Phenotypic characterization of EIF2AK4 mutation carriers in a large cohort of patients diagnosed clinically with pulmonary arterial hypertension

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DOI:
10.1161/CIRCULATIONAHA.117.028351

Phenotypic Characterization of *EIF2AK4* Mutation Carriers in a Large Cohort of Patients Diagnosed Clinically With Pulmonary Arterial Hypertension

Editorial, see p 2034

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et al

BACKGROUND: Pulmonary arterial hypertension (PAH) is a rare disease with an emerging genetic basis. Heterozygous mutations in the gene encoding the bone morphogenetic protein receptor type 2 (*BMPR2*) are the commonest genetic cause of PAH, whereas biallelic mutations in the eukaryotic translation initiation factor 2 alpha kinase 4 gene (*EIF2AK4*) are described in pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis. Here, we determine the frequency of these mutations and define the genotype-phenotype characteristics in a large cohort of patients diagnosed clinically with PAH.

METHODS: Whole-genome sequencing was performed on DNA from patients with idiopathic and heritable PAH and with pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis recruited to the National Institute of Health Research BioResource–Rare Diseases study. Heterozygous variants in *BMPR2* and biallelic *EIF2AK4* variants with a minor allele frequency of <1:10 000 in control data sets and predicted to be deleterious (by combined annotation-dependent depletion, PolyPhen-2, and *sorting intolerant from tolerant* predictions) were identified as potentially causal. Phenotype data from the time of diagnosis were also captured.

RESULTS: Eight hundred sixty-four patients with idiopathic or heritable PAH and 16 with pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis were recruited. Mutations in *BMPR2* were identified in 130 patients (14.8%). Biallelic mutations in *EIF2AK4* were identified in 5 patients with a clinical diagnosis of pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis. Furthermore, 9 patients with a clinical diagnosis of PAH carried biallelic *EIF2AK4* mutations. These patients had a reduced transfer coefficient for carbon monoxide (Kco; 33% [interquartile range, 30%–35%] predicted) and younger age at diagnosis (29 years; interquartile range, 23–38 years) and more interlobular septal thickening and mediastinal lymphadenopathy on computed tomography of the chest compared with patients with PAH without *EIF2AK4* mutations. However, radiological assessment alone could not accurately identify biallelic *EIF2AK4* mutation carriers. Patients with PAH with biallelic *EIF2AK4* mutations had a shorter survival.

CONCLUSIONS: Biallelic *EIF2AK4* mutations are found in patients classified clinically as having idiopathic and heritable PAH. These patients cannot be identified reliably by computed tomography, but a low Kco and a young age at diagnosis suggests the underlying molecular diagnosis. Genetic testing can identify these misclassified patients, allowing appropriate management and early referral for lung transplantation.

The full author list is available on page 2031.

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Sources of Funding, see page 2031

Key Words: genetics ■ hypertension, pulmonary ■ mutation ■ prognosis ■ pulmonary veno-occlusive disease

Clinical Perspective

What Is New?

- One percent of patients with a clinical diagnosis of pulmonary arterial hypertension (PAH) carry biallelic *EIF2AK4* mutations.
- Patients diagnosed clinically with PAH who had a transfer coefficient for carbon monoxide (Kco) <50% predicted and age of diagnosis <50 years were more likely to carry biallelic *EIF2AK4* mutations. The diagnostic yield for genetic testing in this group was 53%.
- Radiological assessment was unable to distinguish reliably between these patients and patients with idiopathic PAH.
- Histology from these patients may show predominately pulmonary arteriopathy, with subtle involvement of the pulmonary veins and capillaries.
- Patients with PAH with biallelic *EIF2AK4* mutations had a worse prognosis compared with other patients with PAH.

What Are the Clinical Implications?

- Younger patients diagnosed with idiopathic PAH but with a low Kco have a high frequency of biallelic *EIF2AK4* mutations.
- Such patients should be reclassified as having pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis.
- Similar to patients with pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis, these patients have a poor prognosis compared with other patients with PAH.
- The spectrum of radiological and histological changes associated with biallelic *EIF2AK4* mutations is wider than previously assumed. The presence of only subtle or infrequent features associated with pulmonary veno-occlusive disease may lead to misclassification of these patients as having PAH.
- Genetic testing allows early identification of these patients, facilitating appropriate management.

Pulmonary arterial hypertension (PAH) is a heterogeneous and rare disorder that can be classified into idiopathic and heritable forms, associated with an underlying condition such as connective tissue disease or congenital heart disease or related to specific drugs and toxins.[1,2] In addition, pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are even rarer forms of pulmonary hypertension that are grouped together with PAH under the current classification system.[2]

Clinical features described in patients with PVOD/PCH include a low transfer coefficient for carbon monoxide (Kco) and oxygen desaturation on exertion, as well as the presence of centrilobular ground glass opacification, interlobular septal thickening, and mediastinal lymphadenopathy on high-resolution computed tomography (CT) of the lung parenchyma.[3,4] However, these clinical and radiological features have also been reported in idiopathic PAH.[5–7] Consequently, the clinical distinction between PVOD/PCH and idiopathic PAH can be challenging. It has been estimated that 10% of patients with PVOD/PCH are misdiagnosed as having idiopathic PAH.[8,9] The diagnosis of PVOD/PCH is often confirmed only postmortem or from explanted lungs by histology.

The histological features of PVOD/PCH typically include pulmonary venous obstructions and pulmonary capillary proliferation, although the distribution of these changes within the lung can be heterogeneous.[10,11] Pulmonary artery smooth muscle hypertrophy and intimal hyperplasia, similar to the changes observed in other forms of PAH, may also be present. Furthermore, pulmonary venous changes have been reported in cases of idiopathic PAH, patients with scleroderma-associated PAH, and those with *BMPR2* mutations to varying extents.[12,13]

A major advance in the molecular diagnosis of PVOD/PCH was the finding of biallelic mutations in the gene encoding the eukaryotic translation initiation factor 2 alpha kinase 4 (*EIF2AK4*) in both familial (100%) and sporadic (20% to 25%) cases of PVOD/PCH.[14,15] EIF2AK4 is an activator of the integrated stress response pathway and responds to environmental stresses, including amino acid deprivation, by phosphorylating the α subunit of eukaryotic translation initiation factor 2.[11,16,17] These discoveries suggest that *EIF2AK4* mutations are specific to PVOD/PCH and that finding biallelic *EIF2AK4* mutations in a patient with pulmonary hypertension would be diagnostic of PVOD/PCH. Patients with PVOD/PCH have a poor prognosis and risk fatal pulmonary edema with the use of pulmonary artery vasodilator therapies.[4,18–20] Consequently, early and accurate diagnosis is vital to guide clinical management.

Heterozygous mutations in the gene encoding the bone morphogenetic protein type 2 receptor (*BMPR2*) are the most common genetic cause of PAH. They are found in ≈17% of individuals with idiopathic PAH and 82% with a family history of the disease.[21] However, mutations in *BMPR2* have also been reported in patients with histologically proven PVOD.[4,22–24] Thus, considerable uncertainty remains as to what extent the finding of *EIF2AK4* or *BMPR2* mutations reliably predicts the clinical phenotype and response to therapy in a population of patients with PAH.

Here, we report the genetic and phenotypic characteristics of patients assessed for *BMPR2* and *EIF2AK4* mutations through whole-genome sequencing within a large cohort (n=880) of patients with PAH recruited to the National Institute of Health Research (NIHR) BRIDGE study (BioResource–Rare Diseases) (Table I in the online-only Data Supplement). The frequency of mutations in other previously reported genes associated with PAH will be reported in a future publication. In this study, we identified and characterized patients with a clinical

and radiological diagnosis of idiopathic PAH who were found to possess biallelic *EIF2AK4* mutations. These patients had a low Kco and were diagnosed at a younger age compared with patients with idiopathic PAH without mutations in these genes. We show that, in common with patients diagnosed clinically with PVOD/PCH, patients with PAH with biallelic *EIF2AK4* mutations have a shorter survival. We conclude that clinical assessment alone is inadequate for the accurate diagnosis of PVOD/PCH. Clinical genetic testing in younger patients presenting clinically with PAH but with a low Kco will allow appropriate classification, leading to better risk stratification and management of these patients.

METHODS

Ethics Approval and Consent

UK patients (621 [70.6%]) were recruited prospectively to the BRIDGE study and provided written informed consent for genetic analysis and the capture of clinical data (BRIDGE study 13/EE/0325). In addition, the study included patients recruited retrospectively from non-UK centers (191 [21.7%]) and deceased UK patients (68 [7.7%]) if they had signed local tissue bank consent forms allowing genetic sequencing.

Explanted lung tissue from an individual undergoing lung transplantation for end-stage PAH was collected under Papworth Hospital Research Tissue Bank ethics (08/H0304/56).

Recruitment and Patients

The BRIDGE study is a prospective study recruiting both prevalent and incident patients with selected rare diseases. Recruitment to the BRIDGE PAH study started in January 2013, and the last patient included in this analysis was recruited on June 15, 2016. Patients with idiopathic PAH, heritable PAH, PVOD, and PCH, diagnosed according to international guidelines at specialist pulmonary hypertension centers in the United Kingdom, the Netherlands, and France, were recruited (Figure 1 and Table II in the online-only Data Supplement).[2] This included 14 patients with confirmed mutations in *BMPR2*.

Throughout this article, we classify patients recruited to the study as having idiopathic PAH or familial PAH on the basis of the absence or presence of a family history of the disease. The term heritable PAH does not distinguish between patients with sporadic PAH with a mutation and patients with a mutation who have a family history. Therefore, the term heritable PAH is used only when referring to previous publications and guidelines.

Patients with other rare diseases and their unaffected relatives recruited to the BRIDGE study (Table III in the online-only Data Supplement) acted as control subjects without PAH for the genetic analysis.

Whole-Genome Sequencing and Variant Calling

Next-generation sequencing with 100– to 150–base pair (bp) paired-end sequencing was performed on DNA libraries created from genomic DNA with Illumina HiSeq 2500 and HiSeq X (Illumina Inc, San Diego, CA).

Reads were aligned against the Genome Reference Consortium human genome (build 37), and variants were called with the Isaac Aligner and Variant Caller (version 2, Illumina Inc). Variants in *BMPR2* and *EIF2AK4* were extracted and annotated with the Ensembl Variant Effect Predictor version 84.[25] Deletions (resulting in the loss of >50 bp) were identified by applying Isaac Copy Number Variant Caller (Canvas, Illumina) and Isaac Structural Variant Caller (Manta, Illumina). Further information is provided in the online-only Data Supplement.

Likely causal variants were identified on the basis of minor allele frequency and predicted deleteriousness. Variants were considered further if they had a minor allele frequency of <1 in 10 000 in unrelated BRIDGE control subjects without PAH and the ExAC database.[26] The rare variants that passed the minor allele frequency filtering were then assessed for deleteriousness. Variants were considered pathogenic on the basis of a combined annotation-dependent depletion score of ≥15 and PolyPhen-2 or *sorting intolerant from tolerant* predictions not classified as benign or tolerated, respectively.[27–29]

Overrepresentation Analyses

For comparison of variant frequencies between disease and control groups, only variants from unrelated individuals were used. The PRIMUS software package was used to identify

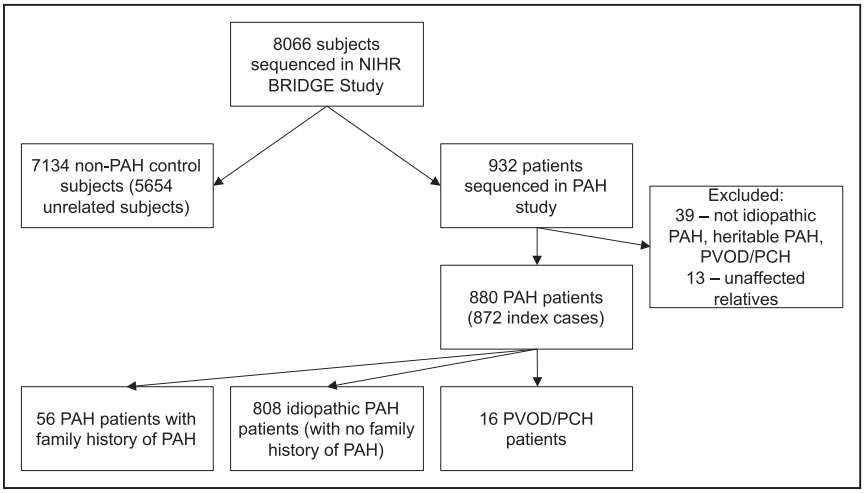

Figure 1. Subjects recruited to the National Institute of Health Research (NIHR) BRIDGE study (BioResource–Rare Diseases) and the clinical diagnostic categories of patients with pulmonary arterial hypertension (PAH) included in this study.
PVOD/PCH indicates pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis.

nonrelated individuals among both BRIDGE control subjects without PAH and patients with PAH.[30] The number of unrelated control subjects was maximized by including either patients with other rare diseases or their unaffected relatives. The frequency of rare and predicted deleterious heterozygous *EIF2AK4* variants in PAH index cases was also compared with publically available information in the ExAC database (http://exac.broadinstitute.org).[26] This analysis provides the maximum estimate of the frequency of heterozygous *EIF2AK4* variants in the ExAC database because variants in ExAC were assumed not to be in a compound heterozygous state.

Phenotypic Data Capture and CT Assessment

Paper and electronic patient records of patients with PAH were reviewed to capture demographic and phenotypic variables from the time of diagnosis and follow-up. Survival data for UK patients were obtained from recruiting centers through the NHS National Spine and local databases. Anonymized information was captured securely online with the free OpenClinica software, adapted for data capture specific to PAH.

CT images of the chest, when available, were reviewed independently by 2 cardiothoracic radiologists (A.S. and N.S.) with specialist imaging experience in pulmonary hypertension who were blinded to the underlying diagnoses with a customized proforma. Further information is provided in the supplemental materials and Tables IV and V in the online-only Data Supplement.

Statistical Analysis

Statistical analysis was performed in R (www.r-project.org). Further information is provided in the online-only Data Supplement.

Semiparametric Cox proportional hazard models were used to assess survival between groups with the survival package in R. Time from diagnosis to both death and death or transplantation was assessed. Age at diagnosis and sex were used as covariates in the models. To avoid immortal time bias arising from the inclusion of retrospectively recruited patients and prevalent patients, a sensitivity analysis was conducted. In this analysis, only prospectively recruited patients from the UK were included, and patients entered the risk set only from the time they consented to the study. Further information is provided in the online-only Data Supplement.

RESULTS

Study Patients

Whole-genome sequencing was performed on 932 patients recruited to the NIHR BRIDGE PAH study and 7134 control subjects without PAH recruited to other NIHR BRIDGE study cohorts. Fifty-two patients were excluded from further analysis because they did not have a clinical diagnosis of idiopathic PAH, heritable PAH, PVOD, or PCH (Figure 1). The remaining 880 patients (of whom 872 were defined as unrelated index cases) consisted of 16 patients (1.8%) with a clinical diagnosis of PVOD/PCH, 56 (6.4%) with PAH and a family history of the disease (referred to as familial PAH), and 808 (91.8%) with idiopathic PAH and no known family history. One of the 16 patients with a clinical diagnosis of PVOD/PCH had an affected sister, whereas the remainder had the sporadic form of the disease.

BMPR2 Mutations in the PAH Cohort

Rare and predicted deleterious *BMPR2* mutations (single-nucleotide variants, indels, and larger deletions) were found in 41 patients (73.2%) with familial PAH and 89 patients (11.0%) with idiopathic PAH. No *BMPR2* mutations were found in patients with a clinical diagnosis of PVOD/PCH.

Rare and Predicted Deleterious *EIF2AK4* Variants in the PAH Cohort

Sixty-nine rare and predicted deleterious *EIF2AK4* single-nucleotide variants and indels were present in the NIHR BRIDGE study. No large deletions were found that affected the *EIF2AK4* gene locus. The variants are summarized in Table VI in the online-only Data Supplement. Five of the 16 patients (31.3%) with clinically diagnosed PVOD/PCH carried biallelic *EIF2AK4* mutations (2 homozygotes and 3 compound heterozygotes).

Twenty-five *EIF2AK4* variants were also found in 19 patients (2.2%) diagnosed clinically with PAH, in whom there was no clinical suspicion of PVOD/PCH (5 homozygotes, 4 compound heterozygotes, and 10 heterozygotes; Table VII in the online-only Data Supplement). One of these patients with a homozygous *EIF2AK4* mutation (c.3097C>T creating a premature stop codon) had a sister who had died of PAH. There was no reported family history of PVOD/PCH.

The remaining rare *EIF2AK4* variants were found in a heterozygous state in 36 control subjects (0.5%). Four of these variants appeared in >1 control subject without PAH, and none were shared with patients with PAH.

Overrepresentation of Rare Heterozygous *EIF2AK4* Variants in Patients With Idiopathic PAH Compared With Control Subjects

The proportion of patients with a clinical diagnosis of idiopathic PAH carrying heterozygous rare *EIF2AK4* variants (1.2%) was significantly greater than the percentage of control subjects without PAH (0.5%; P=0.030). A similar overrepresentation in patients with idiopathic PAH was observed compared with allele frequencies in the ExAC database (0.6%; P=0.042). Two patients with idiopathic PAH with heterozygous rare

EIF2AK4 variants also carried a rare and predicted deleterious *BMPR2* mutation.

Phenotype of Patients With a Clinical Diagnosis of PAH and Biallelic *EIF2AK4* Mutations

Patients with a clinical diagnosis of PAH and biallelic *EIF2AK4* mutations presented at a younger age (median, 29 years; interquartile range, 23–38 years) compared with patients without mutations in the PAH associated genes (51 years; IQR, 37–65 years; *P*=0.024; Table 1). Mean pulmonary artery pressure, cardiac output, and pulmonary vascular resistance were not significantly different between patients with PAH with biallelic *EIF2AK4* mutations and the other groups. As previously reported, hemodynamic variables were significantly worse in patients with *BMPR2* mutations compared with those without any mutations in these genes.

The patients with PAH with biallelic *EIF2AK4* mutations exhibited a reduced Kco (33% [IQR, 30%–35%] predicted) compared with *BMPR2* mutation carriers (81% [IQR, 73%–92%] predicted; *P*<0.001) and patients with PAH with no identified mutation (71% [IQR, 51%–85%] predicted; *P*=0.001). Patients with PAH with biallelic *EIF2AK4* mutations had no obstructive or restrictive deficit on spirometry. These differences remained after the exclusion of patients with abnormal spirometry in the other groups (forced expiratory volume in 1 second of expiration [FEV_1] <80% or forced vital capacity [FVC] <80%; Table VIII in the online-only Data Supplement).

Digital clubbing was overrepresented among patients with biallelic *EIF2AK4* mutations diagnosed clinically with PAH (42%; *P*=0.002). Eleven percent of patients with a clinical diagnosis of PVOD were clubbed.

Only 1 patient with a heterozygous rare and predicted deleterious *EIF2AK4* variant (c.2516T>C) had a reduced Kco (54% predicted) with normal spirometry (FEV_1, 102% predicted; FVC, 98% predicted; and total lung capacity, 100% predicted). There was mild paraseptal emphysema on thoracic CT (<5% of the lung parenchyma affected). This patient, a 44-year-old white man diagnosed with idiopathic PAH, also carried a rare and deleterious *BMPR2* splice acceptor mutation (c.853-2A>G).

We questioned whether Kco was a predictor of biallelic *EIF2AK4* mutations in the wider cohort. However, among patients with PAH with no mutations and normal spirometry (n=255), a reduced Kco (<50% predicted) was present in 65 patients (25.5%). In these patients with a reduced Kco and preserved spirometry, 90.8% were >50 years old at diagnosis, and 69.2% had a history of coronary artery disease, left ventricular dysfunction, or cardiovascular risk factors (diabetes mellitus, systemic hypertension, or hyperlipidemia).

Table 1. Phenotypic Summary of *EIF2AK4* Variant Carriers: Patients With a Clinical Diagnosis of PAH and Biallelic *EIF2AK4* Mutations Were Younger at Diagnosis and Had a Significantly Reduced Kco Compared With Other Groups

	PAH Patients With *BMPR2* Mutations*	PAH Patients With No Mutations in PAH-Associated Genes	PAH Patients With *EIF2AK4* Heterozygous Variants	PAH Patients With Biallelic *EIF2AK4* Mutations	Patients With PVOD/PCH	*P* Value
n	130	704	8	9	16	
Age, y	39 (31–52)	51 (37–65)	49 (36–67)	29 (23–38)	57 (41–69)	<0.001
Female, n (%)	85 (65.4)	494 (70.2)	7 (87.5)	4 (44.4)	9 (56.2)	0.180
White, n (%)	108 (83.1)	551 (78.5)	5 (62.5)	2 (22.2)	13 (81.2)	0.002
Digital clubbing, n (%)	6 (9.7)	10 (3.4)	0 (0)	3 (42.9)	1 (11.1)	0.002
BMI, kg/m^2	28 (24–33)	28 (24–33)	26 (23–28)	24 (20–27)	27 (24–31)	0.216
mPAP, mm Hg	57 (51–69)	52 (44–61)	44 (42–52)	52 (46–65)	48 (40–58)	<0.001
CO, L/min	3 (3–4)	4 (3–5)	3 (3–5)	5 (3–6)	4 (3–4)	<0.001
PVR, WU	15 (11–20)	10 (7–14)	9 (6–10)	9 (8–13)	10 (9–12)	<0.001
Vasoresponders, n (%)	0 (0)	28 (17.5)	0 (0)	0 (0)	0 (0)	0.011
FEV_1, % predicted	90 (78–99)	84 (72–95)	83 (71–94)	94 (85–100)	85 (70–95)	0.031
FVC, % predicted	97 (86–109)	95 (82–106)	96 (75–98)	100 (86–119)	97 (81–103)	0.310
KCO, % predicted	81 (73–92)	71 (51–85)	81 (72–95)	33 (30–35)	37 (32–47)	<0.001
Resting S_AO_2, %	96 (94–97)	96 (93–97)	98 (98–98)	91 (90–94)	94 (91–95)	0.010
S_AO_2 after walk test, %	94 (90–97)	92 (85–96)	94 (84–96)	78 (75–82)	88 (85–89)	<0.001

BMI indicates body mass index; CO, cardiac output; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; Kco, transfer coefficient for carbon monoxide; mPAP, mean pulmonary artery pressure; PAH, pulmonary arterial hypertension; PCH, pulmonary capillary hemangiomatosis; PVOD, pulmonary veno-occlusive disease; PVR, pulmonary vascular resistance; and S_AO_2, arterial oxygen saturation.

*Also includes the 2 patients with a heterozygous *EIF2AK4* variant and a *BMPR2* variant. Data presented as median (interquartile range) unless indicated. Percentages were calculated from the number of patients for whom data were available as the denominator.

Given the high prevalence of a low Kco with preserved spirometry in the wider cohort, we restricted an analysis to patients <50 years of age who at the time of diagnosis had normal spirometry (n=164). Even in this group, a significant proportion (n=15, 9.1%) had a Kco <50% predicted (Figure 2). Eight of these 15 patients carried biallelic *EIF2AK4* mutations. One patient with biallelic *EIF2AK4* mutations was 70 years of age at diagnosis and subsequently did not meet this cutoff.

Among patients with normal spirometry, the presence of a Kco <50% predicted and age at diagnosis <50 years had a high sensitivity (0.889) and specificity (0.977) for identifying patients who carry biallelic *EIF2AK4* mutations; the positive predictive value was low (0.533). Nevertheless, in terms of the diagnostic yield, although genetic testing for biallelic *EIF2AK4* mutations in the entire cohort of patients diagnosed clinically with PAH yielded a 1% detection rate, the presence of biallelic *EIF2AK4* mutations in patients with PAH with a Kco <50% predicted with normal spirometry and <50 years of age at diagnosis was 53%.

CT Features of *EIF2AK4* Mutation Carriers

Centrilobular ground glass opacification extent, mediastinal lymphadenopathy, and interlobular septal thickening are considered suggestive of PVOD/PCH. However, we found subtle or gross centrilobular ground glass opacification in 38% of patients diagnosed clinically with PAH and carrying no mutations (n=21) and 67% of patients with PAH with *BMPR2* mutations (n=21). This was not significantly different compared with patients with a clinical diagnosis of PAH and biallelic *EIF2AK4* mutations (86%, n=7) and patients with a clinical diagnosis of PVOD (50%, n=14). Gross interlobular septal thickening and mediastinal lymphadenopathy were significantly more frequent among patients with PAH and biallelic *EIF2AK4* mutations (29% and 57%, respectively) and those with PVOD (64% and 79%) compared with patients with PAH and no mutation (5% and 0%) or *BMPR2* mutations (5% and 10%). A radiological suspicion of PVOD/PCH was raised in 71% of those with PVOD, 57% of patients with a clinical diagnosis of PAH and biallelic *EIF2AK4* mutations, 14% of patients with PAH with no mutation, and 5% of those with *BMPR2* mutations (Table 2).

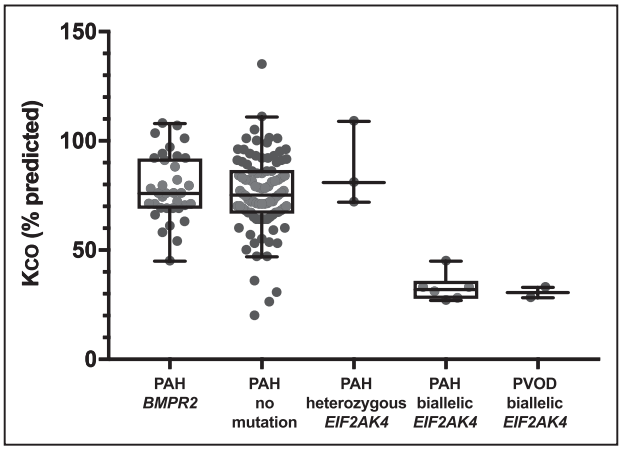

Figure 2. The transfer coefficient for carbon monoxide (Kco) is influenced by genotype in pulmonary arterial hypertension (PAH).
Patients with forced expiratory volume in 1 second of expiration <80% predicted and forced vital capacity <80% predicted and diagnosed with PAH or pulmonary veno-occlusive disease (PVOD)/pulmonary capillary hemangiomatosis after 50 years of age were excluded from the plot.

A further CT analysis comparing patients with biallelic *EIF2AK4* mutations (with a clinical diagnosis of PVOD/PCH or PAH; n=11) and those with a clinical diagnosis of PVOD but not carrying biallelic *EIF2AK4* mutations (n=10) was made (Table IX in the online-only Data Supplement). Patients with biallelic *EIF2AK4* mutations were younger at diagnosis (27 years; IQR, 23–34 years) compared with those with PVOD and no *EIF2AK4* mutations (68 years; IQR, 64–72 years; *P*=0.001). The patients with biallelic *EIF2AK4* mutations also had a lower Kco (32% [IQR, 29%–33%] predicted) compared with patients with PVOD and no *EIF2AK4* mutations (41.4% [IQR, 37%–54%] predicted; *P*=0.013). Centrilobular ground glass opacification appeared more extensive in those with biallelic *EIF2AK4* mutations (82%) compared with those without a mutation (10%; *P*=0.012). However, pleural effusions were more common among those without a mutation (40%) compared with patients with biallelic *EIF2AK4* mutations (0%; *P*=0.035). This may suggest that patients with biallelic *EIF2AK4* mutations have a distinct radiological phenotype compared with patients with PVOD and no biallelic *EIF2AK4* mutations.

Response to Pulmonary Artery Vasodilator Therapies

The response to pulmonary artery vasodilator therapies at 1 and 3 years was assessed for patients with a clinical diagnosis of PAH and biallelic *EIF2AK4* mutations and the other patients with PAH included in the CT analysis. Patients with a clinical diagnosis of PAH and biallelic *EIF2AK4* mutations did not improve their functional class at either 1 or 3 years after diagnosis, unlike the other PAH groups (Table X in the online-only Data Supplement).

Histological Features of a Biallelic *EIF2AK4* Mutation Carrier

The explanted lungs of 1 patient diagnosed with idiopathic PAH but found to have a homozygous *EIF2AK4* missense mutation (c.1795G>C, p.G599R) were as-

Table 2. Radiological Features and Consensus Radiological Diagnosis of Patients With PAH in the CT Substudy

	Group	Patients With PAH With *BMPR2* Mutations (n=21), n (%)	Patients With PAH With No Mutations in the Previously Reported PAH Genes (n=21), n (%)	Patients With PAH With Heterozygous *EIF2AK4* Variants (n=4), n (%)	Patients With PAH With Biallelic *EIF2AK4* Mutations (n=7), n (%)	Patients With PVOD (n=14), n (%)	*P* Value
Centrilobular ground glass opacification density	None	7 (33.3)	13 (61.9)	2 (50.0)	1 (14.3)	7 (50.0)	0.122
	Subtle	12 (57.1)	5 (23.8)	0 (0.0)	2 (28.6)	3 (21.4)	
	Present	2 (9.5)	3 (14.3)	2 (50.0)	4 (57.1)	4 (28.6)	
Centrilobular ground glass opacification extent	None	8 (38.1)	13 (61.9)	2 (50.0)	1 (14.3)	8 (57.1)	0.077
	<5%	0 (0.0)	3 (14.3)	0 (0.0)	1 (14.3)	1 (7.1)	
	5%–25%	2 (9.5)	0 (0.0)	1 (25.0)	2 (28.6)	1 (7.1)	
	25%–50%	2 (9.5)	4 (19.0)	0 (0.0)	0 (0.0)	2 (14.3)	
	50%–75%	5 (23.8)	1 (4.8)	0 (0.0)	2 (28.6)	0 (0.0)	
	75%–100%	4 (19.0)	0 (0.0)	1 (25.0)	1 (14.3)	2 (14.3)	
Interlobular septal thickening	None	17 (81.0)	18 (85.7)	4 (100.0)	5 (71.4)	4 (28.6)	0.001
	Subtle	3 (14.3)	2 (9.5)	0 (0.0)	0 (0.0)	1 (7.1)	
	Present	1 (4.8)	1 (4.8)	0 (0.0)	2 (28.6)	9 (64.3)	
Mediastinal lymphadenopathy	None	19 (90.5)	21 (100.0)	4 (100.0)	3 (42.9)	3 (21.4)	<0.001
	Present	2 (9.5)	0 (0.0)	0 (0.0)	4 (57.1)	11 (78.6)	
Pleural effusion	None	17 (81.0)	21 (100.0)	3 (75.0)	7 (100.0)	10 (71.4)	0.048
	Small	4 (19.0)	0 (0.0)	1 (25.0)	0 (0.0)	4 (28.6)	
Neovascularity	None	12 (57.1)	18 (85.7)	4 (100.0)	6 (85.7)	13 (92.9)	0.077
	Present	9 (42.9)	3 (14.3)	0 (0.0)	1 (14.3)	1 (7.1)	
CT diagnosis	PAH	20 (95.2)	18 (85.7)	3 (75.0)	3 (42.9)	4 (28.6)	
	Possible PVOD/PCH	1 (4.8)	3 (14.3)	1 (25.0)	4 (57.1)	10 (71.4)	

CT indicates computed tomography; PAH, pulmonary arterial hypertension; and PVOD, pulmonary veno-occlusive disease.

sessed. The predominant histological feature was pulmonary arterial vasculopathy. The pulmonary arteries predominantly showed concentric and eccentric intimal fibrosis. No plexiform lesions were observed. Although infrequent, there was some fibrosis of the septal veins and venules, some of which were nearly completely occluded. Although there was evidence of capillary congestion, no capillary hemangiomatosis was observed (Figure 3). The missense variant carried by this patient was not reported in the ExAC database, occurs in a conserved area of the genome (Genomic Evolutionary Rate Profiling score, 5.5), and was predicted to be deleterious (combined annotation-dependent depletion score, 32; PolyPhen-2 prediction of "probably damaging [1]," *sorting intolerant from tolerant* prediction of "deleterious [0]"). The same homozygous mutation was also found in a second unrelated patient with a clinical diagnosis of idiopathic PAH.

Impact of Genotype on Survival

Eight hundred fifty-eight patients were included in the Cox proportional hazards model (Table XI and Figure I in the online-only Data Supplement). Patients diagnosed clinically as having PAH with biallelic *EIF2AK4* mutations had a shorter survival time from diagnosis compared with the *BMPR2* mutation carriers ($P<0.001$) and those without any variants in PAH-associated genes ($P<0.001$). Age ($P<0.001$) and sex ($P=0.001$) also had a significant effect on survival, with male sex and older age at diagnosis associated with shorter survival in the model. Similar results were obtained in the assessment of time to death or transplantation (Table XII in the online-only Data Supplement). In the sensitivity analysis, including only prospectively recruited UK patients, only 2 events occurred in the biallelic *EIF2AK4* group. Thus, no significant difference was observed in mortality between patients diagnosed clinically as having PAH with biallelic *EIF2AK4* mutations and patients with *BMPR2* mutations ($P=0.215$) or patients without any variants in PAH-associated genes ($P=0.282$; Table XIII in the online-only Data Supplement).

DISCUSSION

This is the first study to analyze the frequency of *EIF2AK4* rare variation in a large cohort of patients with PAH and to make detailed phenotypic and radiological assessments. Previously, the presence of biallelic *EIF2AK4* mutations was reported in patients with a clear

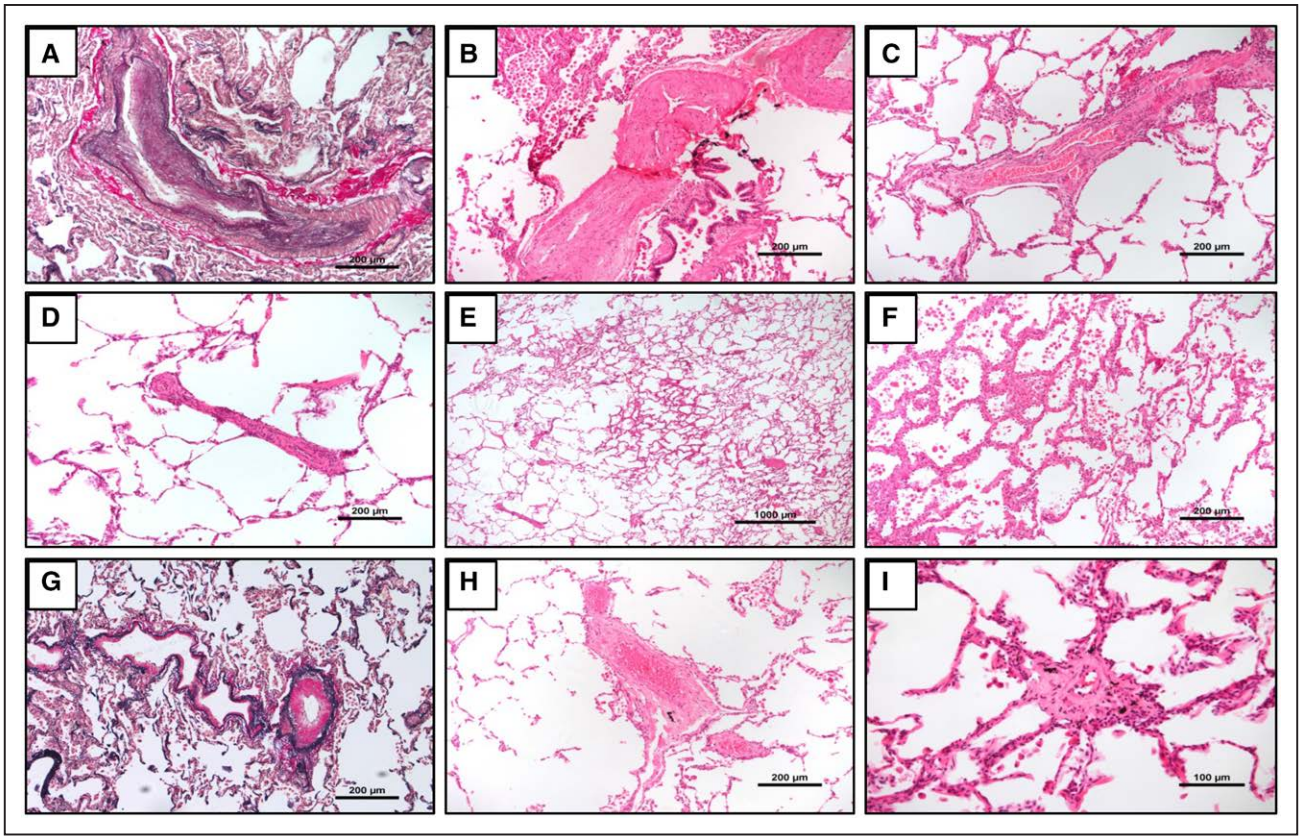

Figure 3. Representative histopathologic images from 1 patient with clinically diagnosed idiopathic pulmonary arterial hypertension (PAH) but found to have a rare (not reported in the ExAC database) and predicted deleterious (combined annotation-dependent depletion score, 32) homozygous *EIF2AK4* missense variant (c.1795G>C). The patient was of Pakistani origin and did not have a family history of PAH or pulmonary veno-occlusive disease (PVOD). At presentation, he was 22 years old and had a reduced transfer coefficient for carbon monoxide (KCO; 31% predicted) despite preserved spirometry. High-resolution computed tomography of his chest showed subtle but extensive (50%–75% involvement) ground glass opacification. No interlobular septal thickening or mediastinal lymphadenopathy was observed. No suspicion of PVOD/pulmonary capillary hemangiomatosis (PCH) was raised from the radiological appearances. Histopathology was reviewed by 2 independent pathologists, each confirming the predominant histological pattern to be one of pulmonary arterial vasculopathy. The pulmonary arteries showed eccentric and concentric intimal fibrosis and medial hypertrophy (**A** and **B**), as well as some lesions with features of recanalized thrombus (**C**). Several concentrically muscularized arterioles were also observed (**D**). No complex plexiform lesions were present. There was patchy thickening of the alveolar septa with capillary congestion and pigmented intra-alveolar macrophages similar to PCH (**E** and **F**). Venous remodeling was difficult to trace and infrequent but present. Fibrous thickening of the intima in septal veins (**G** and **I**) and a microvessel (**H**).

clinical diagnosis of PVOD/PCH and a large kindred and a single family with a possible diagnosis of PAH.[20,31,32] As expected, we identified a high frequency of biallelic *EIF2AK4* mutations in patients with a clear clinical presentation of PVOD/PCH. However, we also found biallelic *EIF2AK4* mutations in patients with a clinical diagnosis of PAH.

The discovery of biallelic *EIF2AK4* mutations in PVOD/PCH raised the possibility of rapid molecular diagnosis in the majority of patients with familial and up to 25% of patients with sporadic PVOD/PCH.[14,15] In the present study, the presence of biallelic *EIF2AK4* mutations was associated with a poor prognosis, even in patients who have a clinical diagnosis of PAH and who did not develop pulmonary edema in response to pulmonary artery vasodilator therapies. Therefore, early identification of these patients through genetic testing may prompt early referral for lung transplantation similar to patients with clinically diagnosed PVOD/PCH.[18]

The presence of biallelic *EIF2AK4* mutations in patients with a clinical diagnosis of PAH raises the question whether *EIF2AK4* mutations can cause classic idiopathic PAH or whether there are cases of PVOD/PCH caused by *EIF2AK4* mutations that are wrongly classified even by expert centers. We further show that phenotypic, radiological, and histological assessments can be difficult to interpret. The presence of subtle or infrequent features may lead to an incorrect diagnosis of PAH in patients with biallelic *EIF2AK4* mutations. This study suggests that patients with pathogenic biallelic

EIF2AK4 mutations may present with a spectrum of phenotypic, radiological, and histological features that can overlap with PAH.

Patients with PAH with biallelic *EIF2AK4* mutations demonstrated a reduced Kco despite normal spirometry, which is characteristic of patients with PVOD/PCH. The reduced Kco likely reflects widespread reduction in alveolar gas exchange caused by endothelial proliferation and patchy thickening of the blood-gas barrier by the process of capillary hemangiomatosis. Ultrastructural thickening of the capillary basement membrane may also play a role.[33] In keeping with previous reports in PVOD/PCH, we also show that patients with PAH with biallelic mutations in *EIF2AK4* are younger at diagnosis than patients with either *BMPR2* mutations or no known mutation.[14,20] However, the presence of these characteristic features has a low positive predictive value for the identification of patients with biallelic *EIF2AK4* mutations.

In contrast to previous descriptions of patients with PVOD, none of the patients with clinically diagnosed PAH and biallelic *EIF2AK4* mutations developed pulmonary edema in response to pulmonary artery vasodilator therapies. For example, intravenous prostanoids were used in 50% of these patients. In patients with classic PVOD, pulmonary edema with intravenous prostanoids has been reported in up to 44% of patients after a median treatment duration of just 9 days.[4] Presumably, the extent and severity of the pulmonary venous involvement in these patients might underlie the differing responses to prostanoids.

It is generally considered that high-resolution CT imaging is a useful noninvasive test to assist in the diagnosis of suspected PVOD/PCH.[11] Although there was an increased prevalence of mediastinal lymphadenopathy and interlobular septal thickening in patients with PAH with biallelic *EIF2AK4* mutations, we found that radiological features at the time of diagnosis could not accurately determine the underlying genotype.[6] The differing radiological features of all patients with biallelic *EIF2AK4* mutations compared with patients with PVOD without mutations is of interest. This may reflect differences between the younger-onset genetic cases of PVOD compared with the predominantly older group of patients without *EIF2AK4* mutations in whom other nongenetic factors such as exposure to inorganic solvents may play an important role.[34]

Histological examination (usually postmortem or from explanted lungs) is often considered essential for diagnostic confirmation of PVOD/PCH but may be confounded by the heterogeneous nature of vascular pathology.[35] Surgical biopsy of the lung in patients with severe PAH is contraindicated, and a limitation of this study is that lung tissue from only 1 patient with biallelic *EIF2AK4* mutations was available for analysis. This patient had a rare and predicted deleterious homozygous missense mutation in *EIF2AK4*. The predominant feature on assessment of the explanted lung tissue was pulmonary arteriopathy, as usually seen in PAH. Although only infrequent, fibrosis of the septal venules and the possible presence of siderophages in the alveolar space were observed. These features are found in patients with PVOD/PCH. This case supports the hypothesis that patients with biallelic *EIF2AK4* mutations may present with a spectrum of venous and arterial involvement.

There are increasing reports of phenotypic, radiological, and histological similarities between PAH and PVOD/PCH.[6,12,13] Tenorio et al[31] reported a homozygous missense mutation in *EIF2AK4* in a large kindred of Iberian Romani with apparent heritable PAH. This kindred is likely to have PVOD/PCH because these diagnoses were not confirmed histologically and PVOD was suspected in half the patients. More recently, Best et al[32] also report 2 sisters with apparent heritable PAH–carrying biallelic *EIF2AK4* mutations. These patients also had a reduced Kco but had not had high-resolution CT assessment of their lung parenchyma, which may have altered their clinical diagnosis. Taken together, these previous reports are compatible with the findings in this larger cohort that patients with a clinical presentation of idiopathic or heritable PAH may in fact have underlying PVOD/PCH as determined by genetic analysis.

A strength of this study is the centralized reporting of radiographic features. However, the data collection was retrospective and incomplete in some cases. Assessing rare diseases such as PAH and PVOD/PCH with a prospective study recruiting incident cases would take a prohibitively long time. This is especially true for the assessment of survival and response to therapy. In this study including prevalent and retrospectively recruited patients, we demonstrated a worse prognosis in patients with a clinical diagnosis of PAH and biallelic *EIF2AK4*. However, the inclusion of prevalent and retrospectively recruited patients can introduce bias such as immortal time bias, when there are long periods between diagnosis and enrollment in the study. The effect of immortal time bias and other confounders such as the inclusion of prevalent and incident cases can be difficult to predict. All groups are likely to include patients who died before study enrollment and thus would not feature in any analysis. When we attempted to eliminate these sources of bias in a sensitivity analysis restricted to prospectively recruited patients from the United Kingdom, the study did not have sufficient power to show a difference in survival between different genotypes. Further studies of survival and response to therapy are needed to definitively show whether misclassified patients with PAH with biallelic *EIF2AK4* mutations and patients with classic PVOD with these mutations have a similarly poor prognosis.

The genetic architecture of idiopathic and heritable PAH remains to be fully elucidated. Ongoing analysis of whole-genome sequence data in our cohort is likely to reveal novel rare variation underlying this condition. Mutations in *BMPR2* account for ≈17% of cases of idiopathic PAH, and other known PAH genes account for ≈1% to 2% of all cases.[21,36] In the present study, *BMPR2* mutations were found in 11% of patients without a family history of PAH. It is worth noting that patients with the sporadic form of the disease with no reported family history represent a higher burden of *BMPR2* mutations (n=89) compared with those with a family history (n=49). This has important implications for clinical genetic testing in patients with sporadic and familial disease.

In previous studies, mutations in both *EIF2AK4* alleles are required to cause PVOD and PCH.[14,15] In autosomal recessive disorders, it is unusual for the heterozygous state to manifest the disease phenotype, and heterozygous *EIF2AK4* variants thus would not be expected to be pathogenic. In this study, we found a significant overrepresentation of heterozygous rare and predicted deleterious *EIF2AK4* variants in patients with PAH compared with control subjects and report 2 patients with rare variants in both *BMPR2* and *EIF2AK4*. Recently, the possibility that heterozygous *EIF2AK4* variants influence the penetrance of *BMPR2* mutations has been raised in a single family with PAH.[37] Further studies are required to determine whether heterozygous *EIF2AK4* variants contribute to pathogenesis in PAH.

CONCLUSIONS

We demonstrate that biallelic *EIF2AK4* mutations are found in patients diagnosed clinically with idiopathic and familial PAH. These patients may have subtle features suggestive of PVOD/PCH on close inspection and are likely to have underlying PVOD/PCH. The spectrum of phenotypic, radiological, and histological features found in patients with biallelic *EIF2AK4* mutations made by current clinical assessments is wider and less clear-cut than previously recognized. This may lead to misclassification of patients as having PAH rather than PVOD and hinders accurate risk stratification. Ascertaining the *EIF2AK4* mutation status of patients through clinical genetic testing provides additional information to aid risk stratification and to guide management. In a young patient presenting with apparent PAH, the presence of a low Kco with normal spirometry strongly suggests the presence of underlying biallelic *EIF2AK4* mutations. Patients with an apparent clinical diagnosis of PAH and biallelic *EIF2AK4* mutations have a worse prognosis compared with patients with *BMPR2* mutations and those without these mutations. Clinical genetic testing should aid identification of this high-risk group and facilitate early referral for lung transplantation and appropriate management.

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ACKNOWLEDGMENTS

The authors acknowledge the help of all the pulmonary hypertension centers, research nurses, and clinical staff involved in the recruitment of patients. They thank the patients and their families who were recruited to this study and the Pulmonary Hypertension Association (United Kingdom).

They acknowledge the support of the NIHR Rare Diseases Translational Research Collaboration, Imperial NIHR Clinical Research Facility, Cambridge NIHR Biomedical Research Center, Netherlands CardioVascular Research Initiative, Dutch Heart Foundation, Dutch Federation of University Medical Centres, Netherlands Organisation for Health Research and Development, and Royal Netherlands Academy of Sciences.

SOURCES OF FUNDING

The NIHR BioResource for Rare Diseases provided funding for sequencing and analysis. The study was supported by a British Heart Foundation Special Project Grant and a Medical Research Council (UK) Experimental Challenge Award.

DISCLOSURES

None.

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FOOTNOTES

Received March 14, 2017; accepted August 25, 2017.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.117.028351/-/DC1.

Circulation is available at http://circ.ahajournals.org.

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