Original Research Article

Pulmonary Circulation

Genetic basis of pulmonary arterial hypertension: a prospective study from a highly inbred population

Abdullah M. Aldalaan[1,*], Khushnooda Ramzan[2,*], Sarfraz A. Saleemi[1], Ihab Weheba[2,3], Laila Alquait[2], Abeer Abdelsayed[1,4], Fatima Alzubi[1], Hamdeia Zaytoun[1], Nadeen Alharbi[1], Mohammed Al-Owain[5] and Faiqa Imtiaz[1]

[1]Department of Medicine, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [2]Department of Clinical Genomics, Centre of Genomic Medicine, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [3]National Research Centre, Cairo, Egypt; [4]Faculty of Medicine, Ain Shams University, Cairo, Egypt; [5]Department of Medical Genetics, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia

Abstract

Pulmonary arterial hypertension (PAH), whether idiopathic PAH (IPAH), heritable PAH, or associated with other conditions, is a rare and potentially lethal disease characterized by progressive vascular changes. To date, there is limited data on the genetic basis of PAH in the Arab region, and none from Saudi Arabian patients. This study aims to identify genetic variations and to evaluate the frequency of risk genes associated to PAH, in Saudi Arabian patients. Adult PAH patients, diagnosed with IPAH and pulmonary veno-occlusive disease, of Saudi Arabian origin, were enrolled in this study. Forty-eight patients were subjected to whole-exome sequencing, with screening of 26 genes suggested to be associated with the disease. The median age at diagnosis was 29.5 years of age, with females accounting for 89.5% of our cohort population. Overall, we identified variations in nine genes previously associated with PAH, in 16 patients. Fourteen of these variants have not been described before. Plausible deleterious variants in risk genes were identified in 33.3% (n = 16/48) of our entire cohort and 25% of these cases carried variants in *BMPR2* (n = 4/16). Our results highlight the genetic etiology of PAH in Saudi Arabia patients and provides new insights for the genetic diagnosis of familial and IPAH as well as for the identification of the biological pathways of the disease. This will enable the development of new target therapeutic strategies, for a disease with a high rate of morbidity and mortality.

Keywords

pulmonary arterial hypertension (PAH), pulmonary veno-occlusive disease (PVOD), mutations, Saudi Arabia

Date received: 17 March 2021; accepted: 16 June 2021

Pulmonary Circulation 2021; 11(3) 1–8
DOI: 10.1177/20458940211032057

Introduction

Pulmonary arterial hypertension (PAH) is a rare and severe disease affecting small pulmonary arteries, caused by abnormal proliferation of their smooth muscle cells and endothelial cells. This results in increased pulmonary vascular resistance and right ventricular failure.[1–3] PAH is nowadays characterized by mean pulmonary artery pressure >20 mmHg, normal left atrial pressure, and pulmonary vascular resistance ≥3 Wood units.[4] Previous estimations indicate an annual incidence of 1–2 cases of PAH per million individuals,[5] but this number has increased, in recent years, to a value between 15 and 60 cases per million individuals.[6] Women have higher risk for PAH, with a 2–3.1 female/male ratio.[4,6,7] Even though PAH mainly affects patients in the third to fourth decade of life, it also manifests in children, most of the times with a more severe prognosis.[8,9]

Despite the improvements regarding the treatment and management of PAH, it remains a fatal disease with poor prognosis and high morbidity and mortality.[10,11] In this

*These authors contributed equally to this study.

Corresponding author:
Abdullah M. Aldalaan, Department of Medicine, King Faisal Specialist Hospital & Research Center, Riyadh 11211, Saudi Arabia.
Email: aldalaan@kfshrc.edu.sa

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
journals.sagepub.com/home/pul

SAGE

context, additional approaches based on a thorough understanding of the pathobiology of PAH are needed to develop therapies to target its molecular etiology.[10]

The pathogenesis of idiopathic PAH (IPAH) appears to be linked to a number of susceptible genes, including the bone morphogenic protein receptor type II (*BMPR2*) gene, coding for a type II receptor member of the transforming growth factor-β family.[12] *BMPR2* mutations are seen in over 80% of cases with heritable PAH. In the idiopathic cases, 25% of the *BMPR2* mutations are *de novo* mutations.[13,14] Only about 20% of the population with *BMPR2* mutations develop the disease, suggesting an incomplete penetrance of the gene and a "second-hit" mechanism based on a major gene and an additional modifier gene.[15,16] In addition, the clinical presentation is variable within the same family, and the incidence is higher in females.[17–20]

Recent studies evidenced that, at least, 30 genes are implicated in the development of PAH, of which 21 showed specific mutations.[21,22] Multiple research studies, including identification of mutations, genes, and gene therapy, are opening new windows for the target treatment of this devastating disease.[23]

To date, apart from a study on the clinical genetic characteristics of 21 PAH patients in Lebanon,[11] the prevalence of genetic abnormalities in patients from the Middle East/Gulf region, including Saudi IPAH patients, has not been reported.

The main objectives of this study are to identify the genetic variations and their frequency in risk genes, in PAH patients of Saudi Arabia, and determine any possible genotype/phenotype correlation. We expect that the results of this study can support extended family screening, genetic counseling, and possible preventive measures in the future.

Methods

Study design

This prospective, non-randomized study included patients from our ongoing SystemAtic prospective follow Up for better unDerstanding of clInical characteristics of patients with Pulmonary Hypertension disease (SAUDIPH) registry.[24] Forty-eight Saudi Arabian PAH patients were enrolled, with full-informed consent, under an Institutional Review Board-approved protocol (RAC# 2161062). The study was approved by Hospital Research Advisory Council and Ethical Committee.

The inclusion criteria were as follows : adult (>18 year of age) patients with diagnosis of PAH, defined as per American College of Chest Physicians (ACCP)/European Respiratory Society (ERS) 2015 guidelines as group I pulmonary hypertension (PH), while excluding all known causes including drugs, connective tissue diseases, portal hypertension, HIV infection, and congenital heart diseases. Patients with PH with associated conditions (Group 1), pulmonary venous hypertension (Group 2), hypoxemic PH (Group 3), chronic thromboembolic PH (Group 4), and Group 5 PH were excluded from the study.

Clinical management

Upon diagnosis, patients were invited to participate in the SAUDIPH registry; those fulfilling the inclusion criteria (IPAH and pulmonary veno-occlusive disease (PVOD)) were included in this study and were followed up, according to routine clinical practice, at the section of Pulmonary Medicine of our hospital, over a period of two years. We performed a review of the electronic medical records and charts of the probands and collected demographic and clinical data of all the patients (World Health Organization Functional Class (WHO FC), six-minute walking distance (6MWD), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and haemodynamic parameters). The history of family members was also taken, if available.

All patient data were collected in a disease-specific electronic medical record (PAH Tool™, Inovultus Lda, Santa Maria da Feira, Portugal).

Sample collection and DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using the Gentra Systems PUREGENE DNA Isolation kit (as per manufacturer's instructions) and quantified spectrophotometrically.

Whole-exome sequencing

Whole-exome sequencing (WES), by the Ion Proton™ System (Life Technologies), was performed according to manufacturer's recommendation. The system combines the Ion TargetSeq™ Exome Kit and the Ion Reporter™ Software. To start, DNA sample were used to build Ion Proton AmpliSeq libraries. DNA was amplified and the amplified Exome targets were ligated with Ion P1 and Ion Xpress Barcode adapters. Purified libraries were then quantified using quantitative real time Polymerase Chain Reaction (qPCR) with the Ion Library Quantification Kit. Reads were mapped using University of California, Santa Cruz (UCSC) genome browser hg19. Generated and aligned data sets were analyzed and filtered using bioinformatics softwares using the Saudi Human Genome Program (SHGP) pipeline.

Variant filtration and prioritization

The quality of each read was checked and confirmed, and low-quality reads (<20) were excluded as a primary quality check. The reads were then aligned to the hg19 human genome reference sequence using the Ion Torrent Suite program (Thermo Fisher; https://github.com/iontorrent/TS). This was followed by variant calling, through the Torrent Suite Variant Caller (TVC) program. All the variants were

annotated using a combination of public and in-house databases. The public databases were obtained from the ANNOVAR package along with other available datasets, such as the Human Gene Mutation Database (HGMD). The analysis pipeline was designed to filter out all non-relevant variants based on quality, functional characteristics, and frequency in the databases including the local SHGP database. Further analysis focused on the identification of plausible pathogenic missense, nonsense, frameshift, and canonical splice-site variants in 26 genes related to PAH including *BMPR2*, *EIF2AK4*, *ACVRL1*, *TBX4*, *GDF2*, *BMPR1B*, *SOX17*, *ENG*, *KCNK3*, *ABCC8*, *ATP13A3*, *SMAD9*, *AQP1*, *CAV1*, *BMP10*, *SMAD4*, *SMAD1*, *KCNA5*, *NOTCH3*, *TOPBP1*, *KLF2*, *FOXF1*, *RASA1*, *ABCA3*, *ACVRL1*, and *GDF2* (Table 1). The candidate risk genes related to PAH were selected as those

Table 1. List of IPAH-associated genes screened using WES in our patient cohort.[a]

Gene	OMIM® number	Associated phenotypes	Inheritance
ABCA3	601615	Surfactant metabolism dysfunction, pulmonary, 3	AR
ABCC8	600509	Hyperinsulinemic hypoglycemia	AD/AR
		Diabetes mellitus (noninsulin-dependent, permanent and transient neonatal); Hypoglycemia, leucine-sensitive	
		Pulmonary arterial hypertension	
ACVRL1	601284	Hereditary hemorrhagic telangiectasia	AD
AQP1	107776	Pulmonary arterial hypertension	AD
ATP13A3	610232	Pulmonary arterial hypertension	AD
BMP10	608748	Suggested role in vascular endothelial quiescence, expression studies, mouse model	
BMPR1B	603248	Acromesomelic dysplasia, Demirhan type	AD/AR
		Brachydactyly type A2	
		Pulmonary arterial hypertension	
BMPR2	600799	Pulmonary hypertension, familial primary	AD
		Pulmonary veno-occlusive disease 1	
CAV1	601047	Lipodystrophy, congenital generalized	AD/AR
		Pulmonary hypertension, primary, 3	
EIF2AK4	609280	Pulmonary veno-occlusive disease	AR
ENG	131195	Juvenile polyposis syndrome	AD
		Hereditary hemorrhagic telangiectasia, type 1	
FOXF1	614975	Alveolar capillary dysplasia with misalignment of pulmonary veins	AD
GDF2	605120	Hereditary hemorrhagic telangiectasia, type 5	AD
		Pulmonary arterial hypertension	
KCNA5	176267	Atrial fibrillation	AD
KCNK3	603220	Pulmonary hypertension, primary, 4	AD
KLF2	602016	Pulmonary arterial hypertension	
NFU1	605711	Multiple mitochondrial dysfunctions syndrome 1	AR
NOTCH3	600276	Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy	AD
		Lateral meningocele syndrome	
RASA1	139150	Parkes Weber syndrome	AD
		Capillary malformation-arteriovenous malformation	
		Spinal arteriovenous anomalies	
SARS2	612804	Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis	AR
SMAD4	600993	Hereditary hemorrhagic telangiectasia	AD
		JPHT syndrome; polyposis, juvenile intestinal	
		Myhre syndrome	
SMAD9	603295	Pulmonary hypertension, primary, 2	AD
SOX17	610928	Vesicoureteral reflux 3	AD
		Pulmonary arterial hypertension	
STRA6	610745	Microphthalmia, syndromic	
		Microphthalmia, isolated, with coloboma	
TBX4	601719	Amelia, posterior, with pelvic and pulmonary hypoplasia syndrome	AD/AR
		Ischiocoxopodopatellar syndrome with or without pulmonary arterial hypertension	
TOPBP1	607760	Idiopathic pulmonary arterial hypertension	AD

Note: AD: autosomal dominant; AR: autosomal recessive; JPHT: juvenile polyposis/hereditary hemorrhagic telangiectasia; OMIM: online mendelian inheritance in man.

[a]The candidate risk genes related to PAH were selected as those screened by the Blueprint Genetics Pulmonary Artery Hypertension Panel (test code CA0601), offered by Blueprint Genetics, a CLIA-certified laboratory, accredited by the College of American Pathologists (Blueprint Genetics PAH panel, test code CA0601; https://blueprintgenetics.com).

screened by the Blueprint Genetics Pulmonary Artery Hypertension Panel (test code CA0601), offered by Blueprint Genetics, a clinical laboratory improvement amendments (CLIA)-certified laboratory, accredited by the College of American Pathologists (Blueprint Genetics PAH panel, test code CA0601; https://blueprintgenetics.com). *BMP10*, *TOPBP1*, and *ABCA3* were also included in our study, as additional candidate genes, based on their association to PAH in the literature.[25–27]

Multiple web-based bioinformatic tools were used to classify the predicted pathogenicity of the identified variants. The predicted effect of each missense variant was studied using Sort Intolerant from Tolerant, Protein Variation Effect Analyzer, and MutationTaster. To analyze the effect of variants in the splice site, in silico analyses were performed using Human Splicing Finder, Spliceview, and Splice Site Prediction by Neural Network. In addition, Combined Annotation Dependent Depletion (CADD), which aggregates several prediction algorithms and conservation scores, allelic frequencies, clinical information, and additional annotations using accessible databases via ANNOVAR, was used to prioritize the variants. The variants identified in this study were also checked against the previously reported variants in HGMD and ClinVar databases.

Sanger sequencing

Polymerase chain reaction (PCR)-based validation, of any potential causative variant identified in the WES, was carried out by Sanger sequencing. PCR amplicons were sequenced using BigDye^{TM} Terminator Cycle Sequencing kit (PE Applied Biosystems, MA, USA) and an ABI 3730xl capillary sequencer. Sequencing data were analyzed using SeqMan II software 6.1 (DNAStar). Segregation of identified sequence variants, that were suspected to be the underlying genetic defect, was performed in the available family members.

Statistical analysis

Data were summarized using descriptive statistics: continuous variables are presented as mean (standard deviation) for normal data or median (interquartile range [Q1, Q3]) for non-normal data, and count (percentage) for categorical variables.

Results

Baseline characteristics of the study population

A cohort of 48 eligible patients was enrolled in the study (Table 1). From these, 46 patients (95.8%) were diagnosed with IPAH and 2 patients (4.2%) with PVOD. From the overall cohort, five patients referred to have familial history of PAH. Most patients (89.6%) were 20 years old or more at diagnosis, with a median age of 29.5 (23.3, 37.5) years. The overall ratio of females to males was 43:5. Most patients (62.5%) were on III/IV WHO FC. The baseline clinical characteristics of the patients are shown in Table 2.

Table 2. Clinical characteristics of the study population.

Characteristics	PAH cohort (n = 48)
Gender, years	
Female	43 (89.6%)
Male	5 (10.4%)
Diagnosis	
IPAH	46 (95.8%)
PVOD	2 (4.2%)
Age at diagnosis, years	29.5 (23.3, 37.5)
Family history of PAH	
Yes	5 (10.4%)
No	43 (89.6%)
Functional class	
I	0 (0)
II	18 (37.5%)
III	26 (54.2%)
IV	4 (8.3%)
6MWD, m	345 (189.7, 434.0)
RAP, mmHg	9.5 (7, 13.8)
PAP, mmHg	51 (50.3, 68.8)
PCWP, mmHg	10 (8, 12.8)
PVR, Wood units	14.1 (10.6, 18.9)
Cardiac output, L/min	3.3 (2.5, 4.0)
Cardiac index, L/min/m^2	2 (1.6, 2.3)
NT-proBNP, pg/mL	714 (185.5, 1842.3)
Uric acid, μmol/L	320 (257.9, 440.3)
DLCO (%)	65.2 (60.3, 70.9)

Note: Results are presented as median (Q1, Q3) for non-normal continuous variables and n (%) for categorical variables. IPAH: idiopathic pulmonary arterial hypertension; PVOD: pulmonary arterial hypertension with overt features of venous/capillaries (PVOD/PCH) involvement (idiopathic); PAH: pulmonary arterial hypertension; 6MWD: six-minute-walk distance; RAP: right atrial pressure; PAP: pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure; PVR: pulmonary vascular resistance; NT-proBNP: N-terminal pro-B-type natriuretic peptide; DLCO: diffusing capacity for carbon monoxide.

Clinical characterization of the patients with molecular variants in risk-associated genes

From the 48 included cases in the study cohort, 16 patients (n = 16/48, 33.3%) had a molecular variant identified in risk-associated genes. From these 16 patients with molecular variants in risk-associated genes, 2 (12.5%) patients were clinically diagnosed with PVOD and 8 (50%) were on III/IV WHO FC. In this group (n = 16), there was a female predominance (75% females vs 25% males) and the median age at diagnosis was 29.5 (20.8, 31.3) years. In this subgroup, 2 (12.5%) patients had a history of PAH within their respective families (P14 and P15). The median 6MWD was 338.5 m (230, 392), and median NT-proBNP was 699.5 pg/mL (222.3, 1081.5).[AAQ: Please spell out "PNB."]

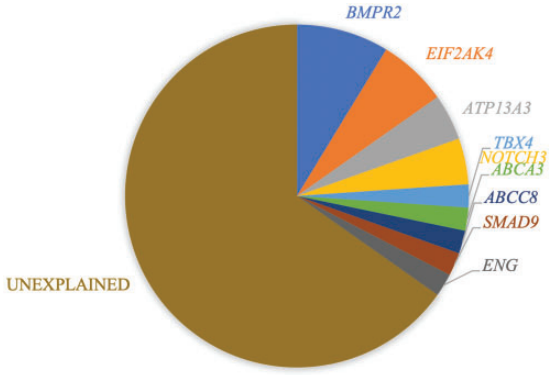

Fig. 1. Contribution of known PAH risk genes in the PAH cohort (n = 48 cases).

Genetic analyses

In this study, we expanded the search for rare variants (allele frequency < 0.01% and CADD score ≥20) in 26 established PAH risk genes (Table 1), in a cohort of 48 patients with PAH. We identified a total of 16 rare variants in 16 patients (P = 16/48; 33.3%), in 9 different risk genes underlying PAH (Fig. 1). Variants were identified in the following genes: *BMPR2* (4/16), *EIF2AK4* (3/16), *ATP13A3* and *NOTCH3* (2/16), *TBX4*, *ABCA3*, *ABCC8*, *SMAD9*, and *ENG* (1/16 each). A complete identified list of the detected variants is provided in Table 3.

BMPR2 mutations were found in four patients (P2, P4, P9, and P14). They were all females with IPAH, with ages at diagnosis of 28, 15, 35, and 32 years, respectively. We identiffour heterozygous variants in *BMPR2*, including two missense (P2, c.1226T>C:p.L409P and P14, c.967G>C:p.D323H), a frameshift (P4, c.1078_1090del: p.G360Cfs*11), and a known splice-site (P9, c.621 + 1G>A) variants. Both the *BMPR2* missense variants were predicted as likely pathogenic/pathogenic by American College of Medical Genetics and Genomics (ACMG) guidelines, with a CADD score of 27.5 and 29.9, respectively (Table 3). The missense amino acid substitutions, p.L409P and p.D323H, occur at highly conserved functionally important sites of the *BMPR2* protein and are located within a domain annotated in UniProt as protein kinase. Genotypes for *CYP1B1* (p.N453S) were determined for all the four female *BMPR2* mutation carriers. We did not identify any patient homozygous for the N/N or S/S CYP1B1 genotypes (p.N453S), while two patients (P4 and P9) were heterozygous for N/S genotype.

We also identified three homozygous pathogenic variants in *EIF2AK4*, including two nonsense (P1, c.2153C>A:p.S718X and P11, c.2488C>T:p.R830X), and a splice-site (P3, c.2686 + 2T>A) variant. Patients P3 and P11 had a diagnosis of PVOD while the P1 is diagnosed as IPAH, with the age of diagnosis as 19, 20, and 25, respectively.

We identified loss of function variants in *ATP13A3* in two patients (P8, c.282C>A:p.Y94X and P13:c.561-4A>G) and missense *NOTCH3* variants in other two patients (P15, c.3548T>C: p.V1183A and P16, c.3308T>C: p.M1103T). The identified variants in *NOTCH3* are located within the epidermal growth factor-like (EGF-like) repeat calcium-binding domains 28 and 30. A novel missense variant c.1274C>T:p.P425L in *TBX4* was identified in patient P6 (IPAH) and in silico analysis suggested a probable pathogenic effect, with a CADD score of 33 for this variant.

In four patients, we identified variants in four additional PAH associated risk genes, three missense variants (P5, *ABCA3*:p.G431S; P7, *ABCC8*:p.R881K; and P12, *ENG*:p.D401Y), and an in-frame deletion in P10, *SMAD9*:p.S214del.

Discussion

In this prospective study, we identified the genetic variations and frequency of risk genes, in IPAH and PVOD patients of Saudi Arabia. Our results highlight the genetic contribution of PAH in Saudi Arabia patients through the identification of variations in 9 genes, previously associated with PAH, in 16 patients, 4 of them with mutations in *BMPR2*.

In the three-dimensional structure of *BMPR2*, the p.D323 residue is located on the surface of the protein, while the p.L409 residue is located in an α-helix and can disturb normal splicing of the transcript. In this context, two *BMPR2* variants that have been previously associated with PAH, c.967G>A:p.D323N and c.1090delG:p.V364Cfs*11, are in same locations as our identified variants (c.967G>C:p.D323H and c.1078_1090del:p.G360Cfs*11), but affecting the protein differently, suggesting possible hotspots for deleterious changes.[29,30] We have identified *BMPR2* mutations only in the female patients, confirming that these variants underlie familial PAH (FPAH), with reduced penetrance and overrepresentation of females.[3,15] In a gene expression study implicating the estrogen-metabolizing enzyme *CYP1B1,* estrogens and/or estrogen metabolites were shown to modify FPAH risk.[31] Even though female *BMPR2* mutation carriers were associated with a four-fold higher penetrance among subjects homozygous for the wildtype genotype (N/N) than those with N/S or S/S genotypes, we could not identify any patient homozygous for the N/N or S/S CYP1B1 genotypes (p.N453S), but found two heterozygous for N/S genotype.

The diagnosis of PVOD, among our patients with homozygous pathogenic variants in *EIF2AK4*, is in agreement with previous association between these mutations and autosomal recessive PVOD-2 or pulmonary capillary haemangiomatosis.[28] Even though we could not find any apparent genotype/phenotype correlation regarding age, the young age at diagnosis of our patients with *EIF2AK4* mutations (19–25 years old) agrees with previous studies.[28]

Our results also showed loss of function variants in *ATP13A3,* which is in agreement with a recent case-control study that revealed a significant overrepresentation of rare variants in *ATP13A3* underlying heritable PAH.[30,32]

Table 3. Variants identified in PAH-related genes in a cohort of Saudi patients.

Patient ID	Gene	Mutation	Zygosity	dbSNP	Mutationtaster	SIFT_ score	PROVEAN	gnomAD exomes (f =)	GERP	CADD	Reference
P1	*EIF2AK4*	NM_001013703:exon12: c.2153C>A:p.S718X	HOM		1A	–	–	0	5.34	46	–
P2	*BMPR2*	NM_001204:exon9: c.1226T>C:p.L409P	HET		1 D	0.001 D	−5.89D	0	5.28	27.5	–
P3	*EIF2AK4*	NM_001013703:exon17: c.2686 + 2T>A	HOM		1 D	–	–	0	5.4699	25.2	–
P4	*BMPR2*	NM_001204:exon8: c.1078_1090del:p.G360Cfs[a]11	HET					0	4.3346		–
P5	*ABCA3*	NM_001089:exon12: c.1291G>A:p.G431S	HET		1 D	0 D	−5.7D	0	5.5199	–	–
P6	*TBX4*	NM_018488:exon8: c.1274C>T:p.P425L	HET		1 D	0.001 D	−2.25N	0	5.4899	33	–
P7	*ABCC8*	NM_000352:exon22: c.2642G>A:p.R881K	HET	rs765857743	1 D	0.008 D	−2.31N	0.00000795	6.17	23.3	–
P8	*ATP13A3*	NM_024524:exon4: c.282C>A:p.Y94X	HET		1 A	–	–	0	−2.8599	25.1	–
P9	*BMPR2*	NM_001204:exon5: c.621 + 1G>A	HET		1 D	–	–	0	5.92	28.6	[27]
P10	*SMAD9*	NM_001127217:exon3: c.640_642del:p.S214del	HET	rs1447247631				0	4.5399		–
P11	*EIF2AK4*	NM_001013703:exon15: c.2488C>T:p.R830X	HOM	rs370269120	1 A			0.00000401	4.82	45	–
P12	*ENG*	NM_000118:exon9: c.1201G>T:p.D401Y	HET		1 P	0.024 D	−3.29D	0	2.98	23.9	–
P13	*ATP13A3*	NM_024524:exon7: c.561-4A>G	HET	rs774703178	–			0.0000193	−6.4099		–
P14	*BMPR2*	NM_001204:exon7: c.967G>C:p.D323H	HET		1 D	0.006 D	−4.77D	0	5.1399	29.2	–
P15	*NOTCH3*	NM_000435:exon22: c.3548T>C:p.V1183A	HET		0.999 D	0.004 D	−3.74D	0	3.9	25.4	[28]
P16	*NOTCH3*	NM_000435:exon20: c.3308T>C:p.M1103T	HET	rs761582318	0.977 D	0.148 T	−2.7D	0.00000797	4.0599	19.34	–

Note: CADD: Combined Annotation Dependent Depletion; dbSNP: Database for Single Nucleotide Polymorphisms and Other Classes of Minor Genetic Variation; GERP: Genomic Evolutionary Rate Profiling; gnomAD: genome aggregation database; PROVEAN: Protein Variation Effect Analyzer; SIFT: Sort Intolerant from Tolerant; HOM: homozygous; HET: heterozygous.

[a]Rare, deleterious variants defined as gnomAD allele frequency (AF) 1.00E−04 and CADD $\geq$ 20.

The identification of missense *NOTCH3* variants within EGF-like 28 and 30 calcium-binding domains is in accordance with previous findings regarding the association of the variant p.V1183M with severity of white-matter lesions, in elderly with hypertension.[33]

We have identified a novel missense variant, *TBX4*, c.1274C>T:p.P425L in one patient P6, in the same position of a different nonsynonymous missense variant c.1274C>A: p.P425Q, previously associated with PAH.[32]

In our study, we could not confirm previous findings that evidenced very young ages at diagnosis, in PAH patients with positive gene mutations, except for those with *EIF2AK4* mutations.[14] The median age at diagnosis of the patients with gene mutations (29.5 years) is in agreement with previous reports on the Saudi Arabia PAH population.[24]

Regarding disease severity, our results showed a tendency for higher disease severity in patients with positive mutations, mainly in those with mutations in *EIF2AK4*. In fact, even though our patients with mutations were equally distributed between WHO FC II and III/IV, 6MWD, NT-proBNP, and hemodynamic parameters (cardiac index (CI), mean pulmonary arterial pressure (mPAP) and mean right atrial pressure (mRAP)) are indicative of a uncontrolled disease, as reported by Pfarr et al.[14] Mutations were identified in 30% of the IPAH patients and in 100% of the PVOD patients (n = 2). These two PVOD patients (P3 and P11) were diagnosed at a very early age (19 and 20), were in WHO FC III and IV, respectively, and were both identified with mutations in *EIF2AK4*, confirming the causal relationship described before.[28] Their clinical condition was clearly worse than that of most IPAH patients with mutations in our study. In particular, the clinical condition of patient P11 was very serious, with a very low 6MWD (47 m), low CI (1.5L/min/m^2), and high NT-proBNP.

The identification of *BMPR2* mutations in four IPAH patients confirmed the high association of these mutations with PAH.[11,13,14,34] Three of these patients were in WHO FC III and one in WHO FC II. They were all women with age at diagnosis between 19 and 35 years and CI higher than those of patients with mutations in *EIF2AK4*. These patients were characterized by a 6MWD ranging between 138 and 437 m and high NT-proBNP values.

These findings highlight the importance of genetic analysis and counseling in PAH patients or their relatives (even the asymptomatic ones), with particular emphasis in PVOD patients with *EIF2AK4* mutations, that can greatly benefit from early diagnosis and specific therapy.[13] The research on this topic has already reported emerging *BMPR2* target therapies that include strategies to rescue the functionality of mutated *BMPR2* genes or to increase signaling, such as ataluren (PTC-124).[23,35] Pre-clinical studies have also reported good results for the rescue of missense mutations with chemical chaperones, such as 4-phenylbutyrate.[36]

In this context, genetic testing can enable not only to identify asymptomatic mutation carriers, contributing to early diagnosis among relatives of symptomatic or asymptomatic patients, but also to detect heritable conditions that can culminate, for instance, on providing pre-pregnancy counseling for couples with familial mutations.

This study presents limitations that must be highlighted. First, the platform used for mutation detection is high throughput but cannot be reliably used for large gene deletions, duplications, or copy number analysis. Although, we cannot exclude that these may also represent novel genetic causes that have yet to be identified. Further functional assessment of all the newly identified potential disease variants is also warranted. The second important limitation of our study is sample size. In fact, we included 48 patients and from these only 16 presented molecular variants in risk genes and, thus, broader studies are necessary to expand the conclusions to the overall Saudi Arabia population. However, in general, our results are in line with other published studies in terms of type and prevalence of mutations. A prevalence of *BMPR2* mutations around 20%–30% has been described by several authors in studies from different geographic regions, followed by a prevalence around 15%–20% of mutations in the *EIF2AK4* gene, mainly in PVOD patients.[11,14,19,28,37]

In conclusion, our study is the first to report the underlying molecular basis of IPAH, in patients of Saudi Arabian origin and highlighted the important contribution of genetic causes for PAH. The approach when applied prospectively is of benefit to personalized diagnosis and the patient's management, while laying the foundations for future prevention. Further studies on a larger number of patients will expand the mutation spectrum in this population.

Acknowledgements

The authors thank Paula Pinto, PharmD, PhD (Pharmaceutical Medicine Academy) for providing medical writing and editorial assistance. The authors also thank the Saudi Human Genome Project and the core facilities at the Department of Clinical Genomics (Centre of Genomic Medicine) at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Author contributions

Abdullah M Aldalaan and Khushnooda Ramzan have contributed equally to the manuscript.

Conflict of interest

The author(s) declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

ORCID iD

Abdullah M. Aldalaan https://orcid.org/0000-0001-9658-081X

References

1. Kiely DG, Elliot CA, Sabroe I, et al. Pulmonary hypertension: diagnosis and management. *Br Med J* 2013; 346: f2028–f2028.
2. Voelkel NF and Tuder RM. Severe pulmonary hypertensive diseases: a perspective. *Eur Respir J* 1999; 14: 1246–1250.
3. Tuder RM, Archer SL, Dorfmüller P, et al. [Relevant issues in the pathology and pathobiology of pulmonary hypertension]. *Turk Kardiyol Dern Ars* 2014; 42: 5–16.
4. Simonneau G, Montani D, Celermajer DS, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2019; 53: 1801913.
5. Peacock AJ, Murphy NF, McMurray JJ V., et al. An epidemiological study of pulmonary arterial hypertension. *Eur Respir J* 2007; 30: 104–109.
6. Galiè N, Humbert M, Vachiery J-L, et al. 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. *Rev Española Cardiol (English Ed)* 2016; 69: 177.
7. Larkin EK, Newman JH, Austin ED, et al. Longitudinal analysis casts doubt on the presence of genetic anticipation in heritable pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2012; 186: 892–896.
8. Ivy D. Pulmonary hypertension in children. *Cardiol Clin* 2016; 34: 451–472.
9. Southgate L, Machado RD, Gräf S, et al. Molecular genetic framework underlying pulmonary arterial hypertension. *Nat Rev Cardiol* 2020; 17: 85–95.
10. Humbert M, Sitbon O, Yaïci A, et al. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. *Eur Respir J* 2010; 36: 549–555.
11. Abou Hassan OK, Haidar W, Nemer G, et al. Clinical and genetic characteristics of pulmonary arterial hypertension in Lebanon. *BMC Med Genet* 2018; 19: 89.
12. Deng Z, Haghighi F, Helleby L, et al. Fine mapping of PPH1, a gene for familial primary pulmonary hypertension, to a 3-cM region on chromosome 2q33. *Am J Respir Crit Care Med* 2000; 161: 1055–1059.
13. Girerd B, Montani D, Jaïs X, et al. Genetic counselling in a national referral centre for pulmonary hypertension. *Eur Respir J* 2016; 47: 541–552.
14. Pfarr N, Szamalek-Hoegel J, Fischer C, et al. Hemodynamic and clinical onset in patients with hereditary pulmonary arterial hypertension and BMPR2 mutations. *Respir Res* 2011; 12: 99.
15. Aldred MA, Comhair SA, Varella-Garcia M, et al. Somatic chromosome abnormalities in the lungs of patients with pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2010; 182: 1153–1160.
16. Machado RD, James V, Southwood M, et al. Investigation of second genetic hits at the BMPR2 locus as a modulator of disease progression in familial pulmonary arterial hypertension. *Circulation* 2005; 111: 607–613.
17. Loyd JE, Butler MG, Foroud TM, et al. Genetic anticipation and abnormal gender ratio at birth in familial primary pulmonary hypertension. *Am J Respir Crit Care Med* 1995; 152: 93–97.
18. Machado RD, Pauciulo MW, Thomson JR, et al. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. *Am J Hum Genet* 2001; 68: 92–102.
19. Rosenzweig EB, Morse JH, Knowles JA, et al. Clinical implications of determining BMPR2 mutation status in a large cohort of children and adults with pulmonary arterial hypertension. *J Hear Lung Transplant* 2008; 27: 668–674.
20. Valencia GA and Krishnan U. Idiopathic pulmonary arterial hypertension in children: a review. *Pulm Ther* 2017; 3: 67–92.
21. Soubrier F, Chung WK, Machado R, et al. Genetics and genomics of pulmonary arterial hypertension. *J Am Coll Cardiol* 2013; 62: D13–D21.
22. Garcia-Rivas G, Jerjes-Sánchez C, Rodriguez D, et al. A systematic review of genetic mutations in pulmonary arterial hypertension. *BMC Med Genet* 2017; 18: 82.
23. Morrell NW, Aldred MA, Chung WK, et al. Genetics and genomics of pulmonary arterial hypertension. *Eur Respir J* 2019; 53: 1–10.
24. Aldalaan AM, Saleemi SA, Weheba I, et al. Pulmonary hypertension in Saudi Arabia: first data from the SAUDIPH registry with a focus on pulmonary arterial hypertension. *Respirology*. Epub ahead of print 15 June 2020. DOI: 10.1111/resp.13879.
25. Hodgson J, Swietlik EM, Salmon RM, et al. Characterization of GDF2 mutations and levels of BMP9 and BMP10 in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2020; 201: 575–585.
26. de Jesus Perez VA, Yuan K, Lyuksyutova MA, et al. Whole-exome sequencing reveals TopBP1 as a novel gene in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2014; 189: 1260–1272.
27. Kunig AM, Parker TA, Nogee LM, et al. ABCA3 deficiency presenting as persistent pulmonary hypertension of the newborn. *J Pediatr* 2007; 151: 322–324.
28. Eyries M, Montani D, Girerd B, et al. EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension. *Nat Genet* 2014; 46: 65–69.
29. Yang H, Zeng Q, Ma Y, et al. Genetic analyses in a cohort of 191 pulmonary arterial hypertension patients. *Respir Res* 2018; 19: 87.
30. Zhu N, Pauciulo MW, Welch CL, et al. Novel risk genes and mechanisms implicated by exome sequencing of 2572 individuals with pulmonary arterial hypertension. *Genome Med* 2019; 11: 69.
31. Austin ED, Cogan JD, West JD, et al. Alterations in oestrogen metabolism: implications for higher penetrance of familial pulmonary arterial hypertension in females. *Eur Respir J* 2009; 34: 1093–1099.
32. Gräf S, Haimel M, Bleda M, et al. Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. *Nat Commun* 2018; 9: 1416.
33. Smith CD, Snowdon DA, Wang H, et al. White matter volumes and periventricular white matter hyperintensities in aging and dementia. *Neurology* 2000; 54: 838–842.
34. Thenappan T, Shah SJ, Rich S, et al. Survival in pulmonary arterial hypertension: a reappraisal of the NIH risk stratification equation. *Eur Respir J* 2010; 35: 1079–1087.
35. Drake KM, Dunmore BJ, McNelly LN, et al. Correction of nonsense BMPR2 and SMAD9 mutations by ataluren in pulmonary arterial hypertension. *Am J Respir Cell Mol Biol* 2013; 49: 403–409.
36. Sobolewski A, Rudarakanchana N, Upton PD, et al. Failure of bone morphogenetic protein receptor trafficking in pulmonary arterial hypertension: potential for rescue. *Hum Mol Genet* 2008; 17: 3180–3190.
37. Ma L and Chung WK. The role of genetics in pulmonary arterial hypertension. *J Pathol* 2017; 241: 273–280.