SUPPLEMENTAL MATERIAL:

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

Hadinnapola et al.

Supplemental Methods:

Whole genome sequencing

Genomic DNA was extracted from whole blood samples prior to assessment of concentration by Qubit, and quality by gel electrophoresis. After fragmentation of DNA into 200bp fragments (Covaris E220, Covaris Inc, Woburn, USA) DNA libraries were created using Tru SeqDNA LT Prep kit (Illumina Inc, San Diego, USA). The libraries underwent next generation sequencing using 100-150 base pair paired-end sequencing using Illumina HiSeq 2500 and HiSeq X (Illumina Inc, San Diego, USA).

Variant calling

Reads were aligned against the Genome Reference Consortium human genome (build 37) (GRCh37) and variants were called using the Issac Aligner and Variant Caller respectively (version 2, Illumina Inc.). Genebuilds for *BMPR2* and *EIF2AK4* genes were based on Ensembl v75. Variants from these genes were extracted and annotated using Ensembl's Variant Effect Predictor (VEP) v84 [1]. VEP was also used to annotate data from the Exome Aggregation Consortium's (ExAC) database [2].

Deletions (resulting in the loss of more than 50bp) were identified by applying Isaac copy number variant caller (Canvas, Illumina) and Isaac Structural Variant Caller (Manta,

Illumina). To be called by both Canvas and Manta deletions required a reciprocal overlap of ≥ 20%. Overlapping deletions represented in the Zarrei dataset with a reciprocal overlap of ≥ 50% and deletions with a non-PAH BRIDGE control frequency of more than 1 in 1,000 were excluded [3].

Analysis of computed tomographic images of the chest

CT images of the chest, where available, were reviewed independently by 2 cardiothoracic radiologists (AS and NS), with specialist imaging experience in pulmonary hypertension, blinded to the underlying diagnoses using a customised proforma (Supplemental Table 4). In addition to CT scans of patients with *EIF2AK4* mutations or with a clinical diagnosis of PVOD in the cohort, CT scans of patients from Papworth Hospital and the Royal Hallamshire Hospital with normal spirometry (FEV_1 > 80% predicted and FVC > 80% predicted) and either *BMPR2* mutations (n=21) or no variants in the known PAH genes (n=21) were analysed (Supplemental Table 5). A consensus read was undertaken for individual CT features and a mutually agreed overall radiological diagnosis was recorded.

Histology

The explanted lung tissue of one patient with a clinical diagnosis of idiopathic PAH and biallelic *EIF2AK4* mutations was available for further analysis. Four micrometre (μm) tissue sections were cut from formalin-fixed paraffin wax embedded blocks from the explanted lung tissue. Representative sections from each lobe of both lungs were stained with Elastic-Van Gieson and Haemotoxylin and Eosin stains. Two expert histopathologists examined the sections independently by light microscopy.

Statistical analysis

Statistical analysis was performed in R (www.r-project.org).

Differences between groups of categorical variables were assessed using the Fisher Exact test. Where one of the variables was an ordinal the Cochran-Armitage test was applied using the chisq_test function from the "coin" package [4]. Differences in continuous variables were assessed using the Mann–Whitney U test (2 comparator groups) and the Kruskal-Wallis test (3 or more comparator groups). Post-hoc pairwise comparisons were performed using Dunn's Test for multiple testing.

Semi-parametric Cox-proportional hazards models were used to assess survival between groups using the "survival" package in R [5]. Survival time from diagnosis to death and diagnosis to death or transplantation was assessed. Patients were censored at the date of transplantation for the primary survival analysis. Age at diagnosis and gender were used as covariates in the models.

The proportional hazards assumptions were tested by assessing Schoenfeld residuals over log time [6]. The goodness of fit of the model was assessed by plotting the log of cumulative hazard of Cox-Snell residuals against the log of time and confirming the simple regression has 0 intercept and slope of 1 [7].

Supplemental Tables

Supplemental Table 1. NIHR BioResource – Rare Diseases Collaboration. See spreadsheet.

Centre	Principle Investigator	Clinicians and research staff
Freeman Hospital, Newcastle, UK	Paul A Corris	Alan Greenhalgh, Debbie Shipley, Margaret Day
Golden Jubilee National Hospital, Glasgow, UK	Andrew Peacock	Colin Church, Val Irvine, Fiona Kennedy
Great Ormond Street Hospital, London, UK	Shahin Moledina	Victoria Cookson
Hammersmith Hospital and Imperial College, London, UK	Martin R Wilkins	Simon Gibbs, John Wharton, Sonia Ali, Larahmie Masati, Sharon Meehan, Ivy Wanjiku, Shokri Othman
Papworth Hospital, Cambridge, UK	Joanna Pepke-Zaba	Mark Toshner, Gary Polwarth
Royal Brompton Hospital, London, UK	Stephen J Wort	Rosa DaCosta, Natalie Dormand, Alice Parker
Royal Free Hospital, London, UK	Gerry Coghlan	Yvonne Tan, Dipa Ghedia
Royal Hallamshire Hospital, Sheffield, UK	David G Kiely	Robin Condliffe, Amanda Creaser-Myers, Stephen Roney, Sara Walker
Royal United Hospitals Bath NHS Foundation Trust, Bath, UK	Jay Suntharalingam	Robert MacKenzie Ross, Mark Grover, Ali Grove, Jill Peel, Ann Coy
University of South Paris	Marc Humbert	David Montani, Florent Soubrier, Barbara Girerd, Mélanie Eyries
VU University Medical Center, Amsterdam, Netherlands	Anton Vonk Noordegraaf	Harm Bogaard, Anna Huis in't Veld, Gwen Schotte, Ale Struiksma

Supplemental Table 2. Specialist pulmonary hypertension centres participating in the study

Recruiting cohorts	n
Genomics England	1965
Specialist Pathology: Evaluating Exomes in Diagnostics	1356
Primary Immune Disorders	1299
Bleeding and Platelet Disorders	978
Pulmonary Arterial Hypertension	932
Multiple Primary Malignant Tumours	376
Hypertrophic Cardiomyopathy	187
Cerebral Small Vessel Diseases	183
Steroid Resistant Nephrotic Syndrome	161
Intrahepatic Cholestasis of Pregnancy	140
Stem Cell & Myeloid Disorders	132
Primary Membranoproliferative Glomerulonephritis	128
Neuropathic Pain Disorder	114
Leber Hereditary Optic Neuropathy	59
Bleeding and Platelet Disorders	26
Control	15
Ehlers-Danlos Syndromes	15

Supplemental Table 3. NIHR BioResource - Rare Diseases Study recruiting cohorts and GEL

Parameter	Response
ID	
Date of birth	
Unenhanced CT	(Y/N)
CTPA	(Y/N)
HRCT	(Y/N)
Expiratory CT	(Y/N)
Pulmonary artery diameter (cm)	
Aorta diameter (cm)	
Ground glass opacification centrilobular pattern DENSITY	(None / Subtle / Present)
Ground glass centrilobular pattern EXTENT	(0, <5%, 5-25, 25-50, >50)
Ground glass DISTRIBUTION	(central (C)/peripheral (P)/zonal (Z) or diffuse (D))
Non-specific mosaic pattern / GGO	
Neovascularity vessels	(Y/N)
Arterio-venous malformations	(Y/N)
Bronchial arteries	(Y/N)
Largest bronchial artery size	
Interlobular septal thickening	(None, Subtle, Present)
Mediastinal lymphadenopathy	(Y/N)
Emphysema	(Y/N) and % of parenchyma involved
Fibrosis	(Y/N) and % of parenchyma involved
Pleural effusion	(Y/N)
Air trapping	(Y/N)
Comments	
Likely diagnosis	Any suspicion of PVOD or PCH / PAH

Supplemental Table 4. Proforma used in analysis of CT scans

Group	n
PAH patients with *BMPR2* variants	21
PAH patients with biallelic *EIF2AK4* variants	7
PVOD patients	14
PAH patients with heterozygous *EIF2AK4* variants	4
PAH patients with no variants in the previously reported PAH genes	21

Supplemental Table 5. CT scans of patients with PVOD and patients with PAH carrying biallelic *EIF2AK4* mutations were reassessed by radiologists blinded to the diagnosis. For comparison CT scans of PAH patients with normal spirometry (FEV_1 > 80 % predicted and FVC > 80 % predicted) who either had no mutations in the previously reported PAH genes or carried *BMPR2* mutations were assessed.

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Project	HGVSc	Consequence	HGVSp	Allele count PAH patients	Allele count non-PAH BRIDGE controls	ExAC MAF	PolyPhen-2	SIFT	CADD Phred Score	*EIF2AK4* genotype
BRIDGE control	c.292C>G	missense variant	p.L98V	0	1	0.00001656	probably damaging (0.999)	deleterious (0)	25.7	Heterozygous variant
BRIDGE control	c.354_355delTG	frameshift variant	p.C118Wfs*7	0	2	Not found in ExAC			35	Heterozygous variant
BRIDGE control	c.745C>T	stop gained & splice region variant	p.R249*	0	1	0.00007451			39	Heterozygous variant
BRIDGE control	c.746G>A	missense variant & splice region variant	p.R249Q	0	1	2.48E-05	probably damaging (0.999)	deleterious (0.02)	34	Heterozygous variant
BRIDGE control	c.767G>T	missense variant	p.C256F	0	1	1.66E-05	possibly damaging (0.904)	deleterious (0.02)	28.4	Heterozygous variant
BRIDGE control	c.985G>A	missense variant	p.E329K	0	1	Not found in ExAC	probably damaging (0.981)	deleterious (0.01)	34	Heterozygous variant
BRIDGE control	c.1153dupG	frameshift variant	p.V385Gfs*30	0	1	0.00003308			32	Heterozygous variant
BRIDGE control	c.1190T>A	missense variant	p.I397N	0	1	Not found in ExAC	possibly damaging (0.67)	deleterious (0)	32	Heterozygous variant

Supplemental Table 6. Summary of rare (MAF < 0.0001) and predicted deleterious (CADD score > 15 and not benign by both PolyPhen-2 and SIFT) *EIF2AK4* variants in NIHR BRIDGE Study. Transcript: ENST00000263791.5. *EIF2AK4* variants are not shared between PAH patients and controls. Biallelic *EIF2AK4* variants are seen only in PAH cases.

Bold - variants identified in more than one patient in the PAH Cohort. MAF - minor allele frequency

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Project	HGVSc	Consequence	HGVSp	Allele count PAH patients	Allele count non-PAH BRIDGE controls	ExAC MAF	PolyPhen-2	SIFT	CADD Phred Score	*EIF2AK4* genotype
BRIDGE control	c.1215C>G	stop gained	p.Y405*	0	2	Not found in ExAC			29.4	Heterozygous variant
BRIDGE control	c.1331A>G	missense variant	p.Y444C	0	1	Not found in ExAC	probably damaging (1)	deleterious (0)	28.7	Heterozygous variant
BRIDGE control	c.1345C>T	missense variant	p.R449C	0	1	0.00001654	probably damaging (1)	deleterious (0)	35	Heterozygous variant
BRIDGE control	c.2249T>A	missense variant & splice region variant	p.L750Q	0	1	Not found in ExAC	probably damaging (1)	deleterious (0)	28	Heterozygous variant
BRIDGE control	c.2298delG	frameshift variant	p.N767Tfs*24	0	1	Not found in ExAC			28.3	Heterozygous variant
BRIDGE control	c.2720A>T	missense variant	p.Y907F	0	4	1.66E-05	probably damaging (1)	deleterious (0)	31	Heterozygous variant
BRIDGE control	c.2828C>T	missense variant	p.T943M	0	1	0.00003311	probably damaging (1)	deleterious (0)	34	Heterozygous variant
BRIDGE control	c.3104_3106delTCT	inframe deletion	p.F1035del	0	1	Not found in ExAC			22	Heterozygous variant

Supplemental Table 6. Summary of rare (ExAC MAF <0.0001) and predicted deleterious (CADD score >15 and not benign by both PolyPhen-2 and SIFT) *EIF2AK4* variants in NIHR BRIDGE Study. Transcript: ENST00000263791.5. *EIF2AK4* variants are not shared between PAH patients and controls. Biallelic *EIF2AK4* variants are seen only in PAH cases.

Bold - variants identified in more than one patient in the PAH Cohort. MAF - minor allele frequency

Supplemental Table 6. Page 3/9										
Project	**HGVSc**	**Consequence**	**HGVSp**	**Allele count PAH patients**	**Allele count non-PAH BRIDGE controls**	**ExAC MAF**	**PolyPhen-2**	**SIFT**	**CADD Phred Score**	***EIF2AK4* genotype**
BRIDGE control	c.3217C>T	missense variant	p.R1073C	0	1	0.0000166	probably damaging (1)	deleterious (0)	35	Heterozygous variant
BRIDGE control	c.3223T>G	missense variant	p.F1075V	0	1	0.0000083	probably damaging (0.997)	deleterious (0)	32	Heterozygous variant
BRIDGE control	c.3344C>T	missense variant	p.P1115L	0	1	8.26E-06	probably damaging (1)	deleterious (0)	35	Heterozygous variant
BRIDGE control	c.3358-3C>T	splice region variant & intron variant	p.NA	0	1	Not found in ExAC			17.15	Heterozygous variant
BRIDGE control	c.3406C>T	stop gained & splice region variant	p.R1136*	0	1	Not found in ExAC			40	Heterozygous variant
BRIDGE control	c.3430A>T	missense variant	p.R1144W	0	1	0.0000248	probably damaging (1)	deleterious (0)	33	Heterozygous variant
BRIDGE control	c.3986T>C	missense variant	p.F1329S	0	1	Not found in ExAC	probably damaging (1)	deleterious (0)	33	Heterozygous variant

Supplemental Table 6. Summary of rare (ExAC MAF <0.0001) and predicted deleterious (CADD score >15 and not benign by both PolyPhen-2 and SIFT) *EIF2AK4* variants in NIHR BRIDGE Study. Transcript: ENST00000263791.5. *EIF2AK4* variants are not shared between PAH patients and controls. Biallelic *EIF2AK4* variants are seen only in PAH cases.

Bold - variants identified in more than one patient in the PAH Cohort. MAF - minor allele frequency

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Project	**HGVSc**	**Consequence**	**HGVSp**	**Allele count PAH patients**	**Allele count non-PAH BRIDGE controls**	**ExAC MAF**	**PolyPhen-2**	**SIFT**	**CADD Phred Score**	***EIF2AK4* genotype**
BRIDGE control	c.3992T>C	missense variant	p.F1331S	0	1	8.28E-06	possibly damaging (0.872)	deleterious (0.01)	28.4	Heterozygous variant
BRIDGE control	c.4039G>A	missense variant	p.A1347T	0	1	8.28E-05	probably damaging (1)	deleterious (0)	34	Heterozygous variant
BRIDGE control	c.4388_4389+12 delAGGTAAAGAC GTCA	splice donor variant & coding sequence variant & intron variant	p.NA	0	1	Not found in ExAC			36	Heterozygous variant
BRIDGE control	c.4397C>A	missense variant	p.S1466Y	0	2	Not found in ExAC	probably damaging (0.988)	deleterious (0)	33	Heterozygous variant
BRIDGE control	c.4729G>A	missense variant & splice region variant	p.V1577M	0	1	Not found in ExAC	probably damaging (0.999)	deleterious (0)	29.6	Heterozygous variant
BRIDGE control	c.4751dupT	frameshift variant	p.L1585Ifs*11	0	1	Not found in ExAC			34	Heterozygous variant
BRIDGE control	c.4920_4931delT AGAGATGACTA	inframe deletion	p.R1641_Y1644 del	0	1	Not found in ExAC			23	Heterozygous variant

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Project	HGVSc	Consequence	HGVSp	Allele count PAH patients	Allele count non-PAH BRIDGE controls	ExAC MAF	PolyPhen-2	SIFT	CADD Phred Score	*EIF2AK4* genotype
PAH	c.44C>T	missense variant	p.P15L	1	0	8.32E-06	unknown (0)	deleterious low confidence (0.03)	23.5	Heterozygous variant
PAH	c.220G>A	missense variant	p.D74N	1	0	1.66E-05	possibly damaging (0.954)	deleterious (0)	32	Heterozygous variant
PAH	c.1072_1073dupGT	frameshift variant	p.V359*	1	0	Not found in ExAC			32	Heterozygous variant
PAH	c.1660G>T	missense variant & splice region variant	p.D554Y	1	0	Not found in ExAC	probably damaging (0.966)	deleterious (0)	28	Heterozygous variant
PAH	c.2446C>T	stop gained	p.Q816*	1	0	Not found in ExAC			41	Heterozygous variant
PAH	c.2516T>C	missense variant	p.I839T	1	0	Not found in ExAC	probably damaging (1)	deleterious (0)	28.9	Heterozygous variant
PAH	c.3218G>T	missense variant	p.R1073L	1	0	Not found in ExAC	probably damaging (0.995)	deleterious (0.01)	35	Heterozygous variant
PAH	c.3604C>T	missense variant	p.H1202Y	1	0	Not found in ExAC	probably damaging (1)	deleterious (0)	29.7	Heterozygous variant

Supplemental Table 6. Summary of rare (ExAC MAF <0.0001) and predicted deleterious (CADD score >15 and not benign by both PolyPhen-2 and SIFT) *EIF2AK4* variants in NIHR BRIDGE Study. Transcript: ENST00000263791.5. *EIF2AK4* variants are not shared between PAH patients and controls. Biallelic *EIF2AK4* variants are seen only in PAH cases.

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Project	HGVSc	Consequence	HGVSp	Allele count PAH patients	Allele count non-PAH BRIDGE controls	ExAC MAF	PolyPhen-2	SIFT	CADD Phred Score	*EIF2AK4* genotype
PAH	c.3711_3713del GAG	inframe deletion	p.R1238del	1	0	0.0000083			21.6	Heterozygous variant
PAH	c.3722A>G	missense variant	p.E1241G	1	0	Not found in ExAC	probably damaging (0.971)	deleterious (0)	27.2	Heterozygous variant
PAH	c.4646G>A	missense variant	p.R1549H	1	0	0.0000910	probably damaging (0.998)	deleterious (0.01)	35	Heterozygous variant
PAH	c.145-2A>G	splice acceptor variant	p.NA	1	0	Not found in ExAC			23.9	Additional second (likely trans) variant identified
PAH	c.257+4A>C	splice region variant & intron variant	p.NA	1	0	8.28E-06			15.5	Additional second (likely trans) variant identified
PAH	c.1392delT	frameshift variant	p.R465Vfs*38	1	0	2.48E-05			35	Additional second (likely trans) variant identified
PAH	c.1739dupA	frameshift variant	p.R581Efs*9	1	0	Not found in ExAC			35	Additional second (likely trans) variant identified

Supplemental Table 6. Summary of rare (ExAC MAF <0.0001) and predicted deleterious (CADD score >15 and not benign by both PolyPhen-2 and SIFT) *EIF2AK4* variants in NIHR BRIDGE Study. Transcript: ENST00000263791.5. *EIF2AK4* variants are not shared between PAH patients and controls. Biallelic *EIF2AK4* variants are seen only in PAH cases.

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Project	HGVSc	Consequence	HGVSp	Allele count PAH patients	Allele count non-PAH BRIDGE controls	ExAC MAF	PolyPhen-2	SIFT	CADD Phred Score	*EIF2AK4* genotype
PAH	c.1820T>G	missense variant & splice region variant	p.V607G	1	0	Not found in ExAC	probably damaging (1)	deleterious (0)	27.3	Additional second (likely trans) variant identified
PAH	c.2727C>G	missense variant	p.S909R	1	0	Not found in ExAC	probably damaging (1)	deleterious (0)	33	Additional second (likely trans) variant identified
PAH	c.2827A>G	missense variant	p.T943A	1	0	Not found in ExAC	probably damaging (1)	deleterious (0)	26.4	Additional second (likely trans) variant identified
PAH	c.2841delG	frameshift variant	p.I948Sfs*35	1	0	Not found in ExAC			35	Additional second (likely trans) variant identified
PAH	c.3055_3064delCTGACCAACG	frameshift variant	p.L1019Wfs*9	1	0	Not found in ExAC			36	Additional second (likely trans) variant identified
PAH	**c.3097C>T**	**stop gained**	**p.Q1033***	**3**	**0**	**8.24E-06**			**45**	**Additional second (likely trans) variant identified**

Supplemental Table 6. Summary of rare (ExAC MAF <0.0001) and predicted deleterious (CADD score >15 and not benign by both PolyPhen-2 and SIFT) *EIF2AK4* variants in NIHR BRIDGE Study. Transcript: ENST00000263791.5. *EIF2AK4* variants are not shared between PAH patients and controls. Biallelic *EIF2AK4* variants are seen only in PAH cases.

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Project	HGVSc	Consequence	HGVSp	Allele count PAH patients	Allele count non-PAH BRIDGE controls	ExAC MAF	PolyPhen-2	SIFT	CADD Phred Score	*EIF2AK4* genotype
PAH	c.3325G>A	missense variant	p.G1109R	1	0	0.0000082	probably damaging (1)	deleterious (0.02)	35	Additional second (likely trans) variant identified
PAH	c.3884T>G	missense variant	p.L1295R	1	0	Not found in ExAC	probably damaging (1)	deleterious (0)	32	Additional second (likely trans) variant identified
PAH	c.4400dupT	frameshift variant	p.E1468Rfs*14	1	0	Not found in ExAC			36	Additional second (likely trans) variant identified
PAH	c.4418_4421delCAGA	frameshift variant	p.T1473Rfs*17	1	0	0.0000083			36	Additional second (likely trans) variant identified
PAH	c.4769delT	frameshift variant	p.L1590*	1	0	0.0000083			33	Additional second (likely trans) variant identified
PAH	c.281dupA	frameshift variant	p.N94Lfs*8	2	0	Not found in ExAC			35	Homozygous variant
PAH	c.1159_1160delCT	frameshift variant	p.L387Cfs*27	2	0	Not found in ExAC			29.6	Homozygous variant

Supplemental Table 6. Summary of rare (ExAC MAF <0.0001) and predicted deleterious (CADD score >15 and not benign by both PolyPhen-2 and SIFT) *EIF2AK4* variants in NIHR BRIDGE Study. Transcript: ENST00000263791.5. *EIF2AK4* variants are not shared between PAH patients and controls. Biallelic *EIF2AK4* variants are seen only in PAH cases.

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Project	HGVSc	Consequence	HGVSp	Allele count PAH patients	Allele count non-PAH BRIDGE controls	ExAC MAF	PolyPhen-2	SIFT	CADD Phred Score	*EIF2AK4* genotype
PAH	**c.1795G>C**	**missense variant**	**p.G599R**	**4**	**0**	**Not found in ExAC**	**probably damaging (1)**	**deleterious (0)**	**32**	**Homozygous variant**
PAH	**c.3097C>T**	**stop gained**	**p.Q1033***	**3**	**0**	**8.24E-06**			**45**	**Homozygous variant**
PAH	c.3605A>T	missense variant	p.H1202L	2	0	Not found in ExAC	probably damaging (1)	deleterious (0)	31	Homozygous variant
PAH	c.4392dupT	frameshift variant & splice region variant	p.K1465*	2	0	Not found in ExAC			35	Homozygous variant

Supplemental Table 6. Summary of rare (ExAC MAF <0.0001) and predicted deleterious (CADD score >15 and not benign by both PolyPhen-2 and SIFT) *EIF2AK4* variants in NIHR BRIDGE Study. Transcript: ENST00000263791.5. *EIF2AK4* variants are not shared between PAH patients and controls. Biallelic *EIF2AK4* variants are seen only in PAH cases.

Bold - variants identified in more than one patient in the PAH Cohort. MAF - minor allele frequency

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Age (years)	Gender	Ethnicity	*EIF2AK4* variant HGVSc	Consequence type	*EIF2AK4* genotype	*BMPR2* mutation	Non-protein coding *EIF2AK4* variant	mPAP (mmHg)	Cardiac output (L/min)	FC	FEV_1 (% pred)	FVC (% pred)	KCO (% pred)	Digital clubbing	CT diagnosis	Family history PAH	Pulmonary artery vasodilator therapy	Pulmonary oedema with treatment	Histology assessed
23	M	British	c.3884T>G	missense variant	C Het			52	3.3	3	97	119	33	Yes	Possible PVOD / PCH		PDE5i + ERA + IV Prostanoid	No	
			c.3055_3064delCTGACCAACG	frameshift variant															
48	M	Other	c.4400dupT	frameshift variant	C Het			46	6.4	3	116	120	45	No	CT not available for analysis		ERA + PDE5i + inhaled Prostanoid	No	
			c.1739dupA	frameshift variant															
38	F	Other Asian	c.2827A>G	missense variant	C Het			40	4.5	2				No	CT not available for analysis		ERA + PDE5i	No	
			c.4418_4421delCAGA	frameshift variant															
			c.145-2A>G	splice acceptor variant															

Supplemental Table 7. Phenotypic and genotypic description of patients with a clinical diagnosis of PAH with *EIF2AK4* variants. mPAP – mean pulmonary artery pressure, FC – functional class, FEV_1 – forced expiratory volume in 1s, FVC - forced vital capacity, KCO – transfer coefficient for carbon monoxide, PDE5i – phosphodiesterase type 5 inhibitor, ERA – endothelin receptor antagonist, C Het – compound heterozygous, Hom – homozygous, Het – heterozygous, Unk – unknown

Supplemental Table 7. Page 2/4

Age (years)	Gender	Ethnicity	*EIF2AK4* variant HGVSc	Consequence type	*EIF2AK4* genotype	*BMPR2* mutation	Non-protein coding *EIF2AK4* variant	mPAP (mmHg)	Cardiac output (L/min)	FC	FEV^1 (% pred)	FVC (% pred)	KCO (% pred)	Digital clubbing	CT diagnosis	Family history PAH	Pulmonary artery vasodilator therapy	Pulmonary oedema with treatment	Histology assessed
70	F	British	c.1392delT c.257+4A>C	frameshift variant splice region variant & intron variant	C Het			76	6.6	3	101	127	33	Unk	Possible PVOD / PCH		PDE5i + ERA + inhaled Prostanoid	No	
36	F	Indian	c.3605A>T	missense variant	Hom			44	2.7	3	73	83	40	Yes	Possible PVOD / PCH		ERA + PDE5i + inhaled Prostanoid	No	
22	M	Pakistani	c.1795G>C	missense variant	Hom			65	3.0	3	92	93	31	Yes	PAH		ERA + PDE5i + IV Prostanoid	No	Yes
29	M	Pakistani	c.3097C>T	stop gained	Hom			50	4.9	3	99	107	27	Unk	PAH	Sister died from PAH	PDE5i	No	
18	M	Not stated	c.1159_1160delCT	frameshift variant	Hom			92		3	86	82	28	No	Possible PVOD / PCH		ERA + IV Prostanoid	No	
25	F	Pakistani	c.1795G>C	missense variant	Hom			57	5.6	3	82	87	33	No	PAH		PDE5i + ERA	No	

Supplemental Table 7. Phenotypic and genotypic description of patients with a clinical diagnosis of PAH with *EIF2AK4* variants. mPAP – mean pulmonary artery pressure, FC – functional class, FEV_1 – forced expiratory volume in 1s, FVC - forced vital capacity, K_{CO} – transfer coefficient for carbon monoxide, PDE5i – phosphodiesterase type 5 inhibitor, ERA – endothelin receptor antagonist, C Het – compound heterozygous, Hom – homozygous, Het – heterozygous, Unk – unknown

Supplemental Table 7. Page 3/4

Age (years)	Gender	Ethnicity	*EIF2AK4* variant HGVSc	Consequence type	*EIF2AK4* genotype	*BMPR2* mutation	Non-protein coding *EIF2AK4* variant	mPAP (mmHg)	Cardiac output (L/min)	FC	FEV_1 (% pred)	FVC (% pred)	KCO (% pred)	Digital clubbing	CT diagnosis	Family history PAH	Pulmonary artery vasodilator therapy	Pulmonary oedema with treatment	Histology assessed
24	F	Not stated	c.2446C>T	stop gained	Het (both on same allele) *			60	5.2	3	96	97	81	Unk	CT not available for analysis	Father and sister died of PAH	Unk	Unk	
			c.3218G>T	missense variant															
39	F	British	c.1072_1073dupGT	frameshift variant	Het			54	3.0	2	87	98	72	No	CT not available for analysis		ERA	No	
40	F	British	c.44C>T	missense variant	Het		c.4303-50delT	43	5.6	2	99	96	109	Unk	Possible PVOD / PCH		ERA	No	
44	M	British	c.2516T>C	missense variant	Het	c.853-2A>G (splice acceptor variant)	c.361-180A>G	53	3.8	3	102	98	54	Unk	PAH		PDE5i + ERA	No	
25	F	British	c.3722A>G	missense variant	Het					3	53	49	41	No	CT not available for analysis		PDE5i + ERA + IV Prostanoid	No	

Supplemental Table 7. Phenotypic and genotypic description of patients with a clinical diagnosis of PAH with *EIF2AK4* variants. mPAP – mean pulmonary artery pressure, FC – functional class, FEV_1 – forced expiratory volume in 1s, FVC - forced vital capacity, KCO – transfer coefficient for carbon monoxide, PDE5i – phosphodiesterase type 5 inhibitor, ERA – endothelin receptor antagonist, C Het – compound heterozygous, Hom – homozygous, Het – heterozygous, Unk – unknown, *maternally inherited

Supplemental Table 7. Page 4/4

Age (years)	Gender	Ethnicity	*EIF2AK4* variant HGVSc	Consequence type	*EIF2AK4* genotype	*BMPR2* mutation	Non-protein coding *EIF2AK4* variant	mPAP (mmHg)	Cardiac output (L/min)	FC	FEV_1 (% pred)	FVC (% pred)	KCO (% pred)	Digital clubbing	CT diagnosis	Family history PAH	Pulmonary artery vasodilator therapy	Pulmonary oedema with treatment	Histology assessed
66	F	Not stated	c.4646G>A	missense variant	Het			44	2.1	3	79	100		Unk	PAH		PDE5i + ERA	No	
72	M	British	c.1660G>T	missense variant & splice region variant	Het			30	2.8	3				No	PAH		IV Prostanoid	No	
59	F	Other	c.3711_3713delGAG	inframe deletion	Het			41	3.4	3	68	68	95	Unk	PAH		ERA + PDE5i	No	
48	F	British	c.3604C>T	missense variant	Het	c.2695C>T (stop gained)		57	4.4	4	90	100	61	Unk	PAH		PDE5i + ERA	No	
70	F	Other White	c.220G>A	missense variant	Het			42	5.4	2				Unk	CT not available for analysis		ERA	Unk	

Supplemental Table 7. Phenotypic and genotypic description of patients with a clinical diagnosis of PAH with *EIF2AK4* variants. mPAP – mean pulmonary artery pressure, FC – functional class, FEV_1 – forced expiratory volume in 1s, FVC - forced vital capacity, KCO – transfer coefficient for carbon monoxide, PDE5i – phosphodiesterase type 5 inhibitor, ERA – endothelin receptor antagonist, C Het – compound heterozygous, Hom – homozygous, Het – heterozygous, Unk – unknown

Supplemental Table 8. Page 1/2						
	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**	**PVOD/PCH patients**	**p**
n	64	255	3	7	5	
Age (years)	42 [31 - 52]	53 [39 - 67]	39 [32 - 40]	25 [23 - 38]	63 [27 - 76]	<0.001
Gender (n female [%])	45 [70.3%]	179 [70.2%]	3 [100%]	2 [28.6%]	4 [80%]	0.161
Ethnicity (n white Caucasian [%])	50 [78.1%]	226 [88.6%]	2 [66.7%]	2 [28.6%]	4 [80%]	<0.001
Digital clubbing (n [%])	5 [13.2%]	3 [2.2%]	0 [0%]	2 [40%]	0 [0%]	0.004
BMI	28 [25 - 33]	27 [24 - 31]	24 [24 - 25]	24 [21 - 27]	27 [24 - 32]	0.202

Supplemental Table 8. Phenotype summary of patients with preserved spirometry (FEV_1 > 80 % predicted and FVC > 80 % predicted). PAH patients with biallelic *EIF2AK4* mutations are still younger at diagnosis and have a significantly reduced KCO compared to other groups.
mPAP – mean pulmonary artery pressure, CO – cardiac output, PVR – pulmonary vascular resistance, FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity, KCO – transfer coefficient for carbon monoxide, BMI – body mass index. * Also includes the 2 patients with heterozygous *EIF2AK4* variants and a *BMPR2* mutation. Data presented as median [IQR] unless indicated. Percentages were calculated using the number of patients for whom data were available as the denominator.

Supplemental Table 8. Page 2/2						
	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**	**PVOD/PCH patients**	**p**
mPAP (mmHg)	56 (15)	51 (18)	54 (8)	57 (20)	57 (7)	0.008
CO (L/min)	3 [3 - 4]	4 [3 - 5]	5 [4 - 5]	5 [4 - 6]	3 [3 - 3]	<0.001
PVR (WU)	14 [10 - 18]	10 [7 - 14]	8 [7 - 9]	9 [8 - 15]	14 [11 - 19]	<0.001
Vasoresponders (n [%])	0 [0%]	18 [21.7%]	0 [0%]	0 [0%]		0.016
FEV_1 (%pred)	97 [88 - 102]	93 [87 - 101]	96 [92 - 97]	97 [89 - 100]	98 [94 - 106]	0.525
FVC (%pred)	102 [96 - 113]	103 [96 - 112]	97 [96 - 98]	107 [90 - 120]	109 [101 - 113]	0.704
KCO (%pred)	80 [71 - 93]	68 [46 - 84]	81 [76 - 95]	33 [30 - 33]	33 [28 - 37]	<0.001
Resting S_AO_2 (%)	96 [94 - 98]	96 [93 - 98]	98 [98 - 99]	91 [90 - 92]	95 [91 - 95]	0.021
S_AO_2 post walk test (%)	95 [90 - 98]	91 [85 - 96]	94 [87 - 96]	80 [75 - 84]	85 [85 - 88]	<0.001

Supplemental Table 8. Phenotype summary of patients with preserved spirometry (FEV_1 > 80 % predicted and FVC > 80 % predicted). PAH patients with biallelic *EIF2AK4* mutations are still younger at diagnosis and have a significantly reduced KCO compared to other groups.
mPAP – mean pulmonary artery pressure, CO – cardiac output, PVR – pulmonary vascular resistance, FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity, KCO – transfer coefficient for carbon monoxide, BMI – body mass index. * Also includes the 2 patients with heterozygous *EIF2AK4* variants and a *BMPR2* mutation. Data presented as median [IQR] unless indicated. Percentages were calculated using the number of patients for whom data were available as the denominator.

Supplemental Table 9. Page 1/2				
Group		**All biallelic *EIF2AK4* mutation carriers**	**PVOD with no *EIF2AK4* mutation**	**p**
n		11	10	
Age (years)		26.8 [22.5 - 34.3]	68.3 [63.9 - 72.1]	0.001
Gender (n female [%])		6 [54.5%]	5 [50.0%]	1.000
Ethnicity (n white Caucasian [%])		5 [45.5%]	9 [90.0%]	0.063
mPAP (mmHg)		52 [47 - 63]	48 [42 - 57]	0.342
PCWP (mmHg)		11 [7.5 - 12]	11.5 [9.0 – 12.2]	0.560
FEV_1 (% pred)		93.1 [82.8 - 98.5]	79.0 [72.3 – 91.0]	0.236
FVC (% pred)		95.5 [84.6 - 108.5]	96.0 [73.0 – 101.0]	0.720
KCO (% pred)		32.0 [28.7 – 33.0]	41.4 [36.8 – 54.0]	0.013
Centrilobular ground glass opacification density	None	2 [18.2%]	6 [60.0%]	0.012
	Subtle	2 [18.2%]	3 [30.0%]	
	Present	7 [63.6%]	1 [10.0%]	

Supplemental Table 9. Phenotypic and radiological characteristics of biallelic *EIF2AK4* mutation carriers compared to patients with a clinical diagnosis of PVOD and no *EIF2AK4* mutation.

mPAP – mean pulmonary artery pressure, PCWP – pulmonary capillary wedge pressure, FEV_1 – forced expiratory volume 1 s, FVC – forced vital capacity, KCO – transfer coefficient for carbon monoxide. Data presented as median [IQR] unless stated.

Supplemental Table 9. Page 2/2				
Group		**All biallelic *EIF2AK4* mutation carriers**	**PVOD with no *EIF2AK4* mutation**	**p**
Centrilobular ground glass opacification extent	None	2 [18.2%]	7 [70.0%]	0.007
	<5%	1 [9.1%]	1 [10.0%]	
	5-25%	2 [18.2%]	1 [10.0%]	
	25-50%	1 [9.1%]	1 [10.0%]	
	50-75%	2 [18.2%]	0 [0.0%]	
	75-100%	3 [27.3%]	0 [0.0%]	
Interlobular septal thickening	None	7 [63.6%]	2 [20.0%]	0.068
	Subtle	0 [0.0%]	1 [10.0%]	
	Present	4 [36.4%]	7 [70.0%]	
Mediastinal lymphadenopathy	None	4 [36.4%]	2 [20.0%]	0.635
	Present	7 [63.6%]	8 [80.0%]	
Pleural effusion	None	11 [100.0%]	6 [60.0%]	0.035
	Small	0 [0.0%]	4 [40.0%]	
Neovascularity	None	10 [90.9%]	9 [90.0%]	1.000
	Present	1 [9.1%]	1 [10.0%]	
CT diagnosis	PAH	4 [36.4%]	3 [30.0%]	
	Possible PVOD/PCH	7 [63.6%]	7 [70.0%]	

Supplemental Table 9. Phenotypic and radiological characteristics of biallelic *EIF2AK4* mutation carriers compared to patients with a clinical diagnosis of PVOD and no *EIF2AK4* mutation.
mPAP - mean pulmonary artery pressure, PCWP - pulmonary capillary wedge pressure, FEV_1 - forced expiratory volume 1 s, FVC - forced vital capacity, KCO - transfer coefficient for carbon monoxide. Data presented as median [IQR] unless stated.

Group	Time to assessment 1 (days)	n	Change in 6mwd (m)	Change in FC	Time to assessment 2 (days)	n	Change in 6mwd (m)	Change in FC	Number on prostanoid therapy before the 2nd assessment [%]
PAH *BMPR2*	357 [314 - 386]	21	+69 [20 - 100]	-1 [-1 - -1]	1120 [1055 - 1174]	18	+45 [31 - 115]	-1 [-1 - -0.5]	5 [23%]
PAH biallelic *EIF2AK4*	358 [335 -388]	9	+28 [-13 - 77]	0 [-1 - 0]	1102 [1090 – 1112]	5	+62 [-8 - 132]	0 [0 - 0]	1 [10%]
PAH no mutation	387 [340 - 414]	16	+81 [61 - 151]	-1 [-1 - 0]	1118 [1105 - 1159]	9	+104 [20 - 144]	-1 [-1 - 0]	4 [17%]
p	0.295		0.343	0.039	0.730		0.748	0.044	0.816

Supplemental Table 10. Response to pulmonary artery vasodilator therapies at 1 and 3 years after diagnosis compared to baseline. 6mwd - six-minute walk test distance, FC - functional class. Drop in number of patients between assessment 1 and 2 due to death, transplantation or lack of sufficient follow up time. Data presented as median [IQR] unless stated.

Variable	Hazard Ratio [95% confidence interval]	p
PAH *BMPR2* mutation*	0.148 [0.055 - 0.396]	<0.001
PAH no mutation*	0.179 [0.073 - 0.440]	<0.001
PVOD*	0.393 [0.075 - 2.065]	0.27
Age at diagnosis	1.043 [1.033 - 1.053]	<0.001
Male gender	1.631 [1.222 - 2.179]	<0.001

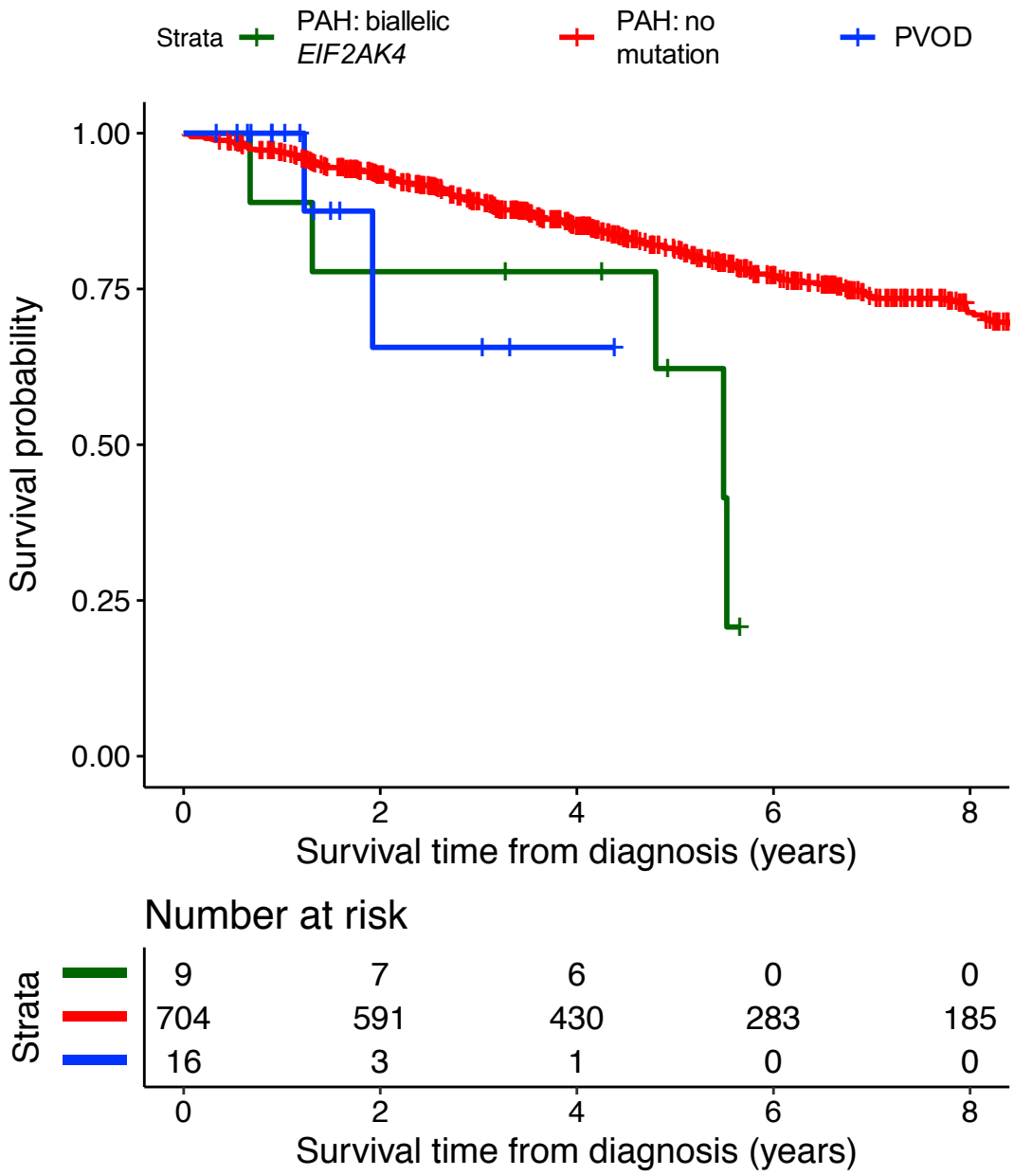
Supplemental Table 11. Cox proportional hazards model assessing time to death. Patients with a clinical diagnosis of PAH and biallelic *EIF2AK4* mutations had an increased risk of death compared to other PAH patients. Number of patients = 858. Events = 194.
* compared to the PAH biallelic *EIF2AK4* mutation carriers

Variable	Hazard Ratio [95% confidence interval]	p
PAH *BMPR2* mutation*	0.175 [0.066 - 0.462]	<0.001
PAH no mutation*	0.203 [0.083 - 0.501]	<0.001
PVOD*	0.840 [0.222 - 3.193]	0.798
Age at diagnosis	1.036 [1.027 - 1.046]	<0.001
Male gender	1.542 [1.165 - 2.042]	0.002

Supplemental Table 12. Cox proportional hazards model assessing time to death or transplantation. Number of patients = 858. Events = 208.
* compared to the PAH biallelic *EIF2AK4* mutation carriers

Supplemental Figures

Figure S1



Supplemental Figure Legends:

Figure S1: Kaplan – Meier survival curves showing survival time (time to death) for patients with a clinical diagnosis of PAH or PVOD.

Supplemental References

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