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 $4\times(TRV)^2 + RAP$.

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 ($V'O_2$), 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 ($P_{(a-ET)}CO_2$), alveolar-arterial oxygen difference ($P_{(A-a)}O_2$), physiologic dead space fraction (V_D/V_T); and two criteria at anaerobic threshold were minute ventilation to carbon dioxide production ratio ($V'E/VCO_2$) and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$).

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 S1. Investigational cardiopulmonary exercise testing probability score of pulmonary hypertension

Variables	Values	Score
$P_{(a-ET)}CO_2$ at peak	Negative value	0
	Positive value	2
$P_{(A-a)}O_2$ at peak	≤29 mmHg	0
	≥30 mmHg	2
V'_E/VCO_2 at AT	≤29	0
	30-34	1
	≥35	2
$P_{ET}CO_2$ 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

$P_{(a-ET)}CO_2$ = arterial-end-tidal PCO_2 difference; $P_{(A-a)}O_2$ = alveolar-arterial oxygen difference; $V'_E/V'CO_2$ = minute ventilation to carbon dioxide production ratio; AT = anaerobic threshold; $P_{ET}CO_2$ = end-tidal partial pressure of carbon dioxide; V_D/V_T = physiologic dead space fraction.

Supplemental Table S2. List of *BMPR2* mutations in the 55 asymptomatic *BMPR2* 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 S3. Characteristics of *BMPR2* mutations carriers (n=55)

Demographic data	
Age, years	37·1 (18-78·1) [25·5-55·6]
Gender, M/F	26/29
Tobacco exposure >5p.y	19 (35%)
BMI, Kg/m²	22·5 (16·8-32. ·2) [21·9-26·0]
Systemic hypertension, n (%)	11 (20%)
Diabetes, n (%)	4 (7%)
Dyslipidemia, n (%)	3 (5%)
Functional parameters	
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]
Cardiopulmonary exercise testing	
V'O2 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]
VO2/HRF, % pred	88·5 (50-125) [78-100]
PaO2 at rest, mmHg	93 (70-118) [85-100]
PaO2 at peak, mmHg	106 (85-124) [97-114]
$P_{(A-a)}O_2$, mmHg	13·8 (-5·6-32·8) [7·8-20·0]
Echocardiography	
TRV, m/s	2 (1·5-2·75) [1·9-2·3] (n=30)
RA surface area, cm²	13 (7.7-27) [10·0-15·0]
LA Surface area, cm²	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]
Hemodynamics	
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]
Biologic tests	
Uricaemia, $\mu mol.L^{-1}$ (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; BMPR2: 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; $P_{(A-a)}O_2$: 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;* $V'O_2$*: oxygen consumption corrected for body weight; 6MWD: 6-minute walk distance; ; % pred : percentage of predicted values.*

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