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

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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

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 >20mmHg, 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

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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), hypoxic 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 ToolTM, 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 PI 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. |
|-----------------|-----------------|---------------------------------|---------------------------------|
| Gene | OMIM® number | Associated phenotypes | Inheritance |
|------|--------------|-----------------------|-------------|
| ABCA3 | 601615 | Surfactant metabolism dysfunction, pulmonary, 3 | AR |
| ABCB8 | 600509 | Hyperinsulinemic hypoglycemia | AD/AR |
| 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 | AD |
| BMPR1B | 603248 | Acromesomelic dysplasia, Demirhan type | AD/AR |
| BMPR2 | 600799 | Pulmonary arterial hypertension | AD |
| CAV1 | 601047 | Lipodystrophy, congenital generalized | AD/AR |
| EIF2AK4 | 609280 | Pulmonary veno-occlusive disease | AD |
| ENG | 131195 | Juvenile polyposis syndrome | AD |
| FOXF1 | 614975 | Alveolar capillary dysplasia with misalignment of pulmonary veins | AD |
| GDF2 | 605120 | Hereditary hemorrhagic telangiectasia, type 5 | AD |
| KCNA5 | 176267 | Atrial fibrillation | AD |
| KCNK3 | 603220 | Pulmonary hypertension, primary, 4 | AD |
| KLF2 | 602016 | Pulmonary arterial hypertension | AD |
| NFU1 | 605711 | Multiple mitochondrial dysfunctions syndrome | AD |
| NOTCH3 | 600276 | Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy | AD |
| RASA1 | 139150 | Parkes Weber syndrome | AD |
| SARS2 | 612804 | Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis | AR |
| SMAD4 | 600993 | Hereditary hemorrhagic telangiectasia | AD |
| SMOH9 | 603295 | Pulmonary hypertension, primary, 2 | AD |
| SOX17 | 601928 | Vesicoureteral reflux | AD |
| STRA6 | 610745 | Microphthalmia, syndromic | AD |
| TBX4 | 601179 | Amelia, posterior, with pelvic and pulmonary hypoplasia syndrome | AD/AR |
| 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.

*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).
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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 Int 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™ 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

| 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/m²              | 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.

(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.

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.”]
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 identified four 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 identified variants in BMPR2 protein are located within a domain annotated in UniProt as protein kinase. Genotypes for CYP1B1 (p.N453S) were determined for all the four female 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 hae-mangiomatosis.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
| Patient ID | Gene      | Mutation | Zygosity | dbSNP | Mutationtaster | SIFT_score | PROVEAN | gnomAD exomes (f =) | GERP | CADD | Reference |
|------------|-----------|----------|----------|-------|----------------|----------|---------|-------------------|------|------|-----------|
| P1         | EIF2AK4   | NM_001013703:exon12: c.2153C>G:Ap.S718X | HOM       | IA    | --             | --       | --      | 0                 | 5.34 | 46   | --        |
| P2         | BMPR2     | NM_001204:exon9: c.1226T>C:Ap.L409P | HET       | I D   | 0.001 D        | -5.89D   | 0       | 5.28              | 27.5 | --   | --        |
| P3         | EIF2AK4   | NM_001013703:exon17: c.2686+2T>A | HOM       | I D   | --             | --       | 0       | 5.4699            | 25.2 | --   | --        |
| P4         | BMPR2     | NM_001204:exon8: c.1078_1090del:G360C6*11 | HET       | --    | --             | --       | --      | 0                 | 4.3346 | --   | --        |
| P5         | ABCA3     | NM_001089:exon12: c.1291G>A:Ap.G431S | HET       | I D   | 0 D            | -5.7D    | 0       | 5.5199            | --   | --   | --        |
| P6         | TBX4      | NM_018488:exon8: c.1274C>T:Ap.P425L | HET       | I D   | 0.001 D        | -2.25N   | 0       | 5.4899            | 33   | --   | --        |
| P7         | ABCB8     | NM_000352:exon22: c.2642G>T:Ap.R881K | HET       | I D   | 0.008 D        | -2.31N   | 0.00000795 | 6.17      | 23.3 | --   | --        |
| P8         | ATP13A3   | NM_024524:exon4: c.282C>G:Ap.Y94X | HET       | I A   | --             | --       | 0       | -2.8599           | 25.1 | --   | --        |
| P9         | BMPR2     | NM_001204:exon5: c.621+1G>A | HET       | I D   | --             | --       | 0       | 5.92              | 28.6 | --   | 27        |
| P10        | SMAD9     | NM_001127217:exon3: c.640_642del:p.S214del | HET       | --    | --             | --       | --      | 0                 | 4.5399 | --   | --        |
| P11        | EIF2AK4   | NM_001013703:exon15: c.2488C>T:Ap.R830X | HOM       | I A   | 0.00000401     | 4.82     | 45      | --                | --   | --   | --        |
| P12        | ENG       | NM_000118:exon15: c.1201G>T:Ap.D401Y | HET       | I 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:Ap.D323H | HET       | I D   | 0.006 D        | -4.77D   | 0       | 5.1399            | 29.2 | --   | --        |
| P15        | NOTCH3    | NM_000435:exon22: c.3548T>C:Ap.V183A | HET       | 0.999 D | 0.004 D        | -3.74D   | 0       | 3.9               | 25.4 | --   | 28        |
| P16        | NOTCH3    | NM_000435:exon20: c.3308T>C:Ap.M1103T | HET       | 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.
aRare, deleterious variants defined as gnomAD allele frequency (AF) $>0.04$ and CADD $>20$. 

Table 3. Variants identified in PAH-related genes in a cohort of Saudi patients.
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 an 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/m²), 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.

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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.

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