Early View

Original research article

An emerging phenotype of pulmonary arterial hypertension patients carrying SOX17 variants

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An emerging phenotype of pulmonary arterial hypertension patients carrying

SOX17 variants

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Summary (255)
PAH due to SOX17 variants is a severe phenotype associated with congenital heart disease, haemoptysis and radiologic anomalies. Histopathology reveals severe pulmonary arterial remodelling and malformations affecting pulmonary vessels and systemic arteries.
ABSTRACT (248 words)

Introduction
The phenotype of pulmonary arterial hypertension (PAH) patients carrying SOX17 pathogenic variants remains mostly unknown.

Methods
We report the genetic analysis findings, characteristics and outcomes of patients with heritable PAH carrying SOX17 variants from the French Pulmonary Hypertension Network.

Results
Twenty patients and eight unaffected relatives were identified. The median (min-max) age at diagnosis was 17 years (2-53), with a female-to-male ratio of 1.5. At diagnosis, most of the patients (74%) were in functional class III or IV with severe hemodynamic compromise, including a median pulmonary vascular resistance (PVR) of 14.0 (4.2-31.5) Wood units (WU). An associated congenital heart disease (CHD) was found in 7 PAH patients (35%). Patients with CHD-associated PAH were significantly younger at diagnosis than PAH patients without CHD. Four patients (20%) suffered from recurrent haemoptysis requiring repeated arterial embolizations. Thirteen out of 16 patients (81%) of whom imaging was available displayed chest computed tomography abnormalities, including dilated, tortuous pulmonary vessels, ground-glass opacities as well as bronchial and non-bronchial arteries anomalies. After a median follow-up of 47 months (1-591 months), 10 patients underwent lung transplantation and one patient benefited from a heart-lung transplantation due to associated CHD. Histopathologic analysis of lung explants showed a congested lung architecture with severe pulmonary arterial remodelling, subpleural vessel dilation and numerous haemorrhagic foci.

Conclusions
PAH due to SOX17 pathogenic variants is a severe phenotype, frequently associated with CHD, haemoptysis and radiologic abnormalities. Pathologic assessment reveals severe pulmonary arterial remodelling and malformations affecting pulmonary vessels and thoracic systemic arteries.
ABBREVIATIONS
6-MWD: 6-minute walk distance
ASD: atrial septal defect
CHD: congenital heart disease
CI: cardiac index
CO: cardiac output
CTPA: computed tomography pulmonary angiography
DLCO: diffusing capacity of the lung for carbon monoxide
ERAs: endothelin receptor antagonists
HMG: high-mobility-group domain
HRCT: high-resolution computed tomography
SvO$_2$: mixed venous oxygen saturation
mPAP: mean pulmonary artery pressure
NYHA: New York Heart Association
PAH: pulmonary arterial hypertension
PaO$_2$: arterial partial pressure of oxygen
PAWP: pulmonary artery wedge pressure
PDE5is: phosphodiesterase type 5 inhibitors
PFTs: pulmonary function tests
PH: pulmonary hypertension
PVR: pulmonary vascular resistance
RAP: right atrial pressure
RHC: right heart catheterisation
SOX17: SRY-box transcription factor 17
TBX4: T-box transcription factor 4
VSD: ventricular septal defect
VUS: variant of uncertain significance
WU: Wood unit
INTRODUCTION

Pulmonary arterial hypertension (PAH) is an uncommon and severe disease affecting small pulmonary arteries. Although the exact pathophysiology of PAH remains obscure, recent advances have provided a better understanding of cellular and molecular drivers of pulmonary vascular remodelling, including endothelial cell dysfunction, smooth muscle cell abnormalities, inflammation and immune system dysregulation and imbalance in receptors and ligands of the transforming growth factor beta superfamily [1–3]. Pulmonary vascular remodelling leads to pre-capillary pulmonary hypertension (PH) and progressive right ventricular failure [4]. The main causes of familial/heritable PAH are pathogenic variants in the bone morphogenetic protein receptor type 2 gene (BMPR2), which were first described in 2000 [5, 6]. Since then, a number of novel pathogenic variants have been identified in several genes (TBX4, ATP13A3, GDF2, SOX17, AQP1, ACVRL1, SMAD9, ENG, KCNK3, CAV1, GDF2, and BMP10) [5].

Gräf et al. recently reported that heterozygous variants in SOX17 gene were overrepresented in a large PAH cohort, with nine patients harbouring SOX17 pathogenic variants among 1,038 PAH index patients [7]. By performing whole-exome sequencing in a PAH cohort with congenital heart diseases (CHD), Zhu et al. identified SOX17 as a candidate risk gene in ~3% of patients, suggesting that rare variants in genes regulated by SOX17 also contribute to PAH-CHD [8, 9]. However, the phenotype of PAH patients carrying SOX17 pathogenic variants remains mostly unknown.

In the present study, we report the clinical, functional, radiologic, histologic and hemodynamic characteristics, as well as the long-term outcomes of PAH patients and relatives from the French PH Network carrying SOX17 variants.
METHODS

Patient selection

We conducted a retrospective population-based study of PAH patients carrying SOX17 variants from the French PH Network and its National Registry (adult coordinating PH reference centre, Hôpital Bicêtre, Assistance-Publique Hôpitaux de Paris (AP-HP); paediatric PH competence centre, Hopital Necker, AP-HP; and 25 PH competence centres across France). This Registry was set up in agreement with French bioethics laws (Commission Nationale de l’Informatique et des Libertés agreement n°842063). As previously described [10], genetic counselling was offered to all patients with idiopathic, familial, or drug-induced PAH and to first-degree relatives of PAH patients with identified pathogenic variants. All patients provided written informed consent prior to genetic analysis.

Genetic analysis

PAH-predisposing genes, including the SOX17 gene, were screened by next-generation sequencing (NGS)-based gene panel analysis as previously described [11]. Briefly, a custom gene panel including established PAH and pulmonary veno-occlusive disease (PVOD) genes (BMPR2, TBX4, EIF2AK4, CAV1, KCNK3, SMAD9, ACVRL1, ENG, GDF2, KDR, SOX17, KDR, BMP10, AQP1, ATP13A3 and ABCC8) was used for NGS-targeted capture using the HyperCap workflow (Roche/NimbleGen) following the manufacturer’s protocol and sequenced on Illumina platforms (Illumina). Data were analysed by a bioinformatic pipeline developed by GenoDiag Inc., which allowed the identification of variants and copy number variants (CNVs). Each base must be covered by at least 30 reads to be validated. All variants of interest were validated by Sanger sequencing. The SOX17 DNA sequence was compared to the reference sequence (NM_0022454.3).
Clinical, functional, hemodynamic and radiologic characteristics

We reviewed clinical data (age, medical history and physical examination); dyspnoea assessed by the modified New York Heart Association (NYHA) functional class; 6-minute walk distance (6-MWD); and pulmonary function tests (PFTs) results, including the diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (DLCO).

Hemodynamic measurements obtained during right heart catheterisation (RHC) included mean pulmonary artery pressure (mPAP), pulmonary artery wedge pressure (PAWP), right atrial pressure (RAP). Cardiac output (CO) was measured by the standard thermodilution technique or by both the Fick principle and thermodilution when an associated CHD was present. The cardiac index (CI) was calculated as CO divided by body surface area. Pulmonary vascular resistance (PVR) was calculated as [(mPAP-PAWP)/CO]. Precapillary PH was defined as mPAP > 20 mmHg, PAWP ≤ 15 mmHg and PVR ≥ 3 Wood units (WU) [6]. We a priori hypothesised that CHD-associated PAH patients had a different phenotype compared to non-CHD associated patients and consequently compared the two subgroups.

High-resolution computed tomography (HRCT) of the chest and CT pulmonary angiography (CTPA) in 16 PAH patients and 8 relatives carrying a SOX17 pathogenic variant were analysed by an expert radiologist (MRJ) blinded to the clinical diagnosis (PAH patient or healthy SOX17 variant carrier).

Outcomes, lung transplantation and pathologic assessment

Medical therapies approved for PAH (prostacyclin derivatives, endothelin receptor antagonists (ERAs) and phosphodiesterase-5 inhibitors (PDE5is)) were administered according to the clinical judgement and discretion of treating physicians. Time to death or lung transplantation was recorded. Eight lung samples, comprising seven lung explants and one surgical biopsy, from seven patients were analysed.

Statistical analysis

Quantitative variables were expressed as medians (min-max), and categorical variables were expressed as numbers (percentages). Comparisons of continuous values were performed using the Mann–Whitney test. Time to death or transplantation was calculated from the time of the diagnosis
of PAH and was estimated by the Kaplan–Meier method. The analyses were performed using GraphPad Prism 8 (GraphPad Software Inc.).

RESULTS

Genetic testing

We analysed the SOX17 gene by targeted panel sequencing in a series of 452 PAH index patients referred to our clinical molecular laboratory for genetic diagnosis of PAH. A SOX17 pathogenic or likely pathogenic variant (American College of Medical Genetics and Genomics (ACMG) class 4 and 5) was identified in 14 (3.1%) index patients, corresponding to 12 sporadic idiopathic PAH and two familial PAH with cases identified over two or three generations (Figure 1A). Parental DNA samples were available for four index cases and SOX17 variants were identified de novo in two patients (Table 1). Genetic counselling and testing was offered to 18 first-degree relatives, and 8 of them (44.4%) carried SOX17 pathogenic variants (Table 1).

Thirteen distinct SOX17 pathogenic or likely pathogenic variants were identified. Among these, 9 had never been described (Table 1). Two were truncating variants, c.788dup, p.(Glu264Glyfs*101) and c.499_520del p.(Leu167Trpfs*213), leading to a loss of the SOX C-terminal domain of the SOX17 protein, which includes the β-catenin binding domain. The c.499_520del p.(Leu167Trpfs*213) variant was identified in two unrelated index patients and was previously described [7]. The c.788dup, p.(Glu264Glyfs*101) variant had never been described, but a deletion of this same amino acid leading to a truncated protein, p.(Pro263Argfs*124), was previously described [12]. The remaining eleven pathogenic or likely pathogenic variants were missense variants. Ten were classified as likely pathogenic variants according to ACMG guidelines (ACMG class 4) [13, 14] since they are located in the HMG box, a conserved region known as a mutational hotspot of the SOX17 gene (Figure 1B). In silico prediction favours a deleterious effect, and they are not found or are found at a very low frequency in the GnomAD database (Table 1). One variant, c.416C>T, p.(Pro139Leu), was classified as a pathogenic variant (ACMG class 5) since parental DNA analysis established that it was a de novo variant.
Three additional SOX17 variants were identified in three patients with sporadic idiopathic PAH. These variants were classified as ACMG class 3 since they were not located in the HMG box (Figure 1B).

**Patient population**

Twenty PAH patients carrying a SOX17 variant were identified in the French PH Registry: 15 sporadic PAH and five familial PAH from two families (four SOX17 variants confirmed by genetic testing and one obligate carrier) (Table 2). The median (min-max) age at diagnosis was 17 years (2-53), with a female predominance (female-to-male ratio of 1.5). Half of the patients carrying a SOX17 variant (n=10) presented with childhood-onset PAH (<18 years). The distribution of ages at diagnosis is presented in Figure 2A. Associated CHD was found in 35% of SOX17 variant carriers (n=7), including 4 atrial septal defects (ASD) of the ostium secundum type and 3 ventricular septal defects (VSD). SOX17 variant carriers with CHD-associated PAH were significantly younger at PAH diagnosis, as compared to patients without CHD (median age at diagnosis of 10 (2-21) versus 26 (8-53); p = 0.004, Figure 2B). A patent foramen ovale (PFO) was found in three additional patients. Recurrent haemoptysis was reported in 4 (20%) patients requiring multiple bronchial artery embolizations in three of them (Table 2). Eight first-degree relatives carrying a SOX17 pathogenic variant were identified. All of them were asymptomatic and reported no medical history of cardiorespiratory disease. In those healthy subjects, echocardiography did not reveal any sign suggesting PH or CHD.

**Clinical, functional and hemodynamic assessment findings at PAH diagnosis**

At diagnosis, 74% of patients carrying a SOX17 variant were in NYHA functional classes III or IV. 6-MWD at diagnosis was available in nine patients older than 13 years, showing a median distance of 460 (285-600) metres. PFTs were available at diagnosis in 13 patients, showing a reduced DLCO (63 (44-97) % of predicted values) and arterial partial pressure of oxygen (PaO₂) of 73 (49-97) mmHg. RHC at diagnosis was available for all patients and revealed severe precapillary PH, with a markedly elevated mPAP of 64.4 (33-105) mmHg, a normal PAWP of 7 (1-14) mmHg, a CI of 2.9 (1.7-4.4) L/min/m², and increased PVR of 15.6 (4.2-31.5) WU (Table 3). Acute vasodilator testing performed in
15 patients showed a mean PVR decrease of 24% (0-55), but only two fulfilled the criteria for acute vasodilator response according to guidelines [4, 6]. CHD-associated PAH patients had a median PVR of 15.1 WU, compared with 12.1 WU in PAH patients without CHD ($p=0.135$).

**Radiologic findings**

Sixteen patients carrying a SOX17 variant had analysable HRCT and CTPA at PAH diagnosis (Table 4 and Figure 3). Eight unaffected relatives carrying a SOX17 variant had analysable HRCT and CTPA. Thirteen patients (81%) and two healthy relatives (25%) had abnormal radiologic findings. In PAH patients, HRCT showed dilated and tortuous pulmonary vessels in 13 PAH patients (81%), most commonly associated with adjacent ground-glass opacities (75%) and micronodular scissural abnormalities (75%). Bronchial, non-bronchial and mediastinal systemic arteries were dilated in 11 (69%), 6 (38%) and 5 (31%) PAH patients, respectively. Systemic-pulmonary shunts were noticed in 6 (38%) PAH patients. Two healthy relatives (25%) had mild radiologic abnormalities (dilated and tortuous pulmonary vessels) (Table 4).

**Pathologic assessment of the lungs**

Eight lung samples were available in 7 patients carrying a SOX17 variant: one surgical lung biopsy and seven lung explants (Figures 4 and 5, and Supplementary table S1). The main histopathological features were severe pulmonary arterial remodelling with plexiform lesions (8/8, 100%), a congestive parenchyma (8/8, 100%), pleural and subpleural vessel dilation (8/8, 100%) in addition to dilated bronchial arteries (5/8, 63%). The overall architecture of the lungs was preserved, although congested, with numerous foci of alveolar haemorrhages. Singular millimetric fibrovascular lesions (SimFis) were frequently identified adjacent to the plexiform lesions, as previously described in other forms of heritable PAH due to BMPR2 mutations [15]. Septal veins were often dilated, occasionally with thickened vessel walls. Pulmonary venous remodelling was, however, heterogeneous, without signs evocative of pulmonary veno-occlusive disease or pulmonary capillary haemangiomatosis. Significant bronchial systemic arteries dilation was noticed, with enlarged peribronchial vasa vasora. In two lung explants, bronchial artery embolization material was detected (Figure 4H), and the
embolizing agent was identified within a subpleural vein in one patient (Figure 5C). In three patients, there was a thickened pleura associated with haemorrhagic pleural and subpleural lesions, suggesting a haemothorax. Pulmonary infarction sequelae were also noticed in these three patients.

**Follow-up and outcomes**

After a median follow-up of 46 months (1-591 months), more than half of the patients carrying a SOX17 variant (n=11) received a lung transplantation (10 double-lung and one heart-lung transplantation due to uncorrectable CHD). Transplantation was performed with a median delay from diagnosis of 114 (7-167) months. Eight patients (40%) were alive and treated with PAH drugs after a median follow-up of 29 (1-591) months.

At last follow-up or before death or lung transplantation, 45% of the patients carrying a SOX17 variant (n=9) were treated with triple PAH therapy associating an ERA, a PDE5i and a prostacyclin analog, 40% (n=8) were receiving dual combination therapy (ERA, in combination with either PDE5i or prostacyclin analog), and one remained on monotherapy (ERA). Two patients with an acute vasodilator response were initiated on calcium channel blocker monotherapy: the first patient died suddenly within one month of diagnosis, while the second one had a satisfactory long-term response to calcium channel blocker monotherapy.

Lung transplantation procedures were associated with a relatively high frequency of complications. Grade 3 primary graft dysfunction occurred in five of the 11 transplanted patients (45%). Moreover, lung transplantation early outcomes were remarkable with an haemothorax complicating three out of 11 procedures (27%).
DISCUSSION

We report the phenotype and outcomes of 20 childhood-onset and adult-onset PAH patients carrying a SOX17 variant. These patients are remarkable because they present with a variety of cardiovascular characteristics including severe pulmonary arterial remodelling with plexiform lesions, dilated systemic bronchial and non-bronchial arteries, and CHD. Indeed, ASD or VSD was observed in one-third of our patients, mainly in childhood-onset disease, underscoring that PAH patients presenting with an associated CHD may have a heritable disease caused by SOX17 pathogenic variant. Lung HRCT of the chest and CTPA detected ground-glass opacities and abnormal pulmonary arteries, systemic bronchial and non-bronchial arteries. More than half of our patients presented with severe clinical and hemodynamic impairment refractory to PAH therapy justifying lung transplantation, which was associated with frequent complications (notably primary graft dysfunction and haemothorax). Lung histopathology showed severe pulmonary arterial remodeling, subpleural vessel dilation and numerous hemosiderin-laden macrophages.

The radiologic and histopathologic findings depicted in our cohort are likely to be related to the embryologic role of SOX17. Members of the SRY-related high mobility group box (SOX) family are shown to be essential for the regulation of numerous developmental processes, where SOX17 acts as a crucial regulator in pulmonary vascular morphogenesis [16, 17] and cardiovascular development [18]. SOX17 is a transcription factor implicated in the regulation of various cell developmental processes, notably endoderm formation and tumour angiogenesis [16, 19] but also required for the normal development of the pulmonary vasculature [17, 20]. Although SOX17 has a well demonstrated role in embryogenesis, PAH occurrence at adult age in some patients suggests that SOX17 is also implicated in pulmonary vascular remodelling in adults. Approximately 20 SOX genes have been characterised, and 3 SOX group F genes (SOX7, SOX17, and SOX18) are coexpressed in vascular endothelial cells [21]. In animal models, SOX7, SOX17, and SOX18 have overlapping roles in postnatal neovascularisation and vascular-endothelial-specific deletion of all three lead to massive oedema [22]. However, SOX17 plays a key role in developing and maintaining arterial endothelial cell
specificity and integrity [16]. SOX17 is selectively expressed in arteries (but not in veins) and SOX17 knockout reduces the expression of arterial-specific genes and induces the expression of venous-specific genes [16]. In a mouse postnatal retina model, the inhibition of SOX17 expression in endothelial cells leads to strong vascular hypersprouting, a loss of arterial integrity, and large arteriovenous malformations [23]. These studies support the critical role of SOX17 in angiogenesis, maintenance of vascular homeostasis and arterial specificity [20]. The SOX family is characterised by a highly conserved HMG box, a sequence specific DNA-binding domain, where most of the missense variants are located in the HMG box. Site-directed mutagenesis studies have shown that missense mutations within this region can impair both direct DNA binding [24] and SOX17/β-catenin protein complex interactions [25] demonstrating that sequence alteration within this domain have a strong function impact on the SOX17 protein.

We identified a SOX17 pathogenic variant in two unrelated patients with familial PAH. Segregation analysis was then performed in seven additional family members of these two patients, and the SOX17 variant was found in the two PAH patients and in four unaffected relatives (Figure 1A). Considering these family trees and the higher frequency of the sporadic PAH cases, we conclude that PAH due to SOX17 mutations has an autosomal transmission with incomplete penetrance. This genetic inheritance, as well as the female predominance, are consistent with what has been described previously for other PAH predisposition genes such as BMPR2, the leading cause of heritable PAH [26, 27, 5]. PAH due to SOX17 mutations was relatively rare in previously reported idiopathic or familial PAH cohorts, with SOX17 variants found in ~0.9% and ~0.7% in reports of 1,038 [7] and 413 PAH patients [8], respectively. In our present study, ACMG class 4/5 variants were identified in 3.1% of PAH index patients. We intentionally included in our study ACMG class 3 variants in order to have a more exhaustive cohort, although their pathogenicity is not demonstrated. It appears however that one of the two patients harbouring a class 3 variant (Patient XV), had a severe hemodynamic impairment (PVR 10.3 WU) and underwent double lung transplantation 7 months after diagnosis (with pathologic abnormalities similar to what was noticed in other patients). The role
of SOX17 in PAH was only recently reported and its mutation frequency was undoubtedly underestimated until now. Genetic screening in patients with CHD-PAH was not routinely done before evidence of the role of certain development genes such as TBX4, SOX17, KDR was demonstrated. In the French PH referral centre, children and adults with CHD-PAH are systematically screened with a genetic panel comprising those genes, which explains an increased identification of these mutations in our cohort.

Rare deleterious SOX17 variants have also been identified in ~3.2% of patients with CHD-associated PAH in another cohort of 256 patients, suggesting an overrepresentation of SOX17 variants in this form of PAH [8]. These results are in accordance with those of our report, in which one-third of SOX17 variant carriers with PAH presented with an associated CHD, a finding reminiscent of that observed with other developmental genes associated with heritable PAH, such as TBX4 [26, 28]. Saba et al. demonstrated that Sox17 mesoderm-specific loss of function in mouse embryos is associated with cardiac defects [29], which could explain this frequent association. Interestingly, SOX17 variant carriers with PAH and CHD were significantly younger at PAH diagnosis (childhood onset) as compared to SOX17 variant carriers with isolated PAH (adulthood onset) (Figure 2). We hypothesise that two non-mutually exclusive mechanisms could explain this difference. First, coexisting PAH and CHD may be the consequence of a major development impairment caused by dysfunction of SOX17, this key player of cardiac and pulmonary vascular development [20, 18]. Second, CHD could act as a second hit triggering accelerated pulmonary vascular remodelling in a predisposed dysfunctional pulmonary circulation related to SOX17 mutation.

At diagnosis, SOX17 variant carriers with PAH presented with severely compromised hemodynamic parameters, with a median mPAP of 64.4 mmHg and a median PVR of 14.0 WU. These parameters are in line with the hemodynamic severity reported in large series of PAH patients with a BMPR2 mutation [27, 30]. HRCT of the chest and CTPA identified a SOX17 variant carrier radiologic phenotype with numerous vascular abnormalities (dilated and tortuous vessels associated with micronodular and ground-glass opacities), that is not a usual findings of idiopathic PAH [31].
Pathological examination can explain these radiologic findings, with evidence of a congested architecture, dilated vessels, notably in the subpleural zones, and severe pulmonary arterial remodelling with plexiform lesions. Moreover ground-glass opacities seen at HRCT are most likely explained by the micro-haemorrhagic foci depicted in lung histopathology.

SOX17-associated vascular malformations are associated with recurrent haemoptysis, frequently requiring bronchial artery embolization and eventually listing for lung transplantation. Increased haemoptysis risk was already described in BMPR2 mutation carriers [15]. Considering the risk of severe life-threatening haemoptysis in SOX17 mutation carriers, further research is needed to evaluate the interest of preventive systemic arteries embolizations once such vascular malformations are identified. However, such preventive procedures would not be exempt from potential risks at least in part due to the presence of arteriovenous shunts. These shunts are seen on CTPA and further supported by identification of embolization material in subpleural veins in a lung explant following embolization, supporting the presence of an anatomical communication between bronchial arteries and pulmonary veins (Figure 5).

The overall prognosis of PAH patients carrying a SOX17 mutation is poor, with more than half of our patients receiving transplantation at follow-up and one early death in a patient treated only with calcium channel blockers. Transplant-free survival is however difficult to evaluate since diagnosis was made in several prevalent PAH cases. Our data suggest a high risk of complications following lung transplantation, notably haemothorax and primary graft dysfunction, that should be firmly confirmed in larger multicentre studies. Periprocedural haemorrhagic complications may be explained at least in part by lung histopathology showing dilated and fragile vessels, notably within the subpleural space (Table S1). Patients carrying a SOX17 pathogenic variant should thus be managed by a trained lung transplantation team with extreme caution before and immediately after lung transplantation, highlighting the relevance of genetic screening and imaging of the chest of such PAH patients. Healthy carriers of pathogenic SOX17 variants had no or mild radiologic abnormalities and they had no evidence of pre-symptomatic cardiac malformation. This wide phenotypic variability within the
same family harbouring the same mutation has already been observed in PAH associated with mutations in developmental genes, especially in small patella syndrome associated with \textit{TBX4} mutations [26]. Genetic counselling and screening of first-degree relatives is recommended in PH guidelines [4, 6]. This is particularly true in healthy carriers of \textit{SOX17} variants when the risks of PAH and CHD are considered. Longitudinal follow-up of healthy \textit{SOX17} variant carriers will reveal whether pulmonary vascular anomalies and PH may occur with time. Screening procedures remain to be defined in these individuals since the penetrance of PAH and CHD in \textit{SOX17} variant carriers is unknown. We previously demonstrated that echocardiography and NT-pro BNP blood level analysis may be insufficient tools to effectively screen for PH in asymptomatic relatives harbouring \textit{BMPR2} mutations [32]. More exhaustive strategies, including cardiopulmonary exercise testing, DLCO measurement, and ECG, might be useful screening tools in first-degree relatives of patients with heritable PAH [32]. The vascular phenotype associated with \textit{SOX17} pathogenic variants suggests that CTPA should be encouraged in first-degree relatives. Echocardiography screening of CHD of first-degree relatives of patients carrying a \textit{SOX17} pathogenic variant should also be considered, allowing detection of asymptomatic CHD and timely management.

In conclusion, \textit{SOX17} pathogenic variants are associated with a severe form of heritable PAH that can be associated with a variety of cardiac and thoracic vessel anomalies. Although our cohort was of relatively limited size, our data clearly demonstrate a unique phenotype of heritable PAH related to \textit{SOX17} pathogenic variants. International prospective registries are warranted to further reveal and understand the phenotype of rare variants associated with PAH, such as \textit{SOX17} pathogenic variants. Identification of signs highly suggestive of \textit{SOX17} pathogenic variant is critical since this phenotype is associated with an augmented risk of various complications, such as recurrent haemoptysis. Furthermore, the role of \textit{SOX17} in pulmonary vascular development and remodeling is of growing interest, as restoring both its gene expression and signalling might constitute a future therapeutic solution for this severe disease.
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REFERENCES


## TABLES

### Table 1: SOX17 identified variants.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Relative</th>
<th>HGVS c.</th>
<th>HGVS p.</th>
<th>gnomAD MAF</th>
<th>CADD Phred score</th>
<th>Inheritance</th>
<th>ACMG class</th>
<th>Type of PAH</th>
</tr>
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<tbody>
<tr>
<td>I-1/I-2</td>
<td>2</td>
<td>c.422G&gt;A</td>
<td>p.(Arg141Gln)</td>
<td>NF</td>
<td>34</td>
<td>ND</td>
<td>4</td>
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<tr>
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<td></td>
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<td>p.(Ala96Asp)</td>
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<td>32</td>
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<tr>
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<td>p.(Arg70Trp)</td>
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<td>maternal</td>
<td>4</td>
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</tr>
<tr>
<td>IV</td>
<td></td>
<td>c.499_520del*#</td>
<td>p.(Leu167Trpfs*213)</td>
<td>NF</td>
<td>ND</td>
<td>de novo</td>
<td>5</td>
<td>Sporadic</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>c.610C&gt;T</td>
<td>p.(Arg204Cys)</td>
<td>NF</td>
<td>33</td>
<td>ND</td>
<td>3</td>
<td>Sporadic</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td>c.499_520del*#</td>
<td>p.(Leu167Trpfs*213)</td>
<td>NF</td>
<td>ND</td>
<td>ND</td>
<td>5</td>
<td>Sporadic</td>
</tr>
<tr>
<td>VII</td>
<td></td>
<td>c.440G&gt;A</td>
<td>p.(Arg147Gln)</td>
<td>NF</td>
<td>29.8</td>
<td>ND</td>
<td>4</td>
<td>Sporadic</td>
</tr>
<tr>
<td>VIII</td>
<td>2</td>
<td>c.199C&gt;G</td>
<td>p.(Arg67Gly)</td>
<td>0.0032%</td>
<td>26.8</td>
<td>maternal</td>
<td>4</td>
<td>Sporadic</td>
</tr>
<tr>
<td>IX</td>
<td></td>
<td>c.416C&gt;T</td>
<td>p.(Pro139Leu)</td>
<td>NF</td>
<td>29.9</td>
<td>de novo</td>
<td>5</td>
<td>Sporadic</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>c.422G&gt;T</td>
<td>p.(Arg141Leu)</td>
<td>NF</td>
<td>33</td>
<td>ND</td>
<td>4</td>
<td>Sporadic</td>
</tr>
<tr>
<td>XI</td>
<td></td>
<td>c.788dup</td>
<td>p.(Glu264Glyfs*101)</td>
<td>NF</td>
<td>ND</td>
<td>ND</td>
<td>5</td>
<td>Sporadic</td>
</tr>
<tr>
<td>XII-1/XII-2</td>
<td>2</td>
<td>c.413G&gt;C</td>
<td>p.(Arg138Pro)</td>
<td>NF</td>
<td>32</td>
<td>ND</td>
<td>4</td>
<td>Familial</td>
</tr>
<tr>
<td>XIII</td>
<td></td>
<td>c.419G&gt;C*</td>
<td>p.(Arg140Pro)</td>
<td>NF</td>
<td>32</td>
<td>ND</td>
<td>4</td>
<td>Sporadic</td>
</tr>
<tr>
<td>XIV</td>
<td></td>
<td>c.718C&gt;G</td>
<td>p.(Pro240Ala)</td>
<td>NF</td>
<td>15.9</td>
<td>ND</td>
<td>3</td>
<td>Sporadic</td>
</tr>
<tr>
<td>XV</td>
<td></td>
<td>c.44A&gt;C</td>
<td>p.(Gln15Pro)</td>
<td>0.0005%</td>
<td>25.8</td>
<td>ND</td>
<td>3</td>
<td>Sporadic</td>
</tr>
<tr>
<td>XVI</td>
<td></td>
<td>c.418C&gt;T</td>
<td>p.(Arg140Trp)</td>
<td>NF</td>
<td>35</td>
<td>ND</td>
<td>4</td>
<td>Sporadic</td>
</tr>
<tr>
<td>XVII</td>
<td></td>
<td>c.394C&gt;G</td>
<td>p.(His132Asp)</td>
<td>NF</td>
<td>26.2</td>
<td>ND</td>
<td>4</td>
<td>Sporadic</td>
</tr>
</tbody>
</table>

A CADD Phred score equal to or greater than 20 indicates the 1% most deleterious variants in the genome sequence.

*This variant was found in two distinct unrelated patients. *Variants previously described in the literature.

ACMG: American College of Medical Genetics and Genomics, CADD: combined annotation dependent depletion, HGVS: Human Genome Variation Society, gnomAD MAF: Genome Aggregation Database minor allele frequency, NF=not found, ND: not determined, PAH: pulmonary arterial hypertension.
TABLE 2. Clinical characteristics of signs of pulmonary arterial hypertension patients carrying a SOX17 mutation (n=20).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at PAH diagnosis</th>
<th>Congenital heart diseases/patent foramen ovale</th>
<th>Haemoptysis</th>
<th>Other medical conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>M</td>
<td>26</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>I-2</td>
<td>F</td>
<td>13</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>I-3</td>
<td>F</td>
<td>40</td>
<td>PFO</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>F</td>
<td>21</td>
<td>ASD</td>
<td>No</td>
<td>Stroke</td>
</tr>
<tr>
<td>III</td>
<td>F</td>
<td>35</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>F</td>
<td>6</td>
<td>ASD</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>F</td>
<td>24</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>M</td>
<td>32</td>
<td>PFO</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>F</td>
<td>13</td>
<td>VSD</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>F</td>
<td>12</td>
<td>VSD</td>
<td>Yes (2 BAE) Epistaxis</td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>F</td>
<td>36</td>
<td>No</td>
<td>Yes</td>
<td>Recurrent miscarriages, optic neuromyelitis</td>
</tr>
<tr>
<td>X</td>
<td>F</td>
<td>10</td>
<td>ASD</td>
<td>Yes (3 BAE)</td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>F</td>
<td>3</td>
<td>VSD</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>XII-1</td>
<td>M</td>
<td>8</td>
<td>PFO</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>XII-2</td>
<td>M</td>
<td>2</td>
<td>ASD</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>XIII</td>
<td>F</td>
<td>18</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>XIV</td>
<td>M</td>
<td>27</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>XV</td>
<td>M</td>
<td>53</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>XVI</td>
<td>M</td>
<td>10</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>XVII</td>
<td>M</td>
<td>16</td>
<td>No</td>
<td>Yes (4 BAE)</td>
<td></td>
</tr>
</tbody>
</table>

PAH: pulmonary arterial hypertension, ASD: atrial septal defect, VSD: ventricular septal defect, BAE: bronchial artery embolization(s)
**TABLE 3.** Clinical, functional and hemodynamic assessment parameters at the diagnosis of pulmonary arterial hypertension in patients carrying a *SOX17* mutation.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age at PAH diagnosis</th>
<th>NYHA</th>
<th>mPAP</th>
<th>CI</th>
<th>mPAP</th>
<th>Acute NO response</th>
<th>Delt a PVR</th>
<th>6-MWD</th>
<th>PaO2</th>
<th>DLco</th>
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<tbody>
<tr>
<td>I-1</td>
<td>26</td>
<td>2</td>
<td>79</td>
<td>3.0</td>
<td>16.4</td>
<td>Yes</td>
<td>-40%</td>
<td>580</td>
<td>67</td>
<td>44</td>
</tr>
<tr>
<td>I-2</td>
<td>13</td>
<td>3</td>
<td>68</td>
<td>2.65</td>
<td>17.3</td>
<td>No</td>
<td>-</td>
<td>391</td>
<td>93</td>
<td>69</td>
</tr>
<tr>
<td>I-3</td>
<td>40</td>
<td>4</td>
<td>70</td>
<td>1.80</td>
<td>23.5</td>
<td>NA</td>
<td>-</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>21</td>
<td>3</td>
<td>92</td>
<td>1.70</td>
<td>31.5</td>
<td>NA</td>
<td>-</td>
<td>54</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>4</td>
<td>66</td>
<td>1.70</td>
<td>17.7</td>
<td>No</td>
<td>-5%</td>
<td>373</td>
<td>57</td>
<td>72</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>3</td>
<td>81</td>
<td>2.90</td>
<td>29.6</td>
<td>No</td>
<td>-28%</td>
<td>322</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>3</td>
<td>54</td>
<td>2.96</td>
<td>9.0</td>
<td>Yes</td>
<td>-55%</td>
<td>491</td>
<td>97</td>
<td>74</td>
</tr>
<tr>
<td>VI</td>
<td>32</td>
<td>3</td>
<td>58</td>
<td>2.10</td>
<td>12.0</td>
<td>No</td>
<td>-28%</td>
<td>460</td>
<td>61</td>
<td>62</td>
</tr>
<tr>
<td>VII</td>
<td>13</td>
<td>2</td>
<td>76</td>
<td>3.00</td>
<td>15.1</td>
<td>No</td>
<td>-27%</td>
<td>285</td>
<td>49</td>
<td>60</td>
</tr>
<tr>
<td>VIII</td>
<td>12</td>
<td>3</td>
<td>105</td>
<td>4.40</td>
<td>12.8</td>
<td>No</td>
<td>-28%</td>
<td>70</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>36</td>
<td>3</td>
<td>41</td>
<td>3.80</td>
<td>4.2</td>
<td>NA</td>
<td>-</td>
<td>79</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>10</td>
<td>3</td>
<td>87</td>
<td>1.90</td>
<td>30.0</td>
<td>NA</td>
<td>-31%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>3</td>
<td>3</td>
<td>51</td>
<td>3.90</td>
<td>12.8</td>
<td>No</td>
<td>-29%</td>
<td>78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XII-1</td>
<td>8</td>
<td>2</td>
<td>49</td>
<td>3.71</td>
<td>8.2</td>
<td>No</td>
<td>-20%</td>
<td>370</td>
<td>87</td>
<td>97</td>
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<tr>
<td>XII-2</td>
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<td>38</td>
<td>5.38</td>
<td>9.6</td>
<td>No</td>
<td>-9%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XIII</td>
<td>18</td>
<td>3</td>
<td>80</td>
<td>2.30</td>
<td>20.3</td>
<td>No</td>
<td>-</td>
<td>460</td>
<td>84</td>
<td>63</td>
</tr>
<tr>
<td>XIV</td>
<td>27</td>
<td>2</td>
<td>37</td>
<td>3.00</td>
<td>7.5</td>
<td>No</td>
<td>-10%</td>
<td>525</td>
<td>92</td>
<td>66</td>
</tr>
<tr>
<td>XV</td>
<td>53</td>
<td>3</td>
<td>43</td>
<td>2.20</td>
<td>10.3</td>
<td>NA</td>
<td>0%</td>
<td>54</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>XVI</td>
<td>10</td>
<td>3</td>
<td>80</td>
<td>3.25</td>
<td>17.3</td>
<td>No</td>
<td>-20%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XVII</td>
<td>16</td>
<td>2</td>
<td>33</td>
<td>2.5</td>
<td>6.2</td>
<td>No</td>
<td>-24%</td>
<td>600</td>
<td>92</td>
<td>81</td>
</tr>
</tbody>
</table>

TABLE 4: Characteristics of high-resolution computed tomography of the chest in 16 pulmonary arterial hypertension patients and 8 asymptomatic relatives carrying a SOX17 mutation.

<table>
<thead>
<tr>
<th></th>
<th>PAH patients (n=16)</th>
<th>Relatives (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilated and tortuous pulmonary vessels</td>
<td>13 (81%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Ground-glass halos adjacent to pulmonary vessels</td>
<td>12 (75%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Micronodular scissural abnormalities</td>
<td>12 (75%)</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse ground-glass opacities</td>
<td>11 (69%)</td>
<td>0</td>
</tr>
<tr>
<td>Dilated bronchial arteries</td>
<td>11 (69%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Dilated nonbronchial systemic arteries</td>
<td>6 (38%)</td>
<td>0</td>
</tr>
<tr>
<td>Systemic-pulmonary shunts</td>
<td>6 (38%)</td>
<td>0</td>
</tr>
<tr>
<td>Mediastinal systemic hypervascularisation</td>
<td>5 (31%)</td>
<td>0</td>
</tr>
</tbody>
</table>

PAH: pulmonary arterial hypertension.
TABLE S1: Lung histopathological analysis in patients with SOX17 variants (n=8)

<table>
<thead>
<tr>
<th>Patient</th>
<th>I-1</th>
<th>I-2</th>
<th>II</th>
<th>VIII</th>
<th>X</th>
<th>XII-1</th>
<th>XIII</th>
<th>XVII</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe pulmonary arterial remodelling</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>Congestive and/or haemorrhagic parenchyma</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>Pleural and subpleural vessel dilation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>Dilated bronchial arteries*</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>5/8 (63%)</td>
</tr>
</tbody>
</table>

Main histological features included severe pulmonary artery remodelling, congestion or parenchymal haemorrhage, dilation of subpleural vessels and dilation of bronchial arteries. Pulmonary arteries were severely abnormal with major vascular wall thickening and plexiform lesions. Lung architecture was overall preserved, although congestion, parenchymal collapse (secondary to haemorrhagic infarction) as well as peripheral vessel dilation were constantly noticed in examining haematoxylin & eosin slides. Bronchial vessels also appeared altered, with dilation being observed in 5 out of 8 lung explants. *Dilation of bronchial vessels could only be observed in central lung samples.
A. Family trees of the familial cases (families I and XII, Table 1).

Family I included three PAH patients (filled shapes), two of whom had identified SOX17 mutations. The third PAH patient died at 40 years old before mutation identification. Two asymptomatic carriers were identified.

Family XII included two PAH patients diagnosed at 8 and 2 years of age. ASD was identified in the younger PAH patients. Two asymptomatic carriers were identified.

ASD: atrial septal defect, PAH: pulmonary arterial hypertension.

B. Sox17 gene representation and identified variants.

FIGURE 2. Patient age at pulmonary arterial hypertension diagnosis.

A: Age at diagnosis of the whole cohort (n=20). Median age at diagnosis was 17 years (min-max 2-53 years).

B: Age at diagnosis according to the presence of congenital heart disease (CHD). Patients with CHD-associated PAH were significantly younger.

PAH: pulmonary arterial hypertension, CHD, congenital heart disease.
FIGURE 3: Representative high-resolution computed tomography (CT) of the chest, CT pulmonary angiography (CTPA) and pulmonary angiogram of pulmonary arterial hypertension patients carrying a SOX17 pathogenic variant.

Thin-collimated, HRCT of the chest showing subpleural dilated and tortuous pulmonary vessels (black arrows, panel A, B) and ground-glass opacities (panel C).

In panel D, the black arrow points to direct communication between a dilated distal pulmonary artery and a dilated intercostal artery.

Aneurysmal dilatation identified on CTPA (white arrow, panel B) and pulmonary angiography (head arrow, panel E).

Marked dilatation of proximal bronchial arteries is frequently observed (white and black arrows, panel F). The presence of numerous fissural irregularities (white arrows, panel G) suggests the additional presence of dilated systemic vessels at the pleural surface.

CTPA: computed tomography pulmonary angiography, HRCT: high-resolution computed tomography.
FIGURE 4. Histopathology of lung explants from pulmonary arterial hypertension patients carrying a SOX17 variant.

A. Lung parenchyma overview from a SOX17 mutated PAH explant. Gross architecture is not altered. Alveolar septa are enlarged by congestion, and cell density appears augmented by alveolitis. No capillary hemangiomatosis foci are visible.
B. Foci of capillary congestion and dilation (star) are easily found within the lung parenchyma.

C. Parenchymal scarring (with fibrosis and alveolar collapse), likely subsequent to a haemorrhagic infarction.

D. Subpleural haemorrhagic infarction displaying a necrotic core (arrow) with peripheral resorptive histiocytic infiltrate (star).

E. Plexiform lesions adjacent to the pulmonary artery are easily found (arrow).

F. Enlarged image focused on the plexiform lesion showing fibrosis and vessel dilation.

G. Bronchial vessels and capillaries are dilated.

H. Bronchial vessels are markedly dilated. Embolizing material is seen in the bronchial vessels (arrow).

*PAH: pulmonary arterial hypertension.*
FIGURE 5. Histopathology of lung explants from pulmonary arterial hypertension patients carrying a SOX17 variant.

A-B, D. Subpleural veins are markedly thickened and dilated (arrow in A, star in B, panel D).

C. Bronchial artery embolization material is found in the subpleural vein in this explant (star).