bicêtre, France

⁸ Pôle de Cardiologie pédiatrique et congénitale, Hôpital Marie Lannelongue, Le Plessis Robinson, France

⁹ AH-HP, Service de Physiologie, Georges Pompidou European Hospital

¹⁰ AP-HP, Service de Physiologie, Hôpital Bicêtre, Le Kremlin Bicêtre, France

¹¹ Service de Chirurgie Thoracique, Hôpital Marie Lannelongue, Le Plessis Robinson, France

¹² AP-HP, Département de Génétique, Hôpital Pitié-Salpêtrière, Paris, France

¹³ UMR_S1166, Sorbonne Université, INSERM, and Institute for Cardiometabolism and Nutrition (ICAN), Paris, France

CORRESPONDENCE

Marc Humbert, MD, PhD

Service de Pneumologie et Soins Intensifs Respiratoires, Hôpital Bicêtre

78, Rue du général Leclerc, 94270 Le Kremlin-Bicêtre, France.

Tel: +331 45217972 ; Fax: +331 45217971

mailto: marc.humbert@aphp.fr

TAKE-HOME MESSAGE

Asymptomatic *BMPR2* mutation carriers have a 2.3%/year risk of developing PAH. DELPHI-2 provides the platform for future international multicentre studies to refine multimodal screening algorithms in *BMPR2* mutation carriers.

Key Words: *BMPR2*; Genetics; Genetic counselling; Pulmonary arterial hypertension; Screening

Funding: French Ministry of Social Affairs and Health (PHRC P100175)

ClinicalTrials.gov Identifier: NCT01600898

ABSTRACT

Background: Heritable pulmonary arterial hypertension (PAH) is most commonly due to heterozygous mutations of the *BMPR2* gene. Based on expert consensus, guidelines recommend annual screening echocardiography in asymptomatic *BMPR2* mutation carriers. The main objectives of this study were to evaluate characteristics of asymptomatic *BMPR2* mutation carriers, assess their risk of occurrence of PAH, and detect PAH at an early stage in this high-risk population.

Methods: Asymptomatic *BMPR2* mutation carriers underwent screening at baseline and annually for a minimum of two years (DELPHI-2 study, NCT01600898). Annual screening included clinical assessment, electrocardiogram, pulmonary function tests, 6-minute walk distance, cardiopulmonary exercise test, chest X-ray, echocardiography, and NT pro-BNP level. Right heart catheterisation (RHC) was performed based on predefined criteria. An optional RHC at rest and exercise was proposed at baseline.

Results: Fifty-five subjects (26 males, median age 37 years) were included. At baseline, no PAH was suspected based on echocardiography and NT pro-BNP levels. All subjects accepted RHC at inclusion, which identified two mild PAH cases (3.6%), and 12 subjects with exercise pulmonary hypertension (21.8%). At long term follow-up (118.8 patients.year follow-up), three additional cases were diagnosed, yielding a PAH incidence of 2.3%/year (0.99%/year in men and 3.5%/year in women). All PAH cases have remained at low-risk status on oral therapy at last follow-up.

Conclusions: Asymptomatic *BMPR2* mutation carriers have a significant risk of developing incident PAH. International multicenter studies are needed to confirm that refined multimodal screening programs with regular follow-up allow early detection of PAH.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare condition with a prevalence of 15 to 60 per million, affecting the small pulmonary arteries leading to right ventricular failure.¹⁻³ Due to its rarity and nonspecific clinical presentation, PAH is usually diagnosed late in the course of the disease.¹ Despite the recent approval of targeted drugs, PAH remains a disease associated with a poor prognosis with a median survival of only seven years in the current era.^{2,4}

Mutations of the *BMPR2* gene (encoding Bone Morphogenetic Protein Receptor type 2) have been identified as the most common cause of heritable PAH. PAH due to *BMPR2* mutation is an autosomal dominant disorder with incomplete penetrance (estimated at 42% in females and 14% in males)⁵ and has a worse prognosis compared to idiopathic PAH.⁶⁻⁸ Genetic counselling and testing has become an integral part of PAH care, and allows screening for mutations in PAH predisposing genes in patients as well as carrier detection in asymptomatic relatives.⁶⁻⁸

Studies have shown that PAH therapy instituted at an early stage translates to better long-term outcomes.⁹⁻¹¹ Therefore, it seems essential to establish early diagnosis, particularly in high-risk populations such as *BMPR2* mutation carriers.^{2,4} In the absence of robust evidence, the European Society of Cardiology/European Respiratory Society (ESC/ERS) guidelines recommend that asymptomatic *BMPR2* mutation carriers may be offered an annual screening Doppler echocardiography for the early detection of PAH.^{2,4} However, this recommendation is based on expert consensus, since no screening strategy has ever been evaluated for this population.^{2,4} Furthermore, no previous study has assessed the risk of incident PAH in *BMPR2* mutation carriers using a longitudinal follow-up design.

The main objectives the present longitudinal study (DELPHI-2 study, NCT01600898) were to 1) assess the risk of occurrence of PAH in *BMPR2* mutation carriers; 2) determine their clinical, functional, biological, echocardiographic and haemodynamic characteristics; 3) determine predictive factors for the development of PAH; and 4) detect PAH at an early stage and offer timely management..

METHODS

Subjects

Genetic counselling and screening of mutations in PAH-predisposing genes are offered to all patients with idiopathic or familial PAH followed at the French Pulmonary Hypertension (PH) Referral Centre and subsequently to their at-risk relatives when a mutation has been identified.¹² The DELPHI-2 study (*Screening of Pulmonary Arterial Hypertension in BMPR2 Mutation Carriers*, NCT01600898) was approved by Institutional Ethics Committee on 25th November 2011 (CPP-IDF-VII, approval No. 11-028). From March 2012 to October 2014, asymptomatic relatives carrying a *BMPR2* mutation were offered inclusion in the DELPHI-2 study with a minimum follow-up of two years (Figure 1). Relatives of heritable PAH and methods of screening and genetic testing (confirmation on two independent tests of *BMPR2* mutations) have been previously reported in the experience of genetic counselling in the French referral centre for PH¹². Inclusion criteria were age \geq 18 years and all subjects were asymptomatic in New York Heart Association functional class (NYHA FC) I at inclusion. Subjects with other known conditions associated with PAH (connective tissue disease, portal hypertension, human immunodeficiency virus infection, schistosomiasis and congenital heart disease with the exception of atrial septal defect) or PH (chronic respiratory disease, left heart disease, chronic thromboembolic disease or sickle cell disease) at inclusion were excluded. The other exclusion criteria were adults under guardianship, persons deprived of liberty, in emergency situations, persons who refused or unable to give their informed consent and subjects without affiliation to a regime of social insurance. Pregnant women at inclusion were not eligible.

Study design

Initial evaluation included medical and occupational history, and questionnaire on prior exposure to drugs and toxins. Assessment at initial and subsequent yearly visits included physical examination, functional assessment by NYHA FC, electrocardiogram (ECG), chest X-ray, non-encouraged 6-minute walk distance (6MWD), pulmonary function tests (PFTs), cardiopulmonary exercise test (CPET), Doppler

echocardiography, and brain natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) plasma levels. RHC at rest and exercise was proposed to each participant at inclusion as an optional investigation.^{1,13,14} ECG, Doppler echocardiography, PFTs, and CPET were reviewed by two experts blinded to the results of RHC and other exams. An investigational CPET probability score of PH was predefined based on five criteria adapted from data published by our group and others (Supplemental Table 1).¹⁵⁻²⁰ Complete methodological descriptions of all investigations are presented in Supplementary Methods.

During the study, subjects were asked to contact study team for an urgent assessment in case of unexplained dyspnoea, malaise, or syncope. RHC was indicated if subjects presented with at least one of the following criteria: (1) clinical signs suggestive of PAH (unexplained dyspnoea with worsened NYHA FC, malaise, or syncope), (2) tricuspid regurgitation velocity (TRV) ≥ 2.8 m/s at Doppler echocardiography, or (3) decrease in oxygen consumption at CPET corrected for body weight at peak exercise ($V'O_2$) $> 20\%$ as compared to reference value at inclusion or between two consecutive evaluations. PAH was confirmed if RHC showed mPAP ≥ 25 mmHg with a PawP ≤ 15 mmHg and PVR ≥ 3 WU on RHC at rest. If precapillary PH was diagnosed during the study, patients were managed as recommended by ESC/ERS guidelines² with a complete work-up (including CT pulmonary angiography, high-resolution chest CT, V/Q lung scan, abdominal ultrasound, HIV serology and immunological tests) to exclude other causes of PH. Abnormal haemodynamics at exercise (“exercise PH”) was defined by a mPAP > 30 mmHg and total pulmonary resistance (TPR) > 3 WU^{1,21}.

At the end of the initial mandatory two-year phase of DELPHI-2 study, an optional long-term extension follow-up with the same design was proposed to all subjects. If subjects declined, an annual phone call was proposed, and subjects were informed to contact the study team urgently in case of dyspnoea, malaise, or syncope. If pregnancy occurred during follow-up, a specific follow-up was proposed with monthly Doppler echocardiography during the second and third trimesters followed by a complete assessment within three months of delivery.

Subjects diagnosed with PAH were included in the French PH Registry according to the requirements of the *Commission Nationale Informatique et Liberté* (approval number 842063).²² PAH patients were risk stratified using the REVEAL score 2.0, the ESC/ERS guidelines risk stratification tool and the simplified French PAH risk assessment.^{2,22,23}

Statistical analysis

Continuous data were presented as median (minimum-maximum) and categorical data were presented as numbers and percentages. We used Mann-Whitney test to compare data between subjects with or without PAH. The chi-square test with Yates correction if needed was used to compare categorical data. We considered a p value less than 0.05 as significant. Event-free survival (time to death or lung transplantation) analyses for PAH patients (screened during DELPHI-2 study or their index cases) were made from the time of the first diagnosis of PAH confirmed by RHC and was estimated by the Kaplan-Meier method. We performed statistical analysis with Statview 5.0 (SAS Institute, Cary, NC, USA) and Graphpad prism (GraphPad Software, La Jolla California, USA).

RESULTS

Study population

At the end of the inclusion period (1st October 2014), genetic counselling had been offered to 550 PAH patients, of whom 157 patients from 130 families carried a *BMP2* mutation (Figure 1). Asymptomatic carrier detection had been performed in 274 relatives of these 157 patients. Ninety-four relatives from 45 families carried a *BMP2* mutation. Three relatives (two females and one male) developed symptomatic PAH before the beginning of the study. In total, 55 *BMP2* mutation carriers (from 31 families) were enrolled in DELPHI-2 study from 1st March 2012 to 1st October 2014 followed by a minimum follow-up of 2 years (Figure 2). During the DELPHI-2 two-year mandatory follow-up period, 144 visits have been performed as part of the study (100.9 patients.year follow-up). The *BMP2* mutations from our subjects' cohort are presented in Supplemental Table 2, including 14 families with nonsense mutations, 10 families with missense mutations and 7 families with large rearrangements previously reported by Girerd et al¹². Median age at inclusion was 37 years (min-max 18-78) and the sex ratio was 0.9 (26 males). Nineteen subjects (35 %) had a smoking history > five pack-year, 11 (20%) had systemic hypertension, four (7%) had type 2 diabetes, three (5%) had dyslipidemia, two had a history of chemotherapy for breast cancer or lymphoma, one had lumbar disc hernia, and one had a past history of pacemaker implantation for sinus node dysfunction. Clinical characteristics are presented in Table 1 and Supplemental Table 3.

Evaluation at baseline

Electrocardiogram, chest X-ray, and biological tests

ECG was normal or with no significant abnormalities in 44 subjects (80%) including one paced rhythm. Two ECG were inconclusive with isolated left axis deviation. Nine (16.3%) subjects had ECG with incomplete right bundle branch block (n=5) or signs of right ventricular hypertrophy including inverted T-waves in the right precordial leads (n=4), and/or right axis deviation (n=1). No abnormalities were found in chest X-ray. BNP or NT-proBNP, creatinine and uricaemia were in normal range in all subjects (Table 1, Supplemental Table 3). Uricaemia was significantly associated with gender ($p < 0.0001$) and BMI ($p < 0.05$) but was not different between patients with or without PAH.

Pulmonary function tests and exercise testing

At inclusion, all subjects were in NYHA FC I. Median 6MWD was 533 m (min-max: 368-693). Results of PFTs (n=55) and CPET (n=54, not performed in one relative because of lumbar disc herniation) were expressed in percentage of predicted value (min-max). Median FEV₁ was 105% (69-152) and FVC was 108% (75-162). Median DLCO and DLCO/VA were 75.5% (39-126) and 88% (49-126), respectively (Table 1, Supplemental Table 3).

During CPET (Table 1), the median workload was 120 Watts (min-max: 42-279). At peak exercise, median V'O₂ at peak was 81% (47-132). Median dead-space fraction (VD/VT) was 0.21 (0.06–0.39; n=52). Based on the investigational CPET probability score of PH (Table 1), 31 (57.4%) subjects were classified as “unlikely”, 21 (38.9%) subjects as “possible”, one subject as “likely” and one as “very likely” (Table 2).

Doppler echocardiography

Doppler echocardiography was performed with blinding of the results of RHC or other exams at inclusion and follow-up. A measurable of TRV was obtained in 30 subjects (54.5%) and was lower than 2.8 m/s in all cases (median 2.0 m/s; min-max: 1.5–2.75). Median right atrial surface area was 13.0 cm² (min-max: 7.7-27.0). Median diastolic right ventricle surface area was 16.9 cm² (min-max: 8.0-28.0) and median systolic right ventricle surface area was 9.5 cm² (min-max: 3.7-15.0). Median TAPSE was 23mm (min-max: 16-28). No pericardial effusion was reported. All results are presented in Table 1 and Supplemental Table 3. PAH was not suspected in any subjects based on Doppler echocardiography results at baseline. An atrial septal defect (*ostium secundum*) of 10 mm was detected in one subject.

Haemodynamic evaluation at rest

All subjects (n=55) accepted the optional RHC at inclusion. This identified two asymptomatic subjects with mild PAH (Figure 2). Haemodynamic characteristics of the 53 subjects without PAH and of the two PAH patients diagnosed at inclusion (Patient 1 and 2) are presented in Table 1. Distributions of mPAP and PVR are presented in Figure 3. One subject with uncontrolled systemic hypertension at inclusion had

post-capillary PH. Repeat RHC after treatment of systemic hypertension demonstrated haemodynamic normalisation.

Haemodynamic evaluation at exercise

Fifty-two out of the 53 subjects without PAH at inclusion performed RHC at exercise. Twelve of them (23%) had exercise PH, as defined by a mPAP >30 mmHg and total pulmonary resistance (TPR) >3 WU (Figure 2). Subjects who experienced exercise PH were significantly older (median of 57.8 vs 32.6 years, $p < 0.0001$) and had significant differences in haemodynamics at rest (higher mPAP, PVR, and TPR and lower cardiac index, pulmonary artery wedge pressure (PawP), and stroke volume index, as compared to patient with normal haemodynamics at exercise) (Table 3). Of note, one patient had an increase PVR >3 WU but normal mPAP of 16 mmHg at baseline. Exercise haemodynamics showed exercise PH with mPAP of 39 mmHg and TPR of 5.0 WU, associated with a decrease in PVR (2.3 WU) and an increase in PCWP (21 mmHg) suggesting the absence of pulmonary vascular disease but possible heart failure with preserved ejection fraction.

Description of the screened PAH cases

The first case was a 26 yo woman included three months post-partum. ECG showed right axis deviation (QRS axis +100 degrees) and inverted T-waves in the right precordial leads. 6MWD was 533 m. PFTs were normal except a low DLCO (50% of predicted values). Based on the investigational CPET probability score, she was considered as “possible” PH. Doppler echocardiography showed normal TRV (2 m/s), absence of right atrial or ventricular dilatation and no other sign suggestive of PH. TAPSE was 25 mm. BNP was normal. RHC revealed pre-capillary PH at rest (mPAP 26 mmHg, PawP 8 mmHg, cardiac output $7.27 \text{ L}\cdot\text{min}^{-1}$, cardiac index $4.38 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$, and PVR 2.5 WU) (Table 1) and exercise PH (mPAP at 43 mmHg, and TPR at 3.6 WU). Because of the absence of symptoms in a subject with PVR below 3 WU at rest, no PAH therapy was started, and a careful follow-up was proposed. After four months, the patient had progressed to NYHA FC II and RHC demonstrated haemodynamic deterioration (mPAP 35 mmHg, cardiac index $2.39 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$, PVR 6.8 WU) confirming PAH. Oral dual

combination therapy with an endothelin receptor antagonist and a phosphodiesterase type 5 inhibitor was initiated.

The second case was a 78 yo woman with systemic hypertension and type 2 diabetes. ECG showed inverted T-waves in the right precordial leads. 6MWD was 420 m. PFTs were normal apart from reduced DLCO (63% of predicted values). Based on the investigational CPET probability score, she was considered as “very likely” PH. Doppler echocardiography showed normal TRV (2.65 m/s), absence of right atrial or ventricular dilatation and no other sign suggestive of PH. TAPSE was 16 mm. BNP was normal. RHC at rest revealed precapillary PH (mPAP 25 mmHg, PawP 7 mmHg, cardiac index $2.80 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$, and PVR 4.4 WU) (Table 1). Oral monotherapy with a phosphodiesterase type 5 inhibitor was initiated.

Two-year mandatory follow-up

During follow-up, five additional RHC were performed based on a decrease in $\dot{V}'\text{O}_2$ at peak $> 20\%$ (in isolation in three subjects and associated with unexplained dyspnoea in two). PH was not diagnosed in these five subjects. Eight subjects did not complete all follow-up visits, including two women because of pregnancy. After delivery, these two women were subsequently followed long-term. Phone calls were made for the subjects who missed follow-up visits: none reported clinical signs suggestive of PH. During the two-year initial follow-up, no unscheduled visit was needed, and no additional PAH case was diagnosed.

Long-term follow-up

Thirty-six subjects consented to undergo the optional long-term follow-up as part of usual care. A total of 103 visits as of August first 2019 (118.8 patients.year of follow-up) were completed as part of long term follow-up. Thus, 247 follow-up visits (215.6 patient.year) were performed during a follow-up of 47 ± 27 months for the entire duration of DELPHI-2 study. The 12 subjects who did not accept a clinical long-term follow-up agreed to receive annual phone calls.

Three additional PAH cases were diagnosed six years after the initial visit in two females (aged 50 and 73) and a 47 yo male. Characteristics of these patients at PAH diagnosis are presented in Table 1 (patients

3, 4 and 5). Patients 4 and 5 were from the same family (aunt and nephew). At time of PAH diagnosis, one patient was asymptomatic (diagnosed at a scheduled visit), and two were mildly symptomatic NYHA FC II (diagnosed at unscheduled visits) with a delay between symptom onset and PAH diagnosis of three and six months, respectively. PAH was associated with mild to moderate functional impact on 6MWD (ranging from 397 to 505 meters). NT-proBNP concentrations were in the normal range in the asymptomatic case and mildly elevated in the two symptomatic patients. Doppler echocardiography was abnormal with a TRV > 2.8 m/s in the three patients (3.3 to 4.4 m/s). RHC confirmed PAH with more severe haemodynamic compromise (PVR between 7.3 and 9.2 WU), as compared to the cases screened by RHC in the initial phase of the study. Three patients had signs of PH on ECG, two had “very likely” PH based on the CPET score and the other had a 22% decrease of V'O₂ at peak (CPET score not evaluable because of the absence of arterial blood gases at exercise) and two of them had DLCO <70% of predicted values.

Incidence of PAH in *BMPR2* mutation carriers

In this cohort, 9.1% of asymptomatic *BMPR2* mutation carriers developed PAH at follow-up with a marked female predominance: 13.8% (4/29) in females and 3.8% (1/26) in males (p=0.42). The incidence of PAH in the whole population was 2.3%/year. The incidence of PAH was higher in women: four females diagnosed with PAH during 114.4 patients years of follow-up and one male with PAH during 101.2 patients years of follow-up (3.5%/year and 0.99 %/year, respectively).

At long-term follow-up, two of the 12 subjects with PH at exercise developed PAH (16.7%) versus one of the 40 subjects (2.5%) with normal exercise haemodynamics (p=0.25) (Figure 2).

Characteristics of patients diagnosed with PAH versus subjects without PH at follow-up

Complete work-up performed in the 5 patients with pre-capillary PH identified no other condition known to be associated with PH according to the current classification of PH.^{1,2} Among the 5 patients diagnosed with PAH in the study, 3 patients belonged to families with several identified cases of PAH and 2 patients were relatives from index cases displaying a sporadic form of PAH (Figure 4). As compared to subjects without PH at follow-up (n=50), patient with PAH presented at diagnosis a lower 6-MWD (median of 420

versus 540 m), lower DLCO (median of 52 versus 78% predicted), and an elevated TRV (median of 3.3 versus 2.0 m/s). No individual CPET parameter differed individually.

Additional risk factors might have played a role as second hits in Patient 1 who developed PAH a few months after pregnancy, and in Patient 3 who received several chemotherapies for breast cancer including docetaxel, trastuzumab, bevacizumab, paclitaxel, lapatinib before inclusion, and vinorelbine and trastuzumab emtansine three months before onset of symptoms. Patient 4 had a personal history of non-Hodgkin's lymphoma treated 18 years before by chemotherapy and radiotherapy.

Non-invasive screening criteria

Since NT-proBNP plasma concentrations and Doppler echocardiography were normal in all subjects at inclusion, we assessed the relationship between ECG, DLCO, and CPET parameters with invasive haemodynamics. Table 4 describes the proportion of screened PAH and exercise PH of unknown significance according to three diagnostic criteria: 1) ECG signs suggestive of PH; 2) “possible, likely or very likely” CPET score (from 3 to 10); 3) DLCO <70% of predicted values. Absence of PH at rest or exercise were present in 85.7%, 80%, 60%, and 0% of subjects with no, one, two or three of these criteria respectively (Table 4). In the three subjects who met three of these criteria at inclusion, two were screened with PAH and one had exercise PH. Absence of these three criteria was present in 21 patients (38.1%) at baseline. All five PAH patients had at least one of these 3 criteria at time of diagnosis.

Outcomes of patients diagnosed with PAH

A total of five *BMPR2* carriers (four females and one male) were diagnosed with PAH during the initial screening and long-term follow-up. Table 5 shows the clinical, functional, and haemodynamic characteristics of the five PAH identified in DELPHI-2 study compared to their 31 index PAH cases with *BMPR2* mutations. Patients diagnosed via DELPHI-2 screening program had markedly better functional capacity and milder haemodynamics compared to index cases diagnosed via routine clinical care.

Four patients were treated with oral dual combination therapy (endothelin receptor antagonist and phosphodiesterase type 5 inhibitor), and one patient received monotherapy (phosphodiesterase type 5 inhibitor). Evolution and response to specific PAH therapy are presented in Table 6. All patients attained

or maintained a low-risk profile according to the REVEAL score 2.0, the ESC/ERS risk stratification tool and the simplified French PAH risk assessment (Table 6). No death occurred at follow-up, while survival observed in index cases was 90% and 84% at one year and three years, respectively (Figure 5).

No death occurred in the remaining 50 subjects. Of note, the case of *ostium secundum* atrial septal defect with mild left-to-right shunt diagnosed during the study was closed based on normal haemodynamic at rest. Haemodynamics at rest and exercise controlled four months after closure remained normal. After six years of follow-up, the patient has not developed PAH and is doing well.

Consequences of the new cut-off values for PH diagnosis

Using the new cut-off values proposed to define PAH (mPAP > 20 mmHg, PawP ≤ 15 mmHg, and PVR ≥ 3 WU)¹, no additional case would be diagnosed in our cohort. At baseline, a 67 yo male presented with a mPAP > 20 mmHg but PVR < 3 WU (mPAP 23 mmHg, PawP 6 mmHg, cardiac output 8.63 L.min⁻¹, PVR 2.0 WU), and a 59yo female presented with PVR ≥ 3 WU but with a mPAP ≤ 20 mmHg (mPAP 16 mmHg, PawP 2 mmHg, cardiac output 3.8 L.min⁻¹, PVR 3.7 WU). At peak exercise, these subjects had exercise PH (mPAP of 54 and 39 mmHg, and TPR of 3.4 and 5.0 WU, respectively) associated with an increased PawP (25 and 21 mmHg, respectively). A decrease in PVR was observed at exercise in these two subjects (from 2 to 1.8 WU and from 3.7 to 2.3 WU, respectively). These patients did not develop incident PAH six years after inclusion in the study.

DISCUSSION

Early diagnosis of PAH and timely intervention are associated with better long-term outcomes.⁹⁻¹¹ Therefore, increasing awareness of PAH and promoting strategies to enable early diagnosis are important strategies to improve the outcomes of this severe but treatable condition. This is particularly true in subjects at high risk of developing PAH such as those with systemic sclerosis or unaffected family members of heritable PAH carrying a *BMPR2* mutation.^{9-11,24} Annual screening echocardiography is currently recommended in asymptomatic *BMPR2* mutation carriers,^{2,4} but the utility and performance of this screening strategy is not known. The DELPHI-2 study aimed to assess the risk of occurrence of PAH in asymptomatic *BMPR2* mutation carriers and to evaluate the incorporation of a multimodal screening program into clinical care for early detection of PAH. *BMPR2* mutations have an incomplete penetrance and heritable PAH can occur at any age, which result in a relatively low overall annual incidence of PAH in asymptomatic *BMPR2* mutation carriers. Despite this, our prospective cohort study showed, for the first time, that asymptomatic adult *BMPR2* mutation carriers can develop incident PAH in a relatively short follow-up time span with an incidence of 2.3%/year. This is particularly true in women which showed an incidence of 3.5%/year versus 0.99 %/year in men. Interestingly, patients diagnosed with PAH in DELPHI-2 screening program belong to families with multiple PAH cases but also in relatives of sporadic PAH cases carrying *BMPR2* mutation. This result suggests that screening program should not be restricted to families with several PAH cases. Some of the cases we report have developed incident PAH after pregnancy or chemotherapy, suggesting the potential relevance of second hits for disease to fully evolve in this population. Interestingly, most of the drugs (docetaxel, trastuzumab, bevacizumab, lapatinib) received by the patient who develop PAH, have been reported to be associated with drug-induced PAH.²⁵⁻²⁸ Chemotherapy exposure did not occur in the 50 relatives without PAH. Using a comprehensive multimodal screening program, PAH was diagnosed at a relatively early stage, allowing timely initiation of targeted PAH therapy.

PAH can frequently complicate the course of associated conditions such as systemic sclerosis. Indeed, 8-12% of systemic sclerosis patients will develop PAH with an incidence of around 1%/year in cohort

studies, broadly comparable to the incidence described in the DELPHI-2 study.²⁹ Recent PAH screening programs in systemic sclerosis have underscored the importance of combining a number of modalities to maximise sensitivity (such as DLCO, NT-proBNP, ECG, and Doppler echocardiography), as compared to approaches solely reliant on the use of a single modality such as echocardiography.^{24,30} In DELPHI-2 study, Doppler echocardiography or BNP/NT-proBNP failed to detect PAH patients in asymptomatic *BMP2* mutation carriers. The two patients screened at inclusion has normal echocardiography with normal TRV, absence of right atrial or ventricular dilatation and absence of other signs suggestive of PH. Of note, three patients had isolated enlarged right atrial surface area (>18 cm²) on echocardiogram, but none of these had PAH at baseline RHC or during follow-up, suggesting that these cases were “false-positive” cases. This study demonstrated that RHC is effective to diagnose PAH in asymptomatic *BMP2* mutation carriers. However, because of its invasive nature and associated morbidity (1.1%) and mortality (0.055%) rates,³¹ RHC cannot be considered a routine screening exam that is used repeatedly but should be reserved when non-invasive investigations raise suspicion of PAH or in high risk situations to confirm diagnosis. Combination of three parameters based on ECG, DLCO and CPET score (Table 4) may be of interest to identify PAH patients. The absence of these 3 criteria was helpful to exclude the diagnosis of PAH in this high-risk population and to avoid unnecessary RHC. Moreover, CPET was also abnormal in all screened PAH patients highlighting the importance of measuring indicators of exercise limitation. Since no CPET value alone are conclusive of PAH, we used a predefined probability score of PH on CPET adapted from previous published studies.¹⁵⁻²⁰ Refinements and validation of CPET scores suggestive of PH should be encouraged in future studies evaluating early diagnosis of PAH. It is therefore likely that the current recommendation of annual echocardiography on its own will be outperformed by a comprehensive multimodal approach in asymptomatic *BMP2* mutation carriers. In the long-term follow-up, 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 our data suggests that echocardiography remains a useful screening test in this setting.^{30,32,33}

Previous findings that *BMPR2* mutation carriers have a worse survival suggest that screening for mutations in unaffected relatives followed by a screening and early detection structured program may improve long-term outcomes. Our study shows that all screened PAH patients were less severe than the usual heritable PAH cases.^{7,8} This is highlighted by the relatively mild invasive haemodynamic findings of *BMPR2* carriers diagnosed via DELPHI-2 compared to index *BMPR2* carriers who were diagnosed routinely due to the presence of symptoms (Table 5). In addition, all were successfully treated with oral monotherapy or dual oral combination therapy with the maintenance of ‘low risk status’ on therapy, which is associated with survival above 95% at five years based on current registry data (Table 6).^{22,23}

Of note, one asymptomatic patient did not completely fulfill the current definition of PAH at time of screening (PVR < 3 WU). According to current guidelines², she was not treated but progressed rapidly to moderate PAH within four months (NYHA FC II, mPAP of 35 mmHg and PVR of 6.8 WU). The sixth World Symposium on PH (Nice 2018) provided an opportunity to discuss the haemodynamic definition of pulmonary vascular diseases.¹ This case illustrates that PVR ranging between 2 and 3 WU in the high-risk population of *BMPR2* mutation carriers might represent an early abnormal haemodynamic status, associated with disease progression and clinical outcomes.^{1,34}

Like in systemic sclerosis²⁴, our study suggests that exercise PH may be a possible early marker of pulmonary vascular remodelling in *BMPR2* mutation carriers. Indeed, 16.7% of the 12 subjects with exercise PH at inclusion subsequently developed PAH during long-term follow-up versus 2.5% among the 40 subjects with normal exercise haemodynamics. Exercise PH in this population requires careful phenotyping in order to distinguish the subset of patients with early pulmonary vascular disease from the highly prevalent left-heart disease population, especially in older subjects and/or those with cardiovascular comorbidities. These results are in accordance with previous studies from Grünig et al, based on echocardiography during exercise showing that an abnormal hemodynamic response occurred significantly more often in relatives of PAH, particularly for those carrying a *BMPR2* mutations^{35,36}. Interestingly, Hinderhofer et al reported in a large family of *BMPR2* mutation carriers that the 2 members who manifested with PAH during follow-up displayed an abnormal haemodynamic response

during exercise at baseline assessment³⁷. Nevertheless, our results open up research perspectives on the relevance of this assessment to identify a sub-population at high-risk of developing PAH. Current multicentre studies analysing cohorts of subjects with exercise RHC will provide insights into the clinical relevance of exercise haemodynamics and how best to discriminate between pre- and post-capillary causes of exercise PH²¹.

Because of the relatively high penetrance of PAH in *BMPR2* mutation carriers, we strongly believed that genetic and reproductive counseling should be proposed to all female of childbearing age in order to explain the potential risk of PAH development during pregnancy and disease transmission to offspring. During pregnancy, we proposed a specific follow-up including monthly Doppler echocardiography during the second and third trimesters followed by a complete assessment within three months of delivery. In France, pre-implantation diagnosis can be offered to *BMPR2* mutation carriers in order to avoid transmission to the offspring. However, to date, pre-implantation diagnosis has not been proposed for women who carry a *BMPR2* mutation, because of the risk of PAH development during pregnancy and the unknown effects of ovarian stimulation in women with a *BMPR2* mutation.

Our study has limitations. First, despite a nationwide recruitment in a high-volume reference centre the number of detected incident PAH was relatively small. However, all screened subjects were carefully investigated including an initial RHC. In addition, all subjects were followed for at least two years and longer-term follow-up data was available up to seven years, with no lost to follow-up and more than 200 patients.year of analysis. Long-term follow-up of *BMPR2* mutation carriers supported by the French referral centre for PH is still ongoing and may bring future information on this high-risk population. Secondly, other promising modalities such as cardiac magnetic resonance or exercise Doppler echocardiography could be of interest.^{2,21,35,36} Whether these exams are superior to conventional tests such as ECG, DLCO, NT-proBNP, echocardiography, and CPET remains to be demonstrated. Our cohort study showed that our multimodality screening approach using commonly available investigations allowed early diagnosis of PAH. It should be acknowledged that early pulmonary vascular disease may be present in patients with lower values of mPAP and PVR than the threshold used to define PAH in this

study. DELPHI-2 was performed before the proposal of a new PAH definition¹ but applying the new definition did not identify any additional PAH cases. Definite conclusions on consequence of screening program on long-term outcome is not possible unless a randomised controlled study is performed comparing patients diagnosed via screening versus patients diagnosed via routine care. Even in the systemic sclerosis population which is broader, such a study has not been performed and is unlikely to be ever performed. The psychological impact of participating in a long-term genetic screening program remains to be assessed. Although the median age of occurrence of PAH in *BMPR2* mutation carriers is 35.4 ± 14.8 years⁸, PAH due to *BMPR2* mutation can occur in children³⁸. Predictive genetic testing for children remains a matter of debate in the absence of preventive treatments available for PAH. Furthermore, no genetic testing is currently offered in France for asymptomatic children who are at risk for carrying a PAH-causing mutation¹². This allows individuals to make a choice once they reach adulthood to make an informed decision regarding genetic testing. Thus, according to French laws on bioethics, this study was restricted to adults and it is difficult to extrapolate our results to the pediatric population. Last, we cannot exclude that lead-time bias or length-time bias might have contributed to the observed response to oral therapy and maintenance of low-risk status at last follow-up.

In conclusion, DELPHI-2 study represents the first study to systematically examine screening for PAH in asymptomatic *BMPR2* mutation carriers. We demonstrate that asymptomatic *BMPR2* mutation carriers have a significant risk of developing incident PAH ranging from 0.99 %/year in men to 3.5%/year in women. Data from this study indicate that a multimodal screening program followed by yearly careful follow-up may be implemented in clinical practice in order to allow early detection and treatment of PAH in *BMPR2* mutation carriers. International multicenter studies are needed to confirm that refined multimodal screening programs with regular follow-up allow early detection of PAH.

REFERENCES

- 1 Simonneau G, Montani D, Celermajer DS, *et al.* Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2019; **53**. DOI:10.1183/13993003.01913-2018.
- 2 Galiè N, Humbert M, Vachiery J-L, *et al.* 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 Respir J* 2015; **46**: 903–75.
- 3 Humbert M, Guignabert C, Bonnet S, *et al.* Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J* 2019; **53**. DOI:10.1183/13993003.01887-2018.
- 4 Galiè N, Humbert M, Vachiery J-L, *et al.* 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension – web addenda. The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and of the European Respiratory Society (ERS). ; : https://www.escardio.org/static_file/Escardio/Guidelines/Publications/PAH/2015%20ESC-ERS%20Gles%20PH-Web%20addenda-ehv317.pdf.
- 5 Larkin EK, Newman JH, Austin ED, *et al.* Longitudinal analysis casts doubt on the presence of genetic anticipation in heritable pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2012; **186**: 892–6.
- 6 Morrell NW, Aldred MA, Chung WK, *et al.* Genetics and genomics of pulmonary arterial hypertension. *Eur Respir J* 2019; **53**. DOI:10.1183/13993003.01899-2018.
- 7 Sztrymf B, Coulet F, Girerd B, *et al.* Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. *Am J Respir Crit Care Med* 2008; **177**: 1377–83.
- 8 Evans JDW, Girerd B, Montani D, *et al.* BMPR2 mutations and survival in pulmonary arterial hypertension: an individual participant data meta-analysis. *Lancet Respir Med* 2016; **4**: 129–37.
- 9 Humbert M, Yaici A, de Groote P, *et al.* Screening for pulmonary arterial hypertension in patients with systemic sclerosis: clinical characteristics at diagnosis and long-term survival. *Arthritis Rheum* 2011; **63**: 3522–30.
- 10 Galiè N, Rubin L, Hoeper M, *et al.* Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. *Lancet* 2008; **371**: 2093–100.
- 11 Lau EMT, Humbert M, Celermajer DS. Early detection of pulmonary arterial hypertension. *Nat Rev Cardiol* 2015; **12**: 143–55.
- 12 Girerd B, Montani D, Jaïs X, *et al.* Genetic counselling in a national referral centre for pulmonary hypertension. *Eur Respir J* 2016; **47**: 541–52.
- 13 Herve P, Lau EM, Sitbon O, *et al.* Criteria for diagnosis of exercise pulmonary hypertension. *Eur Respir J* 2015; **46**: 728–37.

- 14 Naeije R, Vanderpool R, Dhakal BP, *et al.* Exercise-induced pulmonary hypertension: physiological basis and methodological concerns. *Am J Respir Crit Care Med* 2013; **187**: 576–83.
- 15 Laveneziana P, Garcia G, Joureau B, *et al.* Dynamic respiratory mechanics and exertional dyspnoea in pulmonary arterial hypertension. *Eur Respir J* 2013; **41**: 578–87.
- 16 Laveneziana P, Humbert M, Godinas L, *et al.* Inspiratory muscle function, dynamic hyperinflation and exertional dyspnoea in pulmonary arterial hypertension. *Eur Respir J* 2015; **45**: 1495–8.
- 17 Weatherald J, Sattler C, Garcia G, Laveneziana P. Ventilatory response to exercise in cardiopulmonary disease: the role of chemosensitivity and dead space. *Eur Respir J* 2018; **51**. DOI:10.1183/13993003.00860-2017.
- 18 Yasunobu Y, Oudiz RJ, Sun X-G, Hansen JE, Wasserman K. End-tidal PCO₂ abnormality and exercise limitation in patients with primary pulmonary hypertension. *Chest* 2005; **127**: 1637–46.
- 19 Higashi A, Dohi Y, Yamabe S, *et al.* Evaluation of end-tidal CO₂ pressure at the anaerobic threshold for detecting and assessing pulmonary hypertension. *Heart Vessels* 2017; **32**: 1350–7.
- 20 Farina S, Bruno N, Agalbato C, *et al.* Physiological insights of exercise hyperventilation in arterial and chronic thromboembolic pulmonary hypertension. *Int J Cardiol* 2018; **259**: 178–82.
- 21 Kovacs G, Herve P, Barbera JA, *et al.* An official European Respiratory Society statement: pulmonary haemodynamics during exercise. *Eur Respir J* 2017; **50**. DOI:10.1183/13993003.00578-2017.
- 22 Boucly A, Weatherald J, Savale L, *et al.* Risk assessment, prognosis and guideline implementation in pulmonary arterial hypertension. *Eur Respir J* 2017; **50**. DOI:10.1183/13993003.00889-2017.
- 23 Benza RL, Gomberg-Maitland M, Elliott CG, *et al.* Predicting Survival in Patients With Pulmonary Arterial Hypertension: The REVEAL Risk Score Calculator 2.0 and Comparison With ESC/ERS-Based Risk Assessment Strategies. *Chest* 2019; **156**: 323–37.
- 24 Coghlan JG, Denton CP, Grünig E, *et al.* Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann Rheum Dis* 2014; **73**: 1340–9.
- 25 Ranchoux B, Günther S, Quarck R, *et al.* Chemotherapy-induced pulmonary hypertension: role of alkylating agents. *Am J Pathol* 2015; **185**: 356–71.
- 26 Kwon Y, Gomberg-Maitland M, Pritzker M, Thenappan T. Telangiectasia and Pulmonary Arterial Hypertension Following Treatment With Trastuzumab Emtansine: A Case Report. *Chest* 2016; **149**: e103-105.
- 27 Liotta M, Rose PG, Escobar PF. Pulmonary hypertension in two patients treated with bevacizumab for recurrent ovarian cancer. *Gynecol Oncol* 2009; **115**: 308–9.
- 28 Alkhatib Y, Albashaireh D, Al-Aqtash T, Awdish R. The role of tyrosine kinase inhibitor ‘Lapatinib’ in pulmonary hypertension. *Pulm Pharmacol Ther* 2016; **37**: 81–4.
- 29 Hachulla E, de Groote P, Gressin V, *et al.* The three-year incidence of pulmonary arterial hypertension associated with systemic sclerosis in a multicenter nationwide longitudinal study in France. *Arthritis Rheum* 2009; **60**: 1831–9.

- 30 Khanna D, Gladue H, Channick R, *et al.* Recommendations for screening and detection of connective tissue disease-associated pulmonary arterial hypertension. *Arthritis Rheum* 2013; **65**: 3194–201.
- 31 Hoepfer MM, Lee SH, Voswinckel R, *et al.* Complications of right heart catheterization procedures in patients with pulmonary hypertension in experienced centers. *J Am Coll Cardiol* 2006; **48**: 2546–52.
- 32 Hachulla E, Gressin V, Guillevin L, *et al.* Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. *Arthritis Rheum* 2005; **52**: 3792–800.
- 33 Parent F, Bachir D, Inamo J, *et al.* A hemodynamic study of pulmonary hypertension in sickle cell disease. *N Engl J Med* 2011; **365**: 44–53.
- 34 Maron BA, Brittan EL, Hess E, Waldo SW, Barón AE, Huang S, Goldstein RH, Assad T, Wertheim BM, Alba GA, Leopold JA, Olschewski H, Galiè N, Simonneau G, Kovacs G, Tedford, RJ, Humbert M, Choudhary G. The association between pulmonary vascular resistance and clinical outcomes in patients with pulmonary hypertension. *Lancet Respir Med* 2020; **in press**.
- 35 Grünig E, Janssen B, Mereles D, *et al.* Abnormal pulmonary artery pressure response in asymptomatic carriers of primary pulmonary hypertension gene. *Circulation* 2000; **102**: 1145–50.
- 36 Grünig E, Weissmann S, Ehlken N, *et al.* 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**: 1747–57.
- 37 Hinderhofer K, Fischer C, Pfarr N, *et al.* 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.
- 38 Eyries M, Montani D, Nadaud S, *et al.* Widening the landscape of heritable pulmonary hypertension mutations in paediatric and adult cases. *Eur Respir J* 2019; **53**: 1801371.

ACKNOWLEDGMENTS

The DELPHI-2 Study (NCT01600898) was funded by French Ministry of Social Affairs and Health (PHRC P100175) and supported by French Pulmonary Hypertension Patient Association "HTaPFrance", Chancellerie des Universités, Legs Poix, France, Pulmonary Hypertension Grants Program 2013 from Bayer, and European Respiratory Society (grant LTRF-2013-1592).

The authors thank all patients, relatives, physicians, and healthcare professionals from the French Pulmonary Hypertension Reference Network (PulmoTension).

The authors thank the Clinical Research Unit Paris Sud (Bicetre hospital - APHP) for monitoring and coordinating this study.

FIGURES

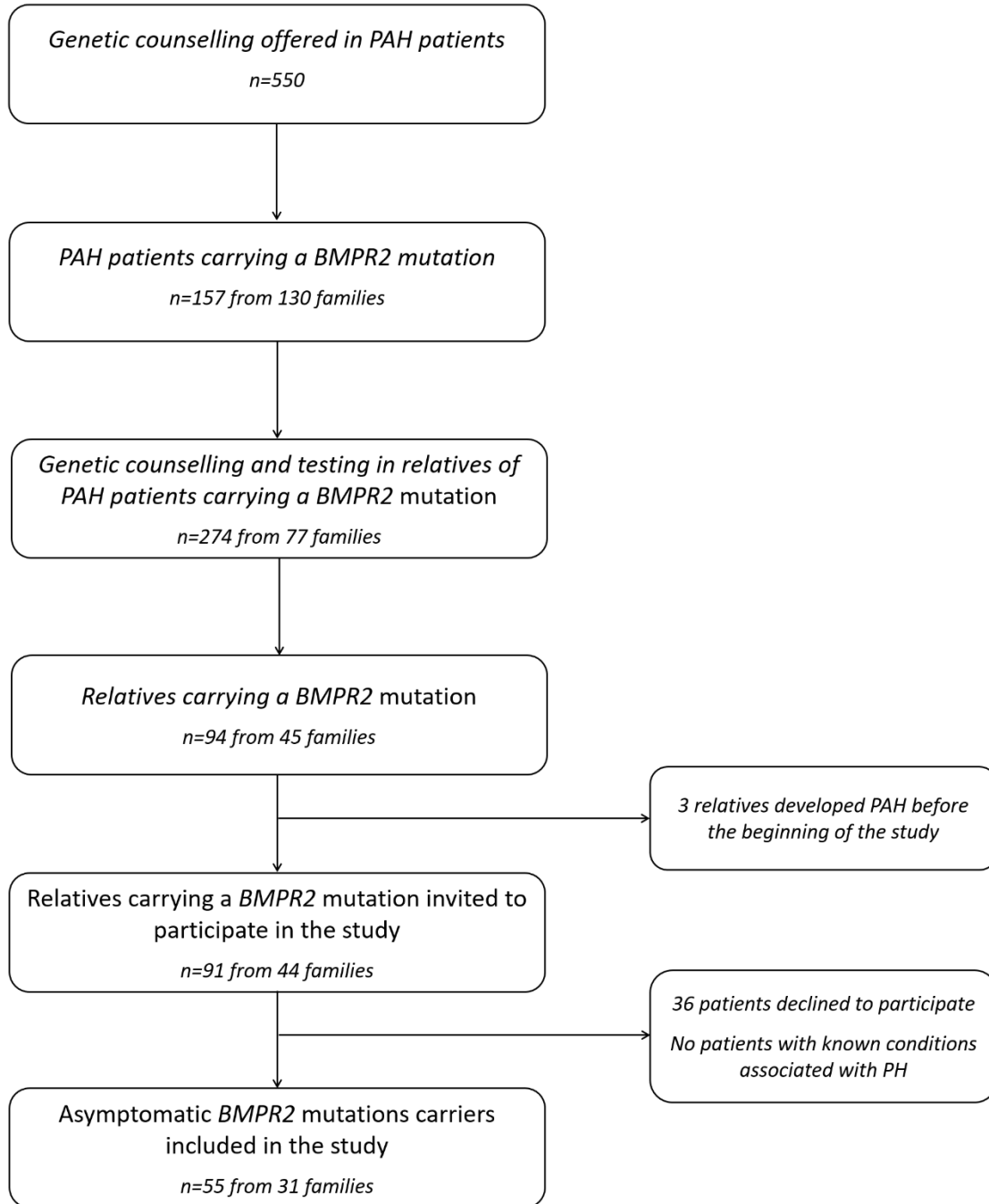


Figure 1. Flow-chart of the DELPHI-2 study

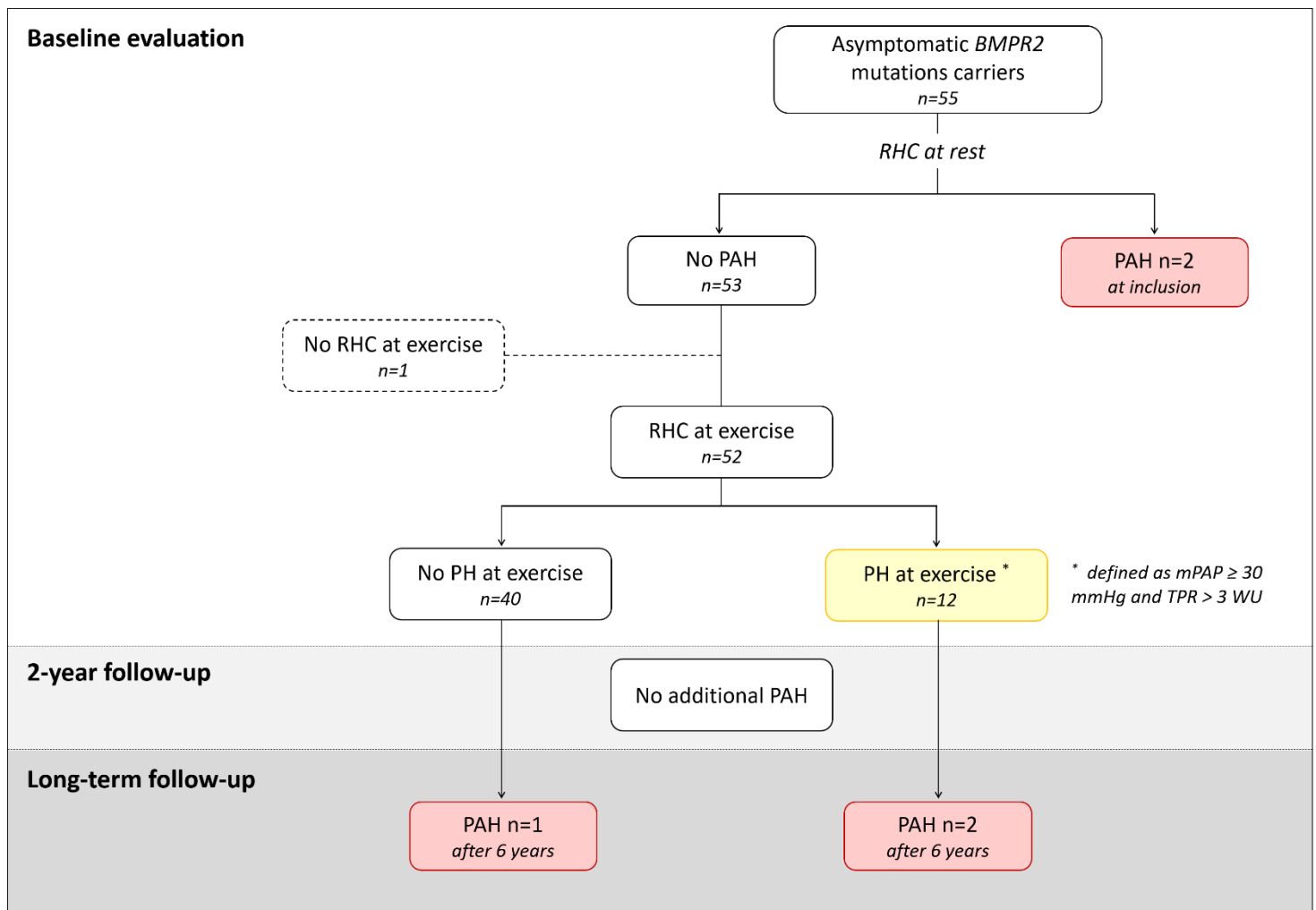


Figure 2. Baseline evaluation and follow-up of the 55 asymptomatic *BMPR2* mutations carriers

PAH: pulmonary arterial hypertension; PH: pulmonary hypertension; RHC: right heart catheterization

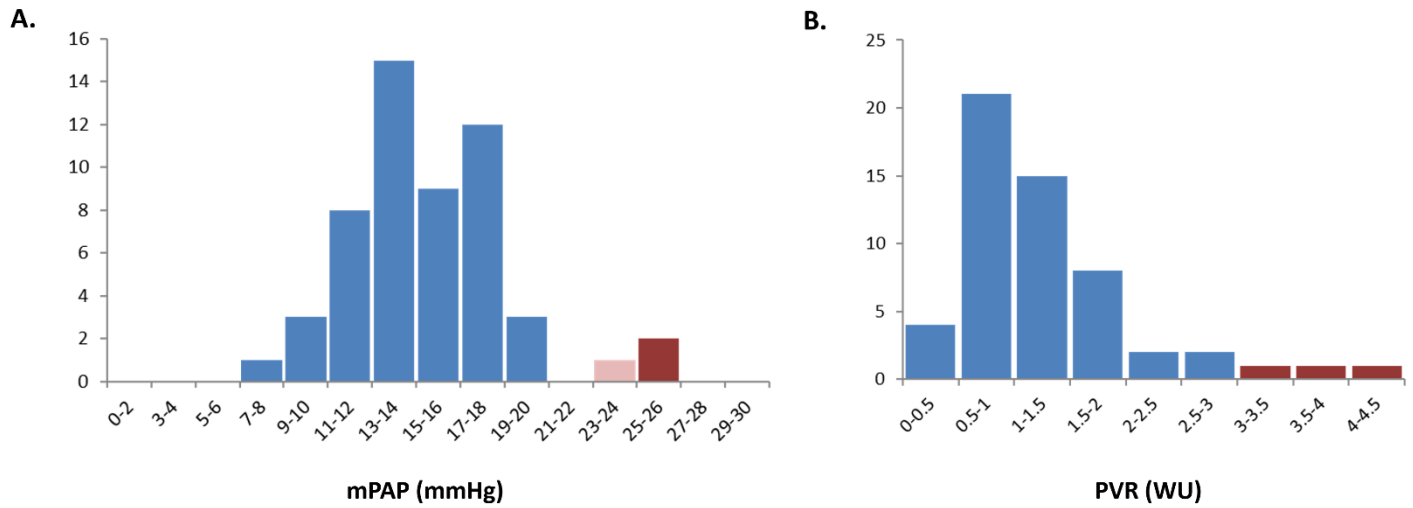


Figure 3. Distribution of mean pulmonary artery pressure (A) and pulmonary vascular resistance (B) at the baseline right heart catheterization amongst 55 asymptomatic *BMPR2* mutations carriers

mPAP: mean pulmonary artery pressure; *PVR*: pulmonary vascular resistance, *WU*: Wood units. Dark red denotes patients with $mPAP \geq 25$ mmHg and/or $PVR > 3$ WU; and light red denotes $mPAP$ 21-24mmHg. Blue denotes patients with normal haemodynamics.

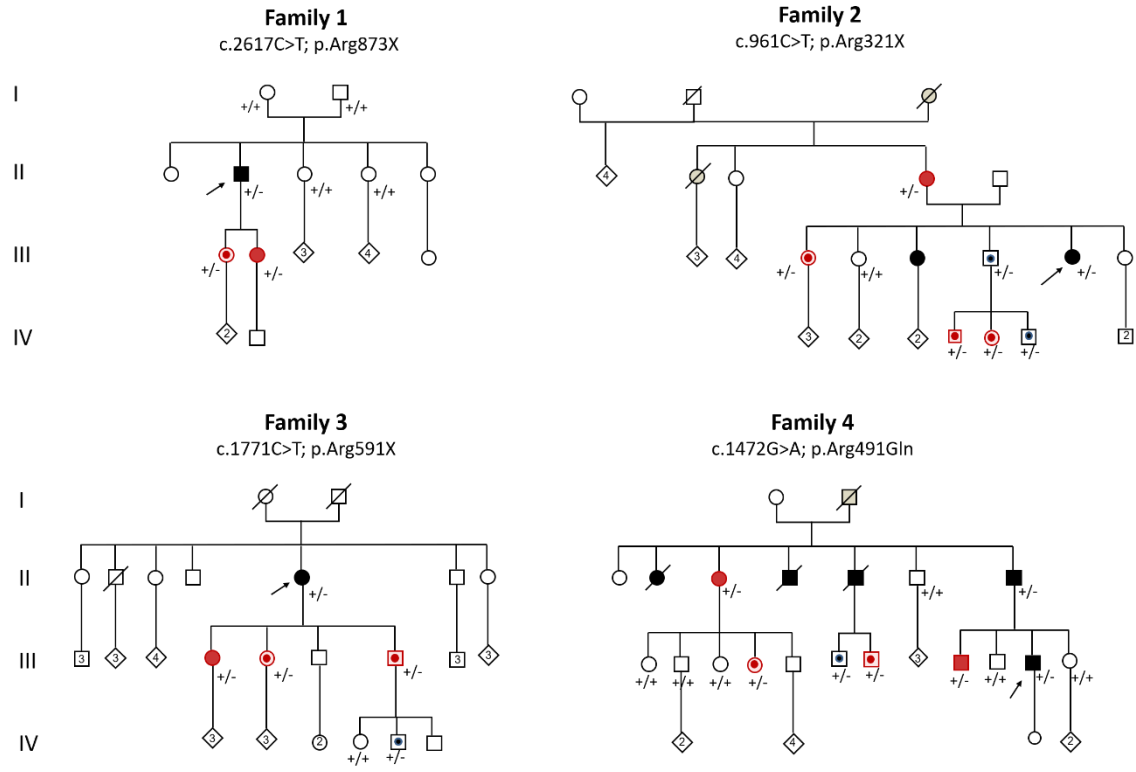
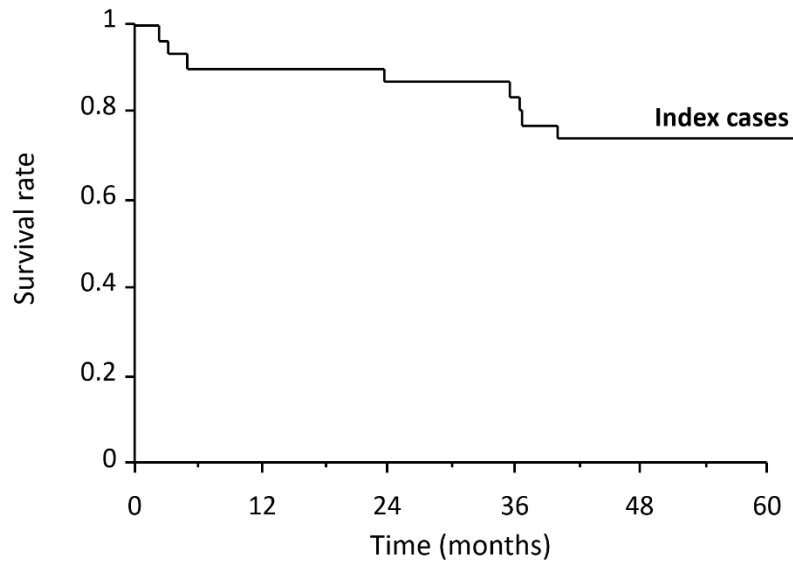


Figure 4: Family trees of *BMPR2* mutation carriers who developed PAH during the DELPHI2 study. Patient 1 belongs to the family 1 (III2). Patient 2 belongs to the family 2 (II7). Patient 3 belongs to the family 3 (III11). Patients 4 and 5 belong to the family 4 (II3 and III11 respectively).

+/+ : mutation non carriers; +/- : *BMPR2* mutation carriers.

- ■ PAH patients
- □ suspected PAH patients
- ■ *BMPR2* mutations carriers who developed PAH during DELPHI2 study
- ⊙ ⊠ Asymptomatic *BMPR2* mutations carriers followed in the DELPHI2 study
- ⊠ Asymptomatic *BMPR2* mutations carriers who declined the follow-up



Number at risk	31	28	27	24	21	20
----------------	----	----	----	----	----	----

Figure 5. Time to death or lung transplantation in index cases (n=31) of subjects included in DELPHI-2 study.

TABLES

Table 1. Characteristics of *BMPR2* mutations carriers without PAH and patients with PAH in DELPHI-2 study and long-term follow-up

	<i>DELPHI-2 study</i> <i>n=55</i>			<i>Long-term follow-up</i> <i>n=36</i>		
	<i>No PAH at inclusion</i> <i>(n=53)</i>	<i>Patient 1</i>	<i>Patient 2</i>	<i>Patient 3</i>	<i>Patient 4</i>	<i>Patient 5</i>
Demographic data						
Age, years	37.1 (18-67.5) [24.9-49.7]	25.5	78.1	49.9	72.5	46.8
Gender, M/F	26/27	F	F	F	F	M
Tobacco exposure >5p.y	18 (34%)	Yes	No	No	No	Yes
BMI, Kg/m²	22.5 (16.8- 32.2) [21.7-26.1]	22.0	25.2	22.9	21.3	24.9
Systemic hypertension, n (%)	10 (19%)	No	Yes	No	No	No
Diabetes, n (%)	3 (6%)	No	Yes	No	No	No
Dyslipidaemia, n (%)	3 (6%)	No	No	No	No	No
Delay between symptoms and diagnosis, months	3	..	3-6
Potential risk factor	..	Pregnancy	None	Chemo therapy	Chemo therapy	None
Functional parameters						
NYHA FC, I-IV	I	I	I	II	I	II
6MWD, m	539 (368-693) [486-599]	533	420	397	412	505
FEV1, % pred	105 (69-141) [97-113]	86	152	91	119	91
FVC, % pred	108 (75-140) [98-117]	89	162	103	144	96
DLCO % pred	76 (39-126) [69-87]	50	63	42	52	82
DLCO/Va, % pred	89 (49-126) [74-100]	62	66	65	58	104
Cardiopulmonary exercise testing						
V'O₂ at peak, % pred	81 (47-132) [65.5-91.5]	70	89	58	81	62
V'E at peak, % pred	76.2 (37.8-156.8) [56.7-101.8]	74	53.9	63.3	63.5	72.4
V_D/V_T, %	0.21 (0.06-0.39) [0.12-0.24]	0.15	0.27	0.53	0.49	-
V'E/VCO₂ at AT	33 (24-66) [23.5-45]	36	54	59	43	39
VO₂/HR, % pred	88 (50-125) [77.5-101]	100	98	60	122	78
PaO₂ at rest, mmHg	93 (75-118) [85-100]	99	70	70	94	63
PaO₂ at peak, mmHg	106 (85-124) [97.5-114]	..	97	49	83	..
P_(A-a)O₂, mmHg	12.7 (-5.6-32.8) [7.7-19.8]	15.7	26	77	40	..
Probability score	2 (0-6)	4	9	10	8	..
Echocardiography						
TRV, m/s	2 (1.5-2.75) n=28	2	2.65	4.2	3.3	4.4

	[1.85-2.3]					
RA surface area, cm²	13 (7.7-27) [10-15]	15	14	19	16	21
LA Surface area, cm²	14 (9.5-23) [12.5-17]	15	9	12	17	13
Diastolic RV, cm²	17.5 (8-28) [14-20]	15.6	16	22.9	15.8	25
Systolic RV, cm²	9.45 (3.7-15) [7-11]	11	8	14.8	8.2	12
RVEF, %	45 (30-71) [40-50]	30	50	35	48	50
TAPSE, mm	23 (16-28) [21-25]	25	16	23	19	21
RV Tei index	0.24 (0.06-0.77) [0.18-0.30]	0.2	..	0.47
Hemodynamics						
mPAP, mmHg	14 (8-23) [13-17]	26	25	43	29	50
PawP, mmHg	8 (2-14) [6-10]	8	7	7	5	8
Cardiac output, L.min⁻¹	6.03 (3.77-10.33) [5.30-7.00]	7.27	4.05	3.93	3.27	4.57
Cardiac index, L.min⁻¹.m²	3.50 (2.20-5.44) [3.11-4.10]	4.38	2.8	2.31	1.94	2.36
Stroke volume index, mL.m²	51.6 (33.7-79.2) [43.6-53.5]	55.4	35.0	46.8	32.3	31.9
TPR, WU	2.5 (1.2- 4.8) [1.9-3.0]	3.58	6.17	10.93	8.88	10.95
PVR, WU	1.0 (0.2-3.7) [0.7-1.4]	2.5	4.4	9.2	7.3	9.2
Acute vasodilator test	not performed	negative	negative	negative	negative	negative
Biological tests						
Uricemia, μmol.L⁻¹ (normal <357)	286 (170-528) [231-374]	250	465	306	270	603
BNP, ng.L⁻¹ (normal < 80)	11 (5-60) [8.8-15.5]	9	19
NT-proBNP, ng.L⁻¹ (normal <300)	484	252	437

All values are expressed as Median (Min-Max) [IQR 25-75]

AT: anaerobic threshold; BMPR2: bone morphogenetic protein receptor type 2; DLCO : diffusing capacity for carbon monoxide; DLCO/Va : DLCO divided by the alveolar volume; FEV1: Forced Expiratory Volume in one second; FVC: forced vital capacity; HR: heart rate; LA: left atrium; NA: non available; NYHA FC: New York Heart Association functional class, PAH: pulmonary arterial hypertension, mPAP= mean pulmonary artery pressure, PaO2: partial pressure of oxygen in arterial blood; PawP : pulmonary capillary wedge pressure; P_(A-a)O₂ : alveolar-arterial oxygen difference; PVR : pulmonary vascular resistance; RA : right atrium; RAP: right atrial pressure; RV: right ventricle; RVEF: right ventricular ejection fraction; TAPSE : tricuspid annular plane systolic excursion; TPR : total pulmonary resistance; TRV: Tricuspid regurgitation velocity; V_D/V_T: physiologic dead space fraction; V_E: minute ventilation; V'O₂ : oxygen consumption corrected for body weight, 6MWD : 6-minute walk distance; % pred : percentage of predicted values.

.. : non available data

Table 2. Baseline results of investigational cardiopulmonary exercise testing probability score of pulmonary hypertension

Total Score	Likelihood	n	No PH	Exercise PH	PAH
0-2	Unlikely	31	28 (90.3%)	3 (9.7%)	0
3-5	Possible	21	11 (52.4%)	9 (42.9%)	1 (4.8%)
6-8	Likely	1	1 (100%)	0	0
9-10	Very likely	1	0	0	1 (100%)

PAH: pulmonary arterial hypertension, PH: pulmonary hypertension

Table 3. Comparison of hemodynamics at rest between patients with normal hemodynamic on exercise (n=40) and subjects with exercise PH (n=12)

	No PH at exercise n=40	PH at exercise* n=12	P value
Female, n (%)	18 (45%)	9 (75%)	0.07
Age, years	26.9 (18-58) [23.6-41.8]	58.7 (43.8-67.5) [52.8-64.1]	< 0.0001
Hemodynamic at rest			
mPAP, mmHg	14 (8-20) [12-16.5]	16.5 (13-23) [14.5-18]	0.020
Cardiac index, L.min⁻¹/m²	3.59 (2.62-5.44) [3.23-4.11]	3.12 (2.20-4.80) [2.62-3.63]	0.0226
SVI, mL.min⁻¹/m²	51.0 (32.3-75.6) [45.3-54.4]	43.9 (32.7-57.9) [37.0-49.5]	0.0076
PawP, mmHg	9 (3-14) [7-11]	6 (2-12) [4.5-9.0]	0.036
PVR, WU	0.8 (0.2-1.5) [0.6-1.2]	1.6 (0.6-3.7) [1.6-2.3]	< 0.0001
TPR, WU	2.3 (1.2-3.7) [1.8-2.8]	3.2 (2.3-4.8) [2.5-3.8]	0.001
Hemodynamic at peak exercise			
Workload, Watt	90 (30-150) [60-100]	70 (30-100) [55-80]	0.015
mPAP, mmHg	20 (8-20) [21-29.5]	40 (34-54) [36.5-47.5]	< 0.0001
TPR, WU	1.6 (0.9-3.1) [1.3-2.1]	3.55 (3-5) [3.3-4.7]	< 0.0001
Cardiac index, L.min⁻¹/m²	8.99 (5.77-11.59) [8.30-9.73]	6.49 (4.75-8.83) [5.8-7.4]	< 0.0001
SVI, mL.min⁻¹/m²	68.5 (48.2-94.5) [59.6-77.2]	53.9 (42.8-66.9) [47.8-59.7]	0.0002
PawP, mmHg	14 (6-25) [12-18]	25 (20-30) [22.5-26.0]	< 0.0001
PVR, WU	0.65 (0.1-1.4) [0.5-0.9]	1.6 (0.8-2.3) [1.3-1.9]	< 0.0001
Long-term follow-up			
PAH, n (%)	1 (2.4%)	2 (16.7%)	

* PH at exercise was defined by an increased in mPAP >30 mmHg and TPR >3 WU.

All values are expressed as Median (Min-Max) [IQR 25-75]

mPAP: mean pulmonary artery pressure; PawP: pulmonary capillary wedge pressure, PH: pulmonary hypertension; PVR: pulmonary vascular resistance; SVI: stroke volume index; TPR: total pulmonary resistance

Table 4. Diagnosis of PAH and exercise PH according to ECG, cardiopulmonary exercise testing, and diffusing lung capacity for carbon monoxide in asymptomatic relatives at inclusion in DELPHI-2 study.

The 3 criteria included:

- 1) *ECG with signs that could be suggestive of PH;*
- 2) *Cardiopulmonary exercise testing possible, likely or very likely for PAH;*
- 3) *Diffusing lung capacity for carbon monoxide (DLCO) <70% of predicted values.*

Number of criteria	All subjects (n=54)	No PH (n=40)	PH at exercise (n=12)	PAH (n=2)
0	21	18 (85.7%)	3 (14.3%)	0
1	20	16 (80%)	4 (20%)	0
2	10	6 (60%)	4 (40%)	0
3	3	0	1 (33.3%)	2 (66.7%)

Table 5. Comparison of clinical and haemodynamics characteristics at diagnosis of PAH patients identified in the DELPHI2 study (n=5) and the index cases in their family (n=31).

	Index cases n=31	DELPHI2 PAH patients n=5	P value
Clinical characteristics			
Female, n (%)	22 (71%)	4 (80%)	0.90
Age, years	38 (5-63) [26-47]	50 (26-78) [41-74]	0.06
NYHA functional class,			
I	0	3	<0.001
II	4	2	
III	18	0	
IV	7	0	
6MWD, m	356 (0-530) [304-400]	420 (397-533) [408-512]	0.02
Hemodynamic characteristics			
mPAP, mmHg	58 (45-95) [51.5-72.3]	29 (25-50) [25.8-44.8]	<0.001
Cardiac index, L.min⁻¹/m²	2.10 (1.14-3.7) [1.61-2.42]	2.36 (1.94-4.38) [2.22-3.20]	0.14
Cardiac output, L.min⁻¹	3.46 (1.85-5.9) [2.65-9.4.13]	4.05 (3.27-7.27) [3.77-5.25]	0.14
PawP, mmHg	8 (1-19) [5-9]	7 (5-8) [6.5-8]	0.6
PVR, WU	15.4 (7.6-31.8) [12.1-22.1]	7.3 (2.5-9.2) [4.0-9.2]	0.001

All values are expressed as Median (Min-Max) [IQR 25-75]

Table 6. Evolution of PAH patients at last follow-up according to risk assessment from REVEAL 2.0 score, ESC/ERS guidelines and simplified risk assessment

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Delay between diagnosis and assessment, months	71	69	5	6	5
PAH therapies	ERA PDE5i	PDE5i	ERA PDE5i	ERA PDE5i	ERA PDE5i
REVEAL 2.0 Score ¹⁶					
Score at diagnosis	2	3	8	4	5
Score at last follow-up	4 (low risk)	4 (low risk)	5 (low risk)	2 (low risk)	3 (low risk)
ESC/ERS Guidelines ²					
Signs of right heart failure	No	No	No	No	No
Progression of symptoms	No	No	No	No	No
Syncope	No	No	No	No	No
NYHA functional class	II	I	II	I	II
6MWD, m	587	358	454	490	533
CPET: $\dot{V}O_2$ at peak, ml/min/Kg (% pred)	15 (81%)	25 (76%)
VE/VCO₂ slope	40	32
NT-proBNP, ng/mL	96	234	66	92	113
Echocardiography: RA area, cm²	< 18	< 18	..	< 18	< 18
Pericardial effusion	No	No	..	No	No
Haemodynamics					
RAP, mmHg	6	7	5	4	11
CI, L.min ⁻¹ .m ²	2.57	2.56	2.83	2.42	2.68
SvO ₂ , %	71	68	73	71	71
Simplified Risk assessment ¹⁵					
NYHA functional class I/II	Yes	Yes	yes	yes	yes
6MWD > 440 m	Yes	No	yes	yes	yes
RAP < 8 mmHg	Yes	Yes	yes	yes	no
CI ≥ 2.5 L.min⁻¹.m²	Yes	Yes	yes	no	yes
Number of low-risk criteria	4	3	4	3	4

For patient 2, haemodynamic reassessment was performed at 6 months of follow-up

SUPPLEMENTARY METHODS

Baseline and follow-up assessment

Electrocardiogram

ECG evaluated signs of possible PH including right bundle branch block, inverted T wave in the right precordial leads or right axis deviation (QRS axis $> +90$ degrees).

Pulmonary function tests

All patients completed standard PFTs with spirometry, whole-body plethysmography, and diffusing lung capacity for carbon monoxide (DLCO), with measurement of alveolar volume (VA) by helium dilution technique according to the recommendations from the American Thoracic Society (ATS)/ERS guidelines.¹ DLCO was measured with a single breath method and a ten-second breathhold and values of DLCO were corrected for hemoglobin.¹

Doppler echocardiography

Measurements included TRV, estimation of right atrial pressure (RAP), right atrial surface, left atrial surface, right ventricular diastolic surface, right ventricular systolic surface, right ventricular ejection fraction, tricuspid annular plane systolic excursion (TAPSE), right ventricular Tei index. Where possible, systolic PAP was estimated as $4 \times (\text{TRV})^2 + \text{RAP}$.

6-minute walk distance and cardiopulmonary exercise test

A non-encouraged 6MWD (expressed in metres) was performed according to the ATS recommendations.² CPET was performed as recommended using an electromagnetically braked cycle ergometer during incremental test.^{3,4} Oxygen consumption ($\dot{V}O_2$), CO_2 production, ventilation, and heart rate were measured breath-by-breath using the Ergocard cardiopulmonary exercise testing system® (Medisoft, Belgium). Arterial blood gases (PaO_2 and PaCO_2) at rest and at peak exercise where possible, were collected with puncture of the radial artery on room air. An investigational CPET probability score of PH was predefined based on five criteria adapted from data published by our group and others.³⁻⁸ (Table 1): three criteria at peak exercise were arterial-end-tidal PCO_2 difference ($P_{(\text{a-ET})\text{CO}_2}$), alveolar-arterial oxygen difference ($P_{(\text{A-a})\text{O}_2}$), physiologic dead space fraction (V_D/V_T); and two criteria at anaerobic threshold were minute ventilation to carbon dioxide production ratio ($\dot{V}E/V\text{CO}_2$) and end-tidal partial pressure of carbon dioxide ($P_{\text{ET}\text{CO}_2}$).

Biological tests

Biological testing included white blood cell counts, haemoglobin, electrolytes, creatinine, liver function tests, BNP at inclusion, BNP or NT-proBNP during follow-up, and uricaemia.

Haemodynamic evaluation at rest and exercise

Haemodynamic evaluation was carried out in supine position and measurements were obtained with a balloon-tipped, double-lumen, fluid-filled 7 Fr Swan Ganz catheter via either the brachial or jugular vein approach, as previously described.⁹ Zero reference was set at the mid-chest level.⁹ Right atrial pressure, systolic, diastolic, and mean PAP were recorded and PawP was measured at end-expiration and end-diastole. Cardiac output (CO) was measured using the thermodilution technique (average of three values differing by <10%), cardiac index (CI) was calculated as CO/body surface area and stroke volume index (SVI) was calculated as CI/heart rate. PVR was calculated as (mPAP–PawP)/CO and total pulmonary resistance (TPR) as mPAP/CO.

Dynamic exercise was performed with subjects in supine position on an electronically braked lower limb cycle ergometer, as previously described.^{10,11} Subjects were encouraged to cycle at a rate of 60 revolutions/min until exhaustion or appearance of exercise-limiting symptoms. Measurements were done at baseline and at the following stages: legs on cycle pedal, unloaded pedaling (0 W) and at constant workload increments of 20–30 W depending on estimated exercise capacity. Pressure measurements were averaged over the respiratory cycle and all measurements were obtained at steady state (i.e. stable mPAP and heart rate) during the last 1–2 min of each exercise step. Exercise PH was defined by an increased in mPAP >30 mmHg and total pulmonary resistance (TPR) >3 WU.^{10,12}

SUPPLEMENTARY TABLES

Supplemental Table 1. Investigational cardiopulmonary exercise testing probability score of pulmonary hypertension

Variables	Values	Score
P_(a-ET)CO₂ at peak	Negative value	0
	Positive value	2
P_(A-a)O₂ at peak	≤29 mmHg	0
	≥30 mmHg	2
V'_E/VCO₂ at AT	≤29	0
	30-34	1
	≥35	2
P_{ET}CO₂ at AT	≥36 mmHg	0
	35-31 mmHg	1
	≤30 mmHg	2
V_D/V_T at peak	decreases from baseline	0
	stable or decreases slightly from baseline	1
	Increases from baseline	2

P_(a-ET)CO₂ = arterial-end-tidal PCO₂ difference; P_(A-a)O₂ = alveolar-arterial oxygen difference; V'_E/VCO₂ = minute ventilation to carbon dioxide production ratio; AT = anaerobic threshold; P_{ET}CO₂ = end-tidal partial pressure of carbon dioxide; V_D/V_T = physiologic dead space fraction.

Supplemental Table 2. List of *BMP2* mutations in the 55 asymptomatic *BMP2* mutations carriers

Family	Patient	Age at inclusion	Gender	Amino acid changes	Protein changes	Type of mutation
1	1A 1B	37.1 42.8	F M	c.418+3A>T	Splice defect	Large rearrangement
2	2A 2B 2C 2D	25.6 32.9 66.4 40.8	F M F M	c.1472G>A	p.Arg491Gln	Missense
3	3A 3B	23.9 40.2	M M	c.1001T>G	p.Leu334X	Nonsense
4	4A	60.5	F	Deletion exon 11 to 13	Deletion exon 11 to 13	Large rearrangement
5	5A 5B	58.2 21.8	F F	c.1277-9A>G	Splice defect	Large rearrangement
6	6A	25.5	F	c.1471C>T	p.Arg491Trp	Missense
7	7A	32.5	M	c.439C>T	p.Arg147X	Nonsense
8	8A	36.8	F	Deletion exon 6	Deletion exon 6	Large rearrangement
9	9A 9B 9C	43.8 46.1 43.6	F M F	c.1771C>T	p.Arg591X	Nonsense
10	10A	26.1	F	c.200A>G	p.Tyr67Cys	Missense
11	11A	26.6	M	c.2618G>A	p.Arg873Gln	Missense
12	12A 12B 12C 12D	25.5 52.3 53.8 55.3	F M M F	c.1171G>A	p.Ala391Thr	Missense
13	13A	35.7	M	c.439C>T	p.Arg147X	Nonsense
14	14A 14B 14C	47.5 38.3 48.8	F F F	c.1348C>T	p.Gln450X	Nonsense
15	15A 15B	27.1 25.6	F F	c.2617C>T	p.Arg873X	Nonsense
16	16A	51.0	F	c.928A>T	p.Arg310X	Nonsense
17	17A	67.5	M	c.1472G>A	p.Arg491Gln	Missense
18	18A	46.4	M	c.528delA	Gly177GlufsX10	Nonsense
19	19A 19B 19C	65.0 36.0 18.0	F F M	c.1019T>C	p.Leu340Pro	Missense
20	20A	44.0	F	Deletion exon 10	Deletion exon 10	Large rearrangement
21	21A	63.1	M	c.994C>T	p.Arg332X	Nonsense
22	22A	58.0	M	c.830T>C	p.Leu277Pro	Missense
23	23A 23B 23C 23D	54.5 78.1 18.4 24.3	F F F M	c.961C>T	p.Arg321X	Nonsense
24	24A 24B	24.2 26.4	F M	c.1471C>T	p.Arg491Trp	Missense
25	25A	21.4	F	Deletion exon 2	Deletion exon 2	Large rearrangement
26	26A 26B 26C 26D	48.6 23.2 22.7 18.6	M M M M	c.2308del	p.Arg770GlyfsX2	Nonsense
27	27A	49.2	M	c.872T>G	p.Leu291X	Nonsense
28	28A	18.0	F	c.968-1G>T	Splice defect	Large rearrangement
29	29A	59.2	F	c.642T>G	p.Tyr214X	Nonsense
30	30A	22.6	M	c.435del	p.Phe145Leufs*7	Nonsense
31	31A 31B	22.3 26.2	M M	c.901T>C	p.Ser301Pro	Missense

Supplemental Table 3. Characteristics of *BMP2* mutations carriers (n=55)

<i>Demographic data</i>	
Age, years	37.1 (18-78.1) [25.5-55.6]
Gender, M/F	26/29
Tobacco exposure >5p.y	19 (35%)
BMI, Kg/m ²	22.5 (16.8-32. .2) [21.9-26.0]
Systemic hypertension, n (%)	11 (20%)
Diabetes, n (%)	4 (7%)
Dyslipidemia, n (%)	3 (5%)
<i>Functional parameters</i>	
6MWD, m	533 (368-693) [479-599]
FEV1, % pred	105 (69 -152) [97-113]
FVC, % pred	108 (75-162) [97-118]
DLCO, % pred	76 (39-126) [69-87]
DLCO/Va, % pred	88 (49-126) [73-99]
<i>Cardiopulmonary exercise testing</i>	
V'O ₂ at peak, % pred	81 (47-132) [66-90]
V'E at peak, % pred	74.8 (37.8-156.8) [56.6-101.4]
V _D /V _T , %	0.21 (0.06-0.39) [0.12-0.25]
V'E/VCO ₂ at AT	33 (24-66) [23-44]
VO ₂ /HRF, % pred	88.5 (50-125) [78-100]
PaO ₂ at rest, mmHg	93 (70-118) [85-100]
PaO ₂ at peak, mmHg	106 (85-124) [97-114]
P _{(A-a)O₂} , mmHg	13.8 (-5.6-32.8) [7.8-20.0]
<i>Echocardiography</i>	
TRV, m/s	2 (1.5-2.75) [1.9-2.3] (n=30)
RA surface area, cm ²	13 (7.7-27) [10.0-15.0]
LA Surface area, cm ²	14 (9.5-23) [13.0-17.0]
Diastolic RV, cm ²	16.9 (8-28) [14.2-20.0]
Systolic RV, cm ²	9.5 (3.7-15) [7.1-11.0]
RVEF, %	45 (30-71) [40-50]
TAPSE, mm	23 (16-28) [21-25]
RV Tei index	0.24 (0.06-0.77) [0.18-0.0]
<i>Hemodynamics</i>	
mPAP, mmHg	15 (8-26) [13-18]
PawP, mmHg	8 (2-14) [6-10]
Cardiac output, L.min ⁻¹	6.03 (3.77-10.33) [5.25-7.02]
Cardiac index, L.min ⁻¹ .m ²	3.50 (3.07-4.10)
Stroke volume index, mL.m ²	51.6 (33.7-79.2) [43.6-53.5]
TPR, WU	2.5 (1.2-6.2) [1.9-3.0]
PVR, WU	1.0 (0.2-4.4) [0.7-1.5]
<i>Biologic tests</i>	
Uricemia, μmol.L ⁻¹ (normal < 357)	286 (170-528) [234-383]
BNP, ng.L ⁻¹ (normal < 80)	11 (5-60) [9-17]

All values are expressed as Median (Min-Max) [IQR 25-75]

AT: anaerobic threshold; *BMP2*: bone morphogenetic protein receptor type 2; DLCO : diffusing capacity for carbon monoxide; DLCO/Va: diffusing capacity for carbon monoxide divided by the alveolar volume; FEV1: Forced Expiratory Volume in the first second; FVC: forced vital capacity; HR: heart rate; LA: left atrium; NYHA FC: New York Heart Association functional class, PAH: pulmonary arterial hypertension, mPAP= mean pulmonary

artery pressure, PaO₂: partial pressure of oxygen in arterial blood; PawP: pulmonary capillary wedge pressure; P_{(A-a)O₂}: alveolar-arterial oxygen difference; PVR: pulmonary vascular resistance; RA: right atrium; RAP: right atrial pressure; RV: right ventricle; RVEF: right ventricular ejection fraction; TAPSE: tricuspid annular plane systolic excursion; TPR: total pulmonary resistance; TRV: Tricuspid regurgitation velocity; V_D/V_T: physiologic dead space fraction; V'_E: minute ventilation; V'O₂: oxygen consumption corrected for body weight; 6MWD: 6-minute walk distance; ; % pred : percentage of predicted values.

REFERENCES

- 1 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. - PubMed - NCBI. <https://www.ncbi.nlm.nih.gov/gate2.inist.fr/pubmed/?term=28049168> (accessed Oct 31, 2019).
- 2 ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; **166**: 111–7.
- 3 Laveneziana P, Garcia G, Joureau B, *et al.* Dynamic respiratory mechanics and exertional dyspnoea in pulmonary arterial hypertension. *Eur Respir J* 2013; **41**: 578–87.
- 4 Laveneziana P, Humbert M, Godinas L, *et al.* Inspiratory muscle function, dynamic hyperinflation and exertional dyspnoea in pulmonary arterial hypertension. *Eur Respir J* 2015; **45**: 1495–8.
- 5 Weatherald J, Sattler C, Garcia G, Laveneziana P. Ventilatory response to exercise in cardiopulmonary disease: the role of chemosensitivity and dead space. *Eur Respir J* 2018; **51**. DOI:10.1183/13993003.00860-2017.
- 6 Yasunobu Y, Oudiz RJ, Sun X-G, Hansen JE, Wasserman K. End-tidal PCO₂ abnormality and exercise limitation in patients with primary pulmonary hypertension. *Chest* 2005; **127**: 1637–46.
- 7 Higashi A, Dohi Y, Yamabe S, *et al.* Evaluation of end-tidal CO₂ pressure at the anaerobic threshold for detecting and assessing pulmonary hypertension. *Heart Vessels* 2017; **32**: 1350–7.
- 8 Farina S, Bruno N, Agalbato C, *et al.* Physiological insights of exercise hyperventilation in arterial and chronic thromboembolic pulmonary hypertension. *Int J Cardiol* 2018; **259**: 178–82.
- 9 Galiè N, Humbert M, Vachiery J-L, *et al.* 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 Respir J* 2015; **46**: 903–75.
- 10 Herve P, Lau EM, Sitbon O, *et al.* Criteria for diagnosis of exercise pulmonary hypertension. *Eur Respir J* 2015; **46**: 728–37.
- 11 Kovacs G, Herve P, Barbera JA, *et al.* An official European Respiratory Society statement: pulmonary haemodynamics during exercise. *Eur Respir J* 2017; **50**. DOI:10.1183/13993003.00578-2017.
- 12 Simonneau G, Montani D, Celermajer DS, *et al.* Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2018; published online Dec 13. DOI:10.1183/13993003.01913-2018.