Case Report

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Pulmonary arterial hypertension-associated genetic variants in combined post-capillary and pre-capillary pulmonary hypertension: a case report

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Abstract

Predisposing factors for the development of a pre-capillary component in pulmonary hypertension associated with left heart disease remain elusive. We report the case of a patient with persistent combined post-capillary and pre-capillary pulmonary hypertension after cardiac transplantation, in whom a rare *BMPR2* variant was found.

Keywords

pulmonary circulation, pulmonary arterial hypertension, heart failure, risk factors, mutations

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Pulmonary hypertension (PH) is common in left heart diseases (LHD-post-capillary PH) and negatively affects outcome.[1,2] The trigger of PH-LHD is the passive increase in left-sided filling pressures.[2] However, a small subset of patients may present with significant pulmonary vascular disease defined as combined post-capillary and pre-capillary PH (CpcPH).[1,2] The interindividual variability of this superimposed pre-capillary component suggests the presence of risk or protective factors, environmental and/or constitutive.[3] Although genetic factors have been identified in pulmonary arterial hypertension (PAH) as well as in some other forms of PH, genetic data in PH-LHD are scarce.[3,4] One study uncovered the presence of 75 variants shared between PAH and CpcPH patients which are present in genes highly expressed in the lungs; however, there is no data on variants in PAH-associated genes in the subgroup of CpcPH patients.[5] We performed targeted gene panel sequencing in a small CpcPH patient cohort and report the case of a patient presenting with persistent CpcPH after cardiac transplantation, in whom a rare *BMPR2* variant of unknown significance (VUS) was found.

Case description

A 64-year-old patient presented with heart failure with reduced ejection fraction and was diagnosed with nonischemic and nonvalvular dilated cardiomyopathy. He was of Northeast African origin and we had no access to relatives. He underwent his first right heart catheterization at our institution during a scheduled hospitalization for heart transplant evaluation (Table 1). According to guidelines,[1] he was diagnosed with CpcPH based on an elevated pulmonary artery wedge pressure (PAWP >15 mmHg), associated with a pulmonary vascular resistance (PVR) >3 Wood units (WU) and a diastolic pulmonary gradient (DPG) ≥ 7 mmHg. He also presented with comorbidities including a metabolic syndrome and continuous positive airway pressure (CPAP)-treated obstructive sleep apnea. He had no history of smoking, no abnormalities on chest imaging,

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Table 1. Hemodynamic evolution and clinical characteristics.

Characteristics	1st RHC	2nd RHC[a]	3rd RHC[b]	RHC 1 year after LVAD	RHC 1 year after transplant	RHC 2 years after transplant	RHC 5 years after transplant
mPAP, mmHg	60	34	34	39	30	57	41
PAWP, mmHg	36	19	20	20	18	20	16
DPG, mmHg	12	5	6	4	0	28	15
RAP, mmHg	18	13	10	15	9	16	10
PVR, WU	11.5	3.6	3.4	4.91	2.14	7.40	4.50
CI, l/min/m^{2c}	1.12	2.30	2.15	2.08	3.10	2.75	3.23
NTproBNP, pg/ml	13,809	NA	1696	NA	NA	807	NA
GFR, ml/min/1.73m^2	32	41	43	34	43	38	49
SaO_2, %	NA	NA	97	97	NA	97	98
SvO_2, %	45	NA	62	NA	NA	63	NA

RHC: right heart catheterization; LVAD: left ventricular assist device; mPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; DPG: diastolic pulmonary gradient; RAP: right atrial pressure; PVR: pulmonary vascular resistance; CI: cardiac index; NTproBNP: N-terminal pro B-type natriuretic peptide; GFR: glomerular filtration rate; SaO_2: arterial oxygen saturation; SvO_2: mixed venous oxygen saturation.
[a]RHC after dobutamine + IV diuretics.
[b]RHC during dobutamine infusion.
[c]Measured by thermodilution.

and a normal carbon monoxide diffusion coefficient. A ventilation/perfusion scintigraphy excluded pulmonary embolism. Based on these findings, group 3 or group 4 PH can be excluded.

His condition worsened until a left ventricular assist device (LVAD) was implanted as a bridge to transplantation. During surgery, he suffered from acute worsening of PH requiring treatment with inhaled nitric oxide. No PAH-approved pulmonary vasodilator was given, as per our standard procedures. One year later, the patient underwent heart transplantation. During the procedure, he suffered from acute right heart failure requiring temporary venoarterial extracorporeal membrane oxygenation. He received a standard immunosuppressive regimen consisting of prednisolone, mycophenolate mofetil, and ciclosporin. After transplantation, his condition greatly improved. However, a CpcPH presentation was observed two years after transplantation, which persists three years later. Of note, chronic treatment regimen remained unchanged. Repeated echocardiograms revealed a normal ejection fraction, no valvular disease, a dilatation of both atriums, and septal dyskinesia. There were no signs of allograft rejection on cardiac biopsies and no significant lesion on his coronary angiogram. There was also no new evidence of group 3 or 4 PH. The patient's hemodynamic evolution is shown in Table 1.

With the approval of our institutional ethics committee and the patient's written informed consent, we performed an in-house designed PAH gene panel consisting of eight genes (*BMPR2*, *BMPR1B*, *KCNK3*, *ENG*, *CAV1*, *SMAD 4*, *SMAD9*, *ACVRL1*). The targeted regions were covered at a mean read depth $\geq 30\times$ in 97% of the exons except for *ENG* exon 1, for which Sanger sequencing was performed. We also looked for copy number variants (CNVs) using multiplex ligation-dependent probe amplification for *BMPR2*, *ENG*, and *ACVRL1*. We filtered the data by selecting genetic variants in coding sequences with an allele frequency (AF) <1% in the reference population database gnomAD.[6] *In silico* analysis was performed using several prediction tools (summarized in Table 2). Variant annotation was achieved using the Highlander software (Helaers et al., under revision, https://sites.uclouvain.be/highlander/) or the VarSome database (https://varsome.com/),[7] both annotating variants using dbNSFP.[8]

Table 2. *In silico* prediction tools used for the analysis of the BMPR2 variant.

Pathogenicity scores		
Tools	Scores	Prediction
SIFT	0.096	Tolerated
PolyPhen-2	0.779	Possibly damaging
Mutation Taster	NA	Disease causing
FATHMM-XF	0.505	Damaging
LRT	0	Deleterious
DEOGEN2	0.4385	Tolerated
CADD	22	Pathogenic
REVEL	0.339	Benign
DANN	0.9816	Pathogenic

Pathogenicity cut-offs: SIFT < 0.05, PolyPhen-2 > 0.446 for possibly damaging and >0.908 for probably damaging, FATHMM-XF > 0.5, LRT < 0.05, DEOGEN > 0.5, CADD > 15, REVEL > 0.5, DANN > 0.5.

Using the above criteria, we found two nonsynonymous variants in *BMPR2* (c.1749C>A, p.Asn583Lys; rs755765731) and in *ENG* (c.1316A>C, p.Lys439Thr; rs368533266), both confirmed by Sanger sequencing. No CNVs were found.

The *ENG* missense variant c.1316A>C (p.Lys439Thr, exon 11) was not predicted to be deleterious by *in silico* analysis (SIFT, Mutation Taster, PolyPhen-2, and LRT).

It was also found in three other patients in our home database (comprising almost 13,000 cases and controls sequenced by gene panel or Mendeliome for various indications). In a study by Mallet et al.,[9] this variant had no effect on protein function or on its cellular localization. In this context, this variant was classified as "likely benign" based on current knowledge and according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[10]

The *BMPR2* missense c.1749C>A (p.Asn583Lys, exon 12) has been reported in gnomAD with an AF of 0.002% (https://gnomad.broadinstitute.org/variant/2-202555414-C-A?dataset=gnomad_r3), although not in individuals of African descent nor in a 12,000 African American genome database (unpublished data, shared by Professor Nathan O. Stitziel, Washington University School of Medicine, St. Louis).[6] However, it was recently found in our home database in a 40-year-old Moroccan female presenting with an autoinflammatory disorder. This rare variant was not described in ClinVar. The variant is located in exon 12, an exon in which causative mutations have been described in PAH including a nonsense mutation in the adjacent amino acid residue (c.1750C>T, p.Arg584Ter).[11] *BMPR2* is intolerant to loss-of-function variants with a pLI of 1 and has an observed/expected (O/E) ratio of 0.76 for missense variants.[6] Tools used for *in silico* prediction are summarized in Table 2. Of note, in a cohort of 49 chronic thromboembolic pulmonary hypertension (CTEPH) cases, 7 exonic *BMPR2* missense variants were found, 6 of them occurring in exon 12.[12] None of these were reported in ClinVar, and four were predicted as probably damaging by PolyPhen-2.[12] According to ACMG guidelines,[10] we classified the *BMPR2* variant we found as a rare VUS due to the absence of strong pathogenic or benign criteria and due to conflicting results of *in silico* predictions.

In the same study, we also recruited all the other CpcPH patients presenting with a DPG $\geq$7 mmHg and a PVR > 3 WU, because this group of patients was shown to present with more severe right heart dysfunction based on previous work by our team.[13] The patients' clinical and hemodynamic characteristics are listed in Table 3. None of them had a positive family history. No variants were found among the five available patients after filtering using the criteria described above.

Discussion

The PH-LHD patient population is heterogeneous, and the factors leading to the development of a pre-capillary component are incompletely understood. The hemodynamic definition of the pre-capillary component in PH-LHD has been debated over time.[2] In the current PH guidelines, CpcPH is defined by a DPG $\geq$7 mmHg and/or a PVR > 3 WU.[1] However, our group recently showed that patients with high DPG *and* PVR presented with more right heart dysfunction compared to DPG *or* PVR.[13] In addition, the first group presented with hemodynamic disturbances closer to PAH.[13] According to these results, we selected CpcPH patients presenting with an elevation of both variables, speculating that these patients might present with more pulmonary vascular remodeling and might be more likely to have an underlying genetic predisposition.

It is now well known that a genetic predisposition underlies some forms of PAH. The transforming growth factor-β superfamily signaling pathway is the main pathway implicated in PAH and *BMPR2* mutations are the most frequently found.[4] So far, the genetics of other forms of PH have not been thoroughly studied and very few genetic studies have implicated PH-LHD patients.[3,5] One study searched for exonic single nucleotide polymorphisms shared between PAH and CpcPH patients using exome-based genotyping arrays.[5] The authors reported 75 shared variants in genes highly expressed in the lungs.[5] However, there are no

Table 3. Characteristics of the other CpcPH patients who underwent genetic testing.

Characteristics	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age, years	70.2	42.1	76.6	63.3	55.6
Gender[a]	F	M	F	F	M
Etiology	HFpEF	HFrEF	HFpEF	Valvular	HFrEF
mPAP, mmHg	33	35	51	59	43
PAWP, mmHg	16	17	19	29	22
DPG, mmHg	7	7	11	13	8
RAP, mmHg	12	12	13	15	7
PVR, WU	3.5	4.1	5.9	12.3	5.2
CI, l/min/m^2	2.9	2.42	2.9	1.5	1.77
NTproBNP, pg/ml	294	2949	615	407	595
GFR, ml/min/1.73m^2	88	51	84	57	95

HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; mPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; DPG: diastolic pulmonary gradient; RAP: right atrial pressure; PVR: pulmonary vascular resistance; CI: cardiac index; NTproBNP: N-terminal pro B-type natriuretic peptide; GFR: glomerular filtration rate.
[a]F: female; M: male.

reports on sequencing of PAH-associated genes in CpcPH patients.

There are rare reports of underlying mutations or polymorphisms in other forms of PH such as CTEPH.[3] In a cohort of 49 CTEPH cases, 7 exonic missense variants were found in *BMPR2* and most were in exon 12.[12] Of note, some patients presented with variants in more than one of the PAH-associated genes. In this same study, a significant enrichment of variants in PAH-associated genes was found in CTEPH patients compared to patients who presented pulmonary emboli without PH.[12]

To our knowledge, this is the first reported study of variants in known PAH-associated genes in a CpcPH patient cohort. We highlighted a rare *BMPR2* missense variant in one CpcPH patient who presented with a severe clinical course and with persistent CpcPH after heart transplantation. In this patient, inotropic support, volume depletion, LVAD implantation, and ultimately heart transplantation, partially reversed the pre-capillary component suggesting that at least part of this component was not "fixed."[2] However, two years after transplantation, an increase in PVR disproportionate to the elevation in left heart filling pressures was observed. Whether this pre-capillary component is due to a genetic determinant or due to confounding factors (long-standing LHD, comorbidities) remains unclear and requires further studies. The current evidence is insufficient to conclude on the pathogenicity of the *BMPR2* variant that we classified as a VUS according to ACMG guidelines.[10] Yet, based on available data, we cannot exclude the hypothesis that this new variant might act as a potential genetic modulator of the disease in a patient presenting with another etiology for PH. In this regard, a very recent study demonstrated more pulmonary vascular remodeling and more severe PH following ascending aortic constriction in *kcnk3*-mutated rats compared to wild-type rats.[14]

Our study has limitations inherent to the small sample size and the lack of a control population. This study is monocentric and took place in a tertiary referral center where only extreme phenotypes undergo genotyping. Also, this study has insufficient power to study frequent variants, potential new genes, or variations in noncoding DNA. Moreover, the fact the *BMPR2* variant was found in another North African patient in our home database could suggest this variant is more frequent in North African populations, as they are underrepresented in global genome databases. In that view, the absence of the variant in a 12,000 African American genome database could be explained by an overrepresentation of West African individuals. Finally, our study lacks functional data.

In conclusion, we did not find causative variants with strong effects in PAH-associated genes in our small CpcPH patient cohort using a candidate gene-panel approach. However, we cannot exclude the hypothesis that rare PAH-associated variants could modulate the pre-capillary component in certain CpcPH patients. Studies in bigger patient cohorts using genome-wide genetic tests and a control population are needed.

Author contributions

JLV, AB, and MA designed the study. LC and AB recruited patients. LC and SC collected clinical data. All authors contributed substantially to the analysis and interpretation of the data. LC drafted the manuscript, and all authors revised it critically for important intellectual content. All authors approved the final version of the article.

Ethical approval

The Hospital-Faculty Ethics Committee of Hôpital Erasme approved this study (no. P2014/461 and P2018/639).

Guarantor

Antoine Bondue.

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Declaration of Conflicting Interests

Outside the submitted work:
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