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Right ventricular and pulmonary vascular reserve in asymptomatic *BMPR2* mutation carriers

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Short title: Exercise hemodynamics in *BMPR2* mutation carriers

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Key words

Cardiac magnetic resonance imaging – Right ventricular function – Pulmonary vascular function – *BMPR2* – Hypoxia – Exercise Physiology

Abstract

Background: Non-invasive estimates suggested that asymptomatic *BMPR2* mutation carriers may have an abnormal pulmonary vascular response to exercise and hypoxia. However, this has not been assessed with gold-standard invasive measures.

Methods: Eight controls and 8 asymptomatic *BMPR2* mutation carriers underwent cardiac magnetic resonance imaging with simultaneous invasive pressure recording during bicycle exercise in normoxia, hypoxia and following sildenafil administration. Abnormal pulmonary vascular reserve was defined as an increase in mean pulmonary artery pressure relative to cardiac output (P/Q slope) >3 mmHg/L/min.

Results: During normoxic exercise, *BMPR2* mutation carriers had a similar P/Q slope compared to healthy subjects. Only, one out of 8 *BMPR2* mutation carriers had a P/Q slope >3 mmHg/L/min. During exercise in hypoxia, 3 out of 8 *BMPR2* mutation carriers had P/Q slopes >3 mmHg/L/min compared to none of the controls. Sildenafil decreased the P/Q slope both in controls and *BMPR2* mutation carriers. The exercise-induced increase in right ventricular ejection fraction was similar between groups. None of the *BMPR2* mutation carriers developed PAH within 2(1.3–2.8) years.

Conclusions: The presence of a BMPR2 mutation per se is not associated with an abnormal pulmonary vascular and RV functional response to exercise in asymptomatic individuals. Longer follow-up will be required to determine whether a P/Q slope > 3 mmHg/L/min during exercise in normoxia or hypoxia is a sign of pre-clinical disease expression.

Introduction

The heritable form of pulmonary arterial hypertension (PAH) has been associated with mutations in the Bone Morphogenetic Protein Receptor II (*BMPR2*) gene. The presence of a *BMPR2* mutation is associated with younger age and more progressive disease at diagnosis (1). Often, these patients are diagnosed when pulmonary artery pressure (PAP) is already markedly elevated at rest and cardiac output (Q) is reduced due to right ventricular (RV) dysfunction (2). It is likely that this advanced stage of the disease is preceded by a pre-clinical phase with an asymptomatic increase in pulmonary arterial constriction and remodeling over several years.

Previously, Grunig et al. showed that an increased proportion of asymptomatic family members of patients with idiopathic PAH have a hypertensive PAP response to exercise or to low-oxygen breathing, suggesting that exercise measures may enable us to identify those subjects at risk of developing PAH in an early phase (3, 4). However, assessment of pulmonary vascular function was determined by a single Doppler echocardiographic estimate of systolic PAP. Given that PAP is expected to increase in all subjects during exercise, recent consensus documents highlighted the importance of expressing PAP during exercise relative to Q to more accurately identify pulmonary vascular pathology (5, 6). Following this rationale, Pavelescu and co-workers used stress echocardiography to assess pressure-flow relationships during exercise and hypoxia in *BMPR2* mutation carriers (7). However, until present, the same approach has not been tested with gold-standard invasive measures. Furthermore, pre-clinical data suggested that *BMPR2* mutations may induce abnormalities in RV structure and function prior to development of PAH (8), but this is yet to be assessed in humans.

We developed a methodology which combines invasive PAP measures with gold-standard cardiac magnetic resonance (CMR) quantification of RV function and Q during exercise and have used this technique to identify sub-clinical pulmonary vascular dysfunction and RV dysfunction (9, 10). Thus, this technique is ideally suited to assess our hypothesis that abnormal pulmonary vascular and RV function would be demonstrable during exercise in asymptomatic subjects with a known *BMPR2* mutation.

Methods

Subjects

Between 2012 and 2014, all consecutive *BMPR2* mutation carriers assessed at our institute who were asymptomatic and had normal resting mean pulmonary artery pressures (mPAP <25 mmHg) were invited to participate in this study. Eleven subjects fulfilled the inclusion criteria and 8 accepted to participate.

Eight control subjects volunteered to participate after responding to local advertisements. All subjects were (1) healthy, (2) had no history of cardiovascular disease, symptoms or risk factors, and (3) had a normal ECG and transthoracic echocardiogram.

The study protocol conformed to the Declaration of Helsinki and was approved by the ethical committee of UZ Leuven (B322201214035). All patients provided informed consent.

Exercise CMR

Firstly, all subjects underwent placement of a pulmonary artery catheter via the internal jugular vein and a systemic arterial catheter via the radial artery. In the CMR suite, these catheters were attached to CMR-compatible pressure transducers that were connected to a PowerLab recording system (AD Instruments, Oxford, United Kingdom).

Subsequently, biventricular volumes were measured during supine cycling exercise using a real-time CMR method with simultaneous invasive pressure measurement that we previously described in detail and have validated against invasive standards (Figure 1).(9) In brief, exercise CMR was performed during normoxia at rest and at 25%, 50% and 66% of maximal exercise power, determined by cardiopulmonary exercise testing (CPET) on the previous day (9). After 15 minutes of recovery, the same exercise measurements were repeated at rest, at 25% and 50% of maximal exercise power whilst subjects were breathing a hypoxic gas mixture (12% oxygen and 88% nitrogen). Finally, subjects took a single oral dose of sildenafil 50 mg and were allowed to rest for 30-60 minutes. Exercise evaluation was then repeated at the same workloads as during normoxia. Pulmonary and systemic arterial

pressures were continuously recorded throughout exercise and analyzed off-line using LabChart v6.1.1 (AD Instruments).

Using an in-house developed software program (RightVol, Leuven, Belgium), end-diastolic and end-systolic volumes were calculated by a summation of disks. From these measurements, stroke volume (SV), Q and ejection fraction (EF) were inferred. The RV end-systolic pressure-volume relationship (RVESPVR), a surrogate of RV contractility, was calculated as mPAP divided by RVESV (2). Pulmonary arterial compliance (C_{PA}) was calculated as the ratio of SV to pulmonary arterial pulse pressure. The relationship between mPAP and Q (subsequently referred to as 'P/Q slope') was determined using linear regression analysis (11). Abnormal pulmonary vascular reserve was defined as a P/Q slope >3.0 mmHg.min.L^{-1} (5), whereas abnormal RV contractile reserve was defined as a ratio of peak-exercise to resting RVESPVR (subsequently referred to as 'RVESPVR ratio') of <2.(12) The distensibility coefficient α was calculated using the equation $mPAP = [((1+\alpha \times PCWP)^5 + 5\alpha R_0 Q)^{1/5} - 1]/\alpha$, where R0 is tPVR at rest and PCWP is pulmonary capillary wedge pressure, which is assumed to be unchanged from the resting state, as previously described (7, 13). Because no PCWP measurements were obtained in control subjects, α was only calculated in the *BMPR2* group.

Blood samples

During normoxia, hypoxia and after sildenafil, arterial and central venous blood samples were collected at rest and at peak exercise and analyzed using an automated blood gas analyzer (ABL 700, Radiometer; Copenhagen, Denmark).

Statistics

Data were analysed using IBM SPSS statistics 22 software. Descriptive data for continuous variables are presented as means±SD or as medians (25% and 75% percentile) when appropriate. Categorical data were compared using the Fisher's exact test. Comparisons between groups for continuous variables were performed using unpaired 2-sample *t* tests or the Mann-Whitney test, as appropriate. A Mixed Linear Model with compound symmetric covariance matrix and the Bonferroni post-hoc test

for multiple comparisons was performed to evaluate the RV functional response to exercise within and between groups. A p-value <0.05 was considered significant.

To determine the sample sizes, the following estimates were used: in a previous study using exercise CMR with invasive pressure measures we demonstrated that healthy subjects had a P/Q slope of 1.1±0.6 L/min. According to our hypothesis, we predicted a P/Q slope of 2.0 L/min in the *BMPR2* mutation carriers. This is a rather conservative estimate compared with the proposed cut-off value of >3 mmHg/L/min for the detection of exercise-induced pulmonary hypertension. Using these assumptions, a sample size of n = 7 was calculated to provide 80% power in detecting abnormal pulmonary vascular reserve in the *BMPR2* mutation carriers (α = 5%, 1-β = 80%, n = 7). A p-value <0.05 was considered statistically significant.

Results

Baseline characteristics

The baseline characteristics of the study population are depicted in Table 1. Controls and *BMPR2* mutation carriers were of similar age and gender. All subjects were asymptomatic and none was receiving negative chronotropic medications or pulmonary vasodilator therapy. Exercise capacity in the *BMPR2* mutation carriers was reduced as reflected by their low peak VO_2 (88±24% of predicted for age and gender) and tended to be lower than in the controls (Table 1). None of the study participants had resting mPAP >25 mmHg.

Blood results

Blood gas changes from rest to peak exercise in normoxia, hypoxia and after sildenafil administration are depicted in Table 2. No different patterns were observed between controls and *BMPR2* mutation carriers.

Pulmonary vascular and right ventricular reserve

During normoxic exercise, *BMPR2* mutation carriers had a similar increase in mPAP per liter of increment in Q than controls (P=0.196; Figure 2). One *BMPR2* carrier had abnormal pulmonary vascular reserve, defined as a P/Q slope >3 mmHg/L/min, compared to none of the controls. Whilst resting C_{PA} was lower in *BMPR2* mutation carriers than controls, no difference was found at peak exercise (Table 3). There was no difference in the RV functional response to exercise between both groups. Both the exercise-induced increase in RVEF (Figure 3) and the RVESPVR ratio from rest to peak exercise were similar (Figure 2).

At rest, mPAP tended to increase during hypoxia in both controls (+4±5 mmHg, P=0.073) and *BMPR2* mutation carriers (+3±4 mmHg, p=0.098). The P/Q slope was similar to normoxic conditions in both groups (Figure 3). Three *BMPR2* mutation carriers had a P/Q slope >3 mmHg/L/min whilst none of the controls reached this threshold (P=0.20). All *BMPR2* mutation carriers with a P/Q slope >3 mmHg/L/min during normoxic or hypoxic exercise were of female gender. Given that only 2 stages of

exercise were performed in hypoxia the RVESPVR ratio was lower compared to normoxic exercise (P<0.0001). However, the RVESPVR ratio during hypoxic exercise was similar in *BMPR2* mutation carriers and controls (Figures 2 and 3). The exercise-induced increase in RVEF was lower in the *BMPR2* mutation carriers, but this was explained by a slightly higher resting RVEF, whereas peak exercise RVEF was similar (Figure 3).

Sildenafil decreased the P/Q slope in controls (-0.38 mmHg/L/min; P=0.029), whereas the change was not significant in *BMPR2* mutation carriers (-0.26 mmHg/L/min; P=0.271). However, none of the *BMPR2* mutation carriers fulfilled criteria of abnormal pulmonary vascular reserve after sildenafil. As depicted in Figures 2 and 3, sildenafil did not alter RV contractile reserve. Also, peak exercise cardiac index was similar during normoxia and post-sildenafil (Table 3).

Within the group of *BMPR2* mutation carriers, the distensibility coefficient α was lower in those subjects with an abnormal P/Q slope to exercise in normoxia or hypoxia compared to those subjects with a normal P/Q slope (Table 4). In contrast, there was no difference in the resting-to-peak exercise RVESPVR ratio between both subgroups.

Clinical follow-up of *BMPR2* mutation carriers

All *BMPR2* mutation carriers were followed for a period of 2 (1.3 – 2.8) years. During follow-up, none of these subjects developed symptoms or clinical signs suggestive of PAH. There were no changes in any of the CPET parameters (Table 5). There was no difference in outcome measures between those subjects with a P/Q slope >3 mmHg/L/min to exercise in normoxia or hypoxia and subjects with a P/Q slope <3 mmHg/L/min.

Discussion

Using contemporary invasive pressure and CMR-derived volume measurements with improved sensitivity to test our hypothesis of an association between *BMPR2* mutation status and sub-clinical pulmonary vascular and RV dysfunction, we found that only a minority of mutation carriers had hemodynamic responses to exercise that might be considered abnormal. Thus, the presence of a mutation in the BMPR2 gene by itself is not sufficient to develop pulmonary vascular disease. During hypoxic exercise, 3 out of 8 *BMPR2* mutation carriers had abnormal pulmonary vascular reserve (as defined by a >3mmHg/L/min criterion) as opposed to none of the controls, which may suggest that the *BMPR2* mutation increases susceptibility to hypoxic pulmonary vasoconstriction. During short term follow-up, none of the *BMPR2* mutation carriers developed clinical PAH.

Pressure/flow relationships in *BMPR2* mutation carriers

It is well established that late diagnosis and initiation of treatment in PAH is associated with hemodynamic impairment and worse survival (14). *BMPR2* mutations are the most common cause of familial PAH, accounting for approximately 75% of heritable cases (15). However, not all carriers of a *BMPR2* mutation eventually develop PAH with an overall penetrance estimated at 27% (16). Previous studies by Grunig et al. suggested that evaluation of the pulmonary vascular response to exercise may be useful to identify those subjects at risk in an early phase of the disease but this echocardiographic study relied on a single PAP estimate without reference to Q (3).

The use of multipoint exercise measurements of PAP with simultaneous reference to Q has significant advantages over single peak exercise measures. Firstly, imaging constraints are greatest at peak exercise, which is particularly true for echocardiography (12). Furthermore, due to the near linear relationship between mPAP and Q, healthy individuals frequently exceed the proposed cut-off value of mPAP >30 mmHg during exercise, especially trained athletes and those aged >50 years (17-19). To account for the flow-dependent characteristics of the pulmonary circulation, previous investigators used echocardiography to assess mPAP/Q relationships in *BMPR2* mutation carriers and controls (7). The same approach, however, has not been performed using invasive techniques in *BMPR2* mutation

carriers. In the current study, using simultaneous invasive pressure and CMR-derived Q measurements we confirm previous data that only a minority of *BMPR2* mutation carriers have an abnormal P/Q slope during exercise. Similarly, the pulmonary arterial distensibility coefficient α was calculated at 2% change in diameter per mmHg transmural pressure in the *BMPR2* group, which is very similar to previously reported values in healthy individuals (13, 20). These results suggest that the *BMPR2* mutation does not cause an abnormal pulmonary vascular response in its own right. Therefore, it is likely that additional mechanisms or factors are necessary to develop PAH, i.e. a 'second hit phenomenon,' as suggested by previous investigators (3, 4).

We also tested the hypothesis that *BMPR2* mutation carriers have an abnormal pulmonary vascular response to exercise in hypoxia. Previous studies showed that *BMPR2* mutation carriers more frequently have a hypertensive pulmonary vascular response to prolonged hypoxia in the rested state than controls (3, 4). However, the hemodynamic response to hypoxia in *BMPR2* mutation carriers has only been assessed non-invasively using echocardiography and only in the resting state (3, 4). Although we did not find a statistical difference in the response to hypoxia between BMPR2 mutation carriers and controls, 3 out of 8 *BMPR2* mutation carriers had a P/Q slope >3 mmHg/L/min to hypoxic exercise, which may hint that the *BMPR2* mutation increases the susceptibility to hypoxic pulmonary vasoconstriction (4, 7).

Right ventricular functional reserve

As an important additional finding in this study, we found that *BMPR2* mutation carriers had preserved RV contractile reserve. The latter is clinically important as previous animal data showed that mutant *BMPR2* expression is associated with intramyocardial lipid deposition in the RV, even in the absence of increased afterload (8). To the best of our knowledge, however, no previous studies have evaluated RV reserve in human carriers of a *BMPR2* mutation. This is at least partly to the technical challenges in assessing the RV during exercise. In a manner akin to pressure-volume analysis, we used a hybrid technique combining invasive PAP and CMR-derived volume measurements to determine the ratio of resting to peak exercise RV ESPVR to detect sub-clinical RV dysfunction (10). Using this

approach, we demonstrate that RV functional reserve is not different in *BMPR2* mutation carriers compared to controls, even in those subjects with an abnormal pulmonary vascular response to exercise and/or hypoxia.

Clinical implications

The results of this study are clinically relevant for the evaluation and follow-up of subjects with a *BMPR2* mutation. Using a combined approach of invasive evaluation of pulmonary vascular function and RV contractile reserve wither higher sensitivity and accuracy to detect latent pulmonary vascular disease compared with echocardiography, our data provide robust evidence that the presence of a *BMPR2* mutation by itself does not cause the disease. This is in keeping with longitudinal data showing that only 27% of *BMPR2* mutation carriers eventually develop PAH (16). In other words, the finding of exercise-induced pulmonary hypertension as defined by a P/Q slope >3 mmHg/L/min is not a baseline feature of *BMPR2* mutation carriers and hence may require closer follow-up to detect clinical disease in an early phase. As exercise-induced pulmonary hypertension typically occurs in the context of latent or early disease, usually there is a preference for non-invasive risk stratification. On the other hand, *BMPR2* mutation carriers developing PAH often have a more aggressive disease, as characterized by younger age and worse hemodynamics at diagnosis, and shorter time to death or transplant (21). Therefore, the use of invasive assessment may be defendable in these subjects as early identification of disease is clinically important and may lead to better long-term outcomes (14, 22). In this study, we showed that none of those subjects with a P/Q relationship >3 mmHg/L/min during normoxia or hypoxia developed signs or symptoms of pulmonary vascular disease within 2 (1.3 – 2.8) years. However, further studies using similar methodology in a larger cohort with longer follow-up are required to more definitively assess the implications of an increased P/Q ratio.

Limitations

The small sample size was an expected limitation of this study given that PAH is a rare disease and familial disease represents only a small proportion of cases (4). This is the largest series to include invasive pressure measurements in healthy subjects and in asymptomatic *BMPR2* mutation carriers.

The small sample size may have increased the probability of type II statistical errors due to lack of power, whereas the use of multiple comparisons increased the chances of type I errors. Nevertheless, the established accuracy of invasive pressure and CMR-derived volume measurements provided sufficient sensitivity to detect meaningful hemodynamic differences even within this modest-sized cohort. Although the difference in gender was not statistically significant, the *BMPR2* group consisted mainly of women, whereas the controls were predominantly men. A previous study using exercise echocardiography showed that P/Q slopes are similar in men and women (23). Furthermore, a recent report by the French PAH network showed that gender did not have a significant effect on age at diagnosis and outcome amongst *BMPR2* mutation carriers (1). Our cohorts are not of sufficient size to support or refute any gender imbalance in pulmonary vascular pathophysiology. Because of the very small but potentially serious risk for adverse events in performing PCWP measurements, no PCWP measurements were obtained in controls. As a consequence, the distensibility coefficient α could only be calculated in the *BMPR2* mutation carriers but not in the controls. Moreover, in the *BMPR2* mutation carriers PCWP was not measured during exercise, but assumed constant, in keeping with previous invasive estimates in normal subjects at exercise showing that increases in PCWP only become significant at very high COs (24). Finally, the cutoff value of a slope of P/Q plots >3 mmHg/L/min for the definition of exercise-induced pulmonary hypertension has only been derived from exercise in normoxia, but not in hypoxia. Further studies in a large population of healthy individuals are necessary to assess whether this value can also applied as abnormal for exercise in hypoxia, and whether it is of prognostic relevance.

Conclusions

The presence of a BMPR2 mutation per se is not associated with an abnormal pulmonary vascular and RV functional response to exercise in asymptomatic individuals. Longer follow-up will be required to determine whether a P/Q slope > 3 mmHg/L/min during exercise in normoxia or hypoxia is a sign of pre-clinical disease expression.

Conflicts of interest

GC: I hereby declare that I have no conflict of interest

ALG: I hereby declare that I have no conflict of interest

TP: I hereby declare that I have no conflict of interest

HG: I hereby declare that I have no conflict of interest

JB: I hereby declare that I have no conflict of interest

MC: I hereby declare that I have no conflict of interest

SD: I hereby declare that I have no conflict of interest

PC: I hereby declare that I have no conflict of interest

MD served as investigator, speaker, consultant, or steering committee member for Actelion, Bayer, Eli Lilly, GlaxoSmithKline, Pfizer and United Therapeutics; and received research grants from Actelion, GlaxoSmithKline and Pfizer.

HH is on the Speakers' Bureau and Advisory Board of Bayer, Boehringer-Ingelheim, Daiichi Sankyo, Bristol-Myers Squibb, Pfizer, Merck, Biotronik, and St. Jude Medical.

Figure legends

Figure 1. Illustration of the **(A)** study workflow, data acquisition and analysis. **(B, C)** A subject is lined up just before the cardiac magnetic resonance imaging bore whilst breathing through a mouthpiece (arrow) connected to a Douglas bag (asterisk) containing a hypoxic gas mixture. Imaging is performed during exercise within the CMR bore whilst invasive pressure monitoring is performed simultaneously. **(D)** A representative mid-ventricular slice obtained during peak-intensity exercise. **(E)** Three-dimensional volume stack generated by the software after manual delineation of left and right ventricular endocardial borders at end-diastole (upper panels) and end-systole (bottom). CPET, cardiopulmonary exercise testing; ExCMRip, Exercise cardiac magnetic resonance imaging with invasive pressure monitoring.

Figure 2. Pulmonary vascular and right ventricular reserve in controls and *BMPR2* mutation carriers**.** Black squares (controls) and red triangles (*BMPR2* mutation carriers) denote individual values during normoxia, hypoxia and post-sildenafil. Dotted lines indicate thresholds for abnormal pulmonary vascular (P/Q slope >3 mmHg.L.min^{-1}) and right ventricular (RVESPVR ratio <2) reserve. P values are shown for comparison of P/Q slopes (left panels) and RVESPVR ratios (right panels) between controls and *BMPR2* mutation carriers.

Figure 3. Changes in right ventricular ejection fraction in normoxia, hypoxia and following sildenafil**.** RVEF, right ventricular ejection fraction; CI, cardiac index. P values are shown for comparison of ΔRVEF between controls and *BMPR2* mutation carriers.

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Table 1. Baseline characteristics

	Healthy controls (n=8)	***BMPR2* mutation carriers (n=8)**	**P-value**
Clinical			
Age, y	40±14	42±16	0.765
BSA, m^2	2.00±0.13	1.71±0.20	0.004
BMI, kg/m^2	25.8±4.6	24.1±5.7	0.526
Sex, M(F)	7(1)	3(5)	0.119
Cardiopulmonary exercise testing			
VO_2 peak, ml/kg/min	35.2±8.8	28.0±6.4	0.089
VO_2, % predicted	108±18%	91±24%	0.148
Peak HR, bpm	177±9	160±22	0.074
Peak Power, W	248±38	133±29	<0.0001
VE/VCO_2	25±4	31±8	0.070
Right heart catheterization			
Heart rate, bpm	65±5	71±16	0.379
mPAP, mmHg	16±2	12±2	0.004
PCWP, mmHg	NA	10±3	
CI, $L/min/m^2$	3.5±0.7	2.8±0.7	0.066
PVR, dynes	NA	89±24	
tPVR, dynes	187±54	192±34	0.845
C_{PA}, ml/mmHg	9.7±3.7	6.3±1.8	0.040

BSA, body surface area; BMI, body mass index; VO_2 peak, peak oxygen consumption; bpm, beats per minute; W, watts; VE/VCO2, minute ventilation-carbon dioxide production relationship; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CI, cardiac index; PVR, pulmonary vascular resistance; tPVR, total pulmonary vascular resistance; C_{PA}, pulmonary arterial compliance

Table 2. Pooled blood gas data of all subjects

		Normoxia	Hypoxia	Sildenafil	P-value
pHa	Rest	7.44±0.04	7.45±0.05	7.41±0.02*	0.016
	Peak ex	7.38±0.02†	7.45±0.04*	7.40±0.04†	<0.0001
PaO2 (mmHg)	Rest	105±19	55±8*	88±13*	<0.0001
	Peak ex	96±13	39±7*†	100±12†	<0.0001
PaCO2 (mmHg)	Rest	36±5	31±5*	37±3	<0.0001
	Peak ex	37±4	30±5*	34±4*†	<0.0001
SaO2 (%)	Rest	98.5±1.2	90.7±4*	97.0±22.2*	<0.0001
	Peak ex	97.8±1.5†	78.0±6.8*†	98.0±1.2†	<0.0001
PcvO2 (mmHg)	Rest	38±4	36±7	39±3	0.138
	Peak ex	31±5†	24±5*†	28±4†	<0.0001
ScvO2 (%)	Rest	74.1±3.3	71.0±6.3	76.0±3.7	0.034
	Peak ex	53.8±11.2†	44.5±12.5*†	49.8±13.2†	0.005
Lactate (mmol/L)	Rest	1.0±0.4	3.6±1.7*	2.2±1.2*	
	Peak ex	4.7±1.9†	5.0±2.1†	5.4 ±2.7†	
$C(a\text{-}cv)O_2$, ml O_2/100 mL	Rest	4.8±0.7	3.8±1.2*	4.2±0.6*	0.018
	Peak ex	9.2±2.2†	6.7±2.3*†	9.9±2.8†	<0.0001

pHa, arterial pH; PaO_2, arterial oxygen tension; $PcvO_2$, central venous oxygen tension; SaO_2, arterial oxygen saturation; $ScvO_2$, central venous oxygen saturation; $C(a\text{-}cv)O_2$, arterial-central venous differences in oxygen content.

P-values from repeated measures ANOVA for difference between normoxia, hypoxia and sildenafil and between rest and peak exercise; *P<0.05 for difference vs. normoxia; † P<0.05 for difference vs. rest

Table 3. Exercise CMR with invasive pressure monitoring data

		REST		PEAK EXERCISE	
		Controls	***BMPR2***	**Controls**	***BMPR2***
HR, bpm	Normoxia	65±5	71±16	149±11	142±13
	Hypoxia	93±7	92±11	150±15	141±14
	Sildenafil	85±8	81±17	160±15	148±16
LVEF, %	Normoxia	60.1±6.3	65.4±9.2	70.7±4.0	69.5±7.8
	Hypoxia	66.4±4.2	70.2±8.2	76.0±4.4	72.1±11.1
	Sildenafil	65.5±6.9	67.6±6.9	73.4±3.5	72.5±8.2
RVEF, %	Normoxia	59.0±6.0	63.2±6.7	72.2±4.9	74.3±5.9
	Hypoxia	60.4±8.5	67.2±10.3	72.0±5.5	72.2±10.1
	Sildenafil	62.7±4.8	65.9±7.9	75.3±6.1	77.4±6.2
CI, L/min/m^2	Normoxia	3.5±0.7	2.8±0.7	9.0±2.2	6.2±0.8*
	Hypoxia	4.8±1.0	3.6±0.4*	9.4±2.1	5.9±0.7*
	Sildenafil	4.4±1.0	3.1±0.5*	9.2±1.9	6.0±1.0*
mPAP, mmHg	Normoxia	16±2	12±2*	30±6	22±6*
	Hypoxia	19±3	14±5*	31±4	23±6*
	Sildenafil	13±3	11±3	22±4	19±6
tPVR/SaO_2, Wood units.$\%^{-1}$	Normoxia	0.018±0.007	0.019±0.006	0.016±0.007	0.018±0.006
	Hypoxia	0.017±0.004	0.019±0.007	0.020±0.006	0.025±0.008
	Sildenafil	0.010±0.005	0.013±0.004	0.010±0.004	0.016±0.006*
C_{PA}, ml.$mmHg^{-1}$	Normoxia	10.3±2.6	7.2±2.8*	5.1±1.9	4.7±2.5
	Hypoxia	8.4±2.0	5.4±2.4*	5.0±2.1	3.4±0.9
	Sildenafil	10.1±3.3	8.4±3.3	5.4±2.4	5.1±2.5

HR, heart rate; LVEF, left ventricular ejection fraction; RVEF, right ventricular ejection fraction; CI, cardiac index; mPAP, mean pulmonary artery pressure; tPVR, total pulmonary vascular resistance; SaO_2: arterial oxygen saturation; C_{PA}, pulmonary arterial compliance

Table 4. Pulmonary vascular distensibility and RV reserve in *BMPR2* mutation carriers

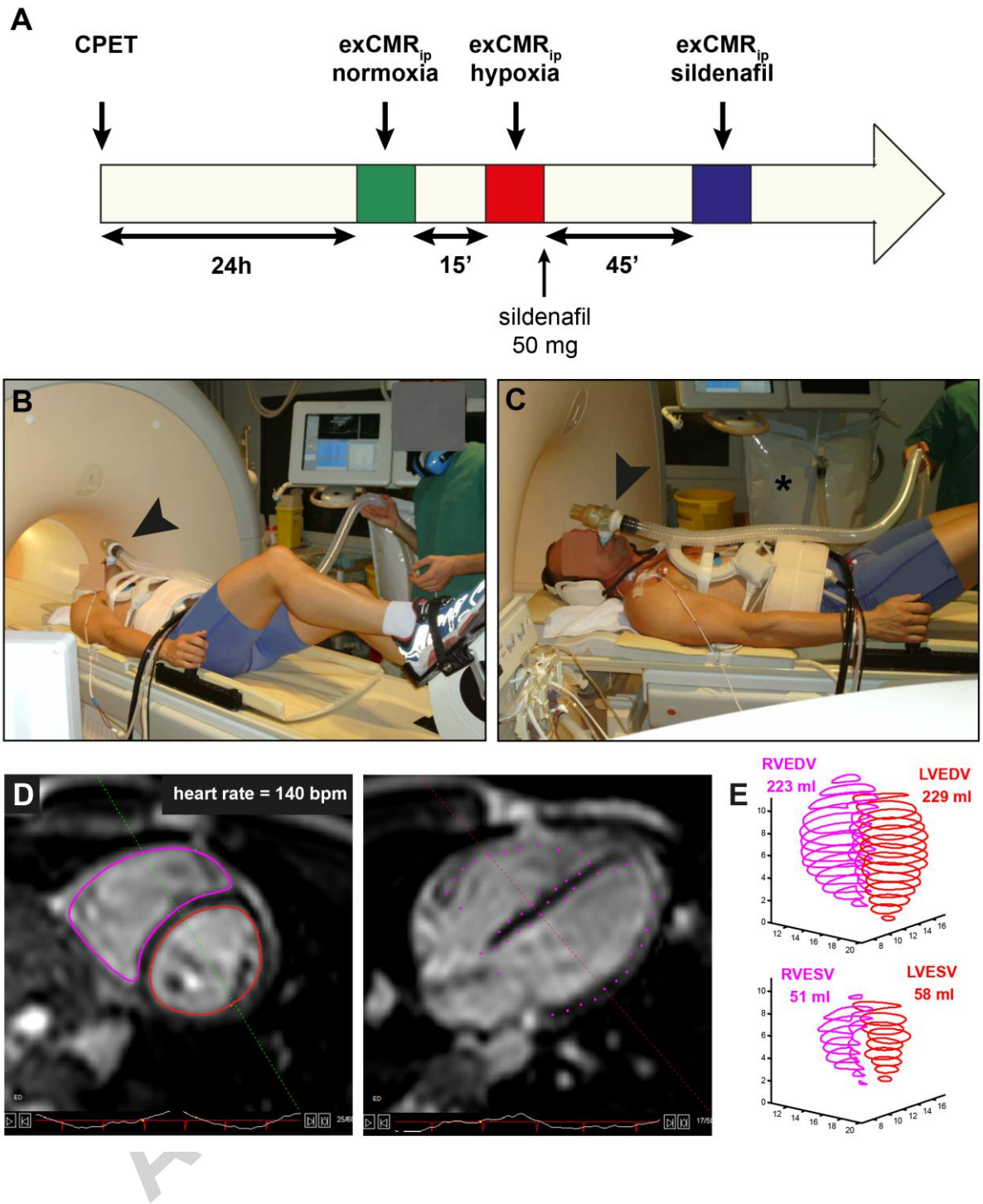
		***BMPR2* mutation carriers**			
		All subjects (n=8)	**P/Q slope < 3 mmHg/L/min to exercise or hypoxia (n=5)**	**P/Q slope > 3 mmHg/L/min to exercise or hypoxia (n=3)**	**P-value**
α, $\%.mmHg^{-1}$	Normoxia	2.0±1.8	2.9±1.7	0.5±0.4	0.053
	Hypoxia	1.5±1.0	2.2±0.7	0.6±0.2	0.013
	Sildenafil	2.9±2.5	4.1±2.5	1.0±0.4	0.050
RVESPVR ratio	Normoxia	3.6±0.9	3.7±1.0	3.4±0.8	0.728
	Hypoxia	2.2±0.6	2.1±0.4	2.3±0.9	0.863
	Sildenafil	4.3±1.6	4.4±1.9	4.0±1.2	0.749

α, distensibility coefficient; RVESPVR ratio, resting to peak exercise right ventricular end-systolic pressure-volume ratio

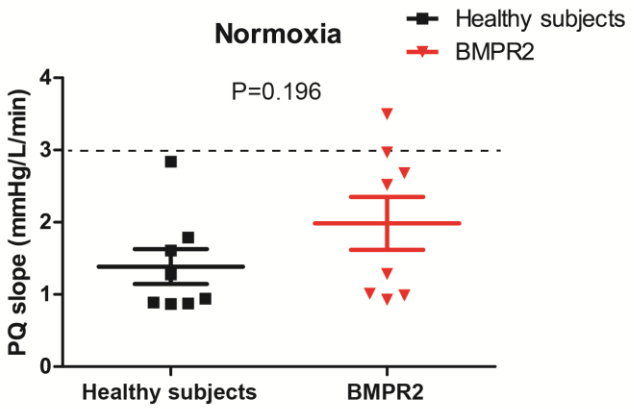
Table 5. Follow-up data of *BMPR2* mutation carriers

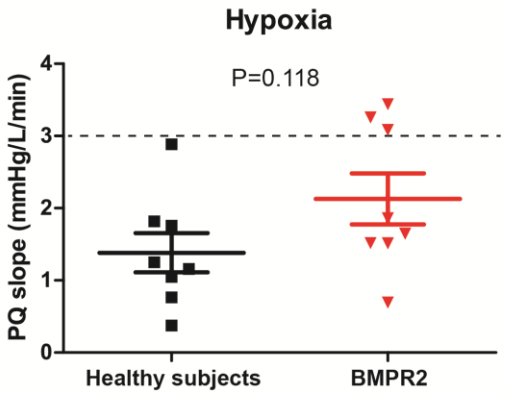
	Baseline (n=8)	Follow-up (n=8)	P-value
Clinical			
Age, y	42±16	44±15	<0.0001
BSA, m^2	1.71±.20	1.72±.20	0.434
BMI, kg/m^2	24.1±5.7	24.5±5.6	0.456
NYHA			
I	8	8	1.0
II	0	0	1.0
III	0	0	1.0
IV	0	0	1.0
Cardiopulmonary exercise testing			
VO_2 peak, ml. min^{-1}. kg^{-1}	28.0±6.9	26.3±6.2	0.248
Peak heart rate, bpm	160±22	158±15	0.564
Peak Power, W	133±29	129±30	0.180
VE/VCO_2 slope	31±8	31±7	0.920
Vd/Vt at peak exercise, %	17.4±1.9	17.6±1.3	0.802
O_2/HR at peak exercise, ml	11.1±1.9	10.7±1.7	0.419

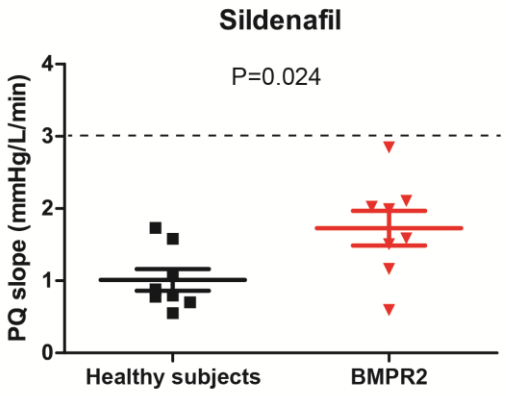
BSA, body surface area; BMI, body mass index; VO_2 peak, peak oxygen consumption; bpm, beats per minute; W, watts; VE/VCO2, minute ventilation-carbon dioxide production relationship; Vd/Vt, ratio of dead-space to tidal volume; O2/HR, oxygen pulse



Pulmonary vascular reserve







Right ventricular reserve