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CASE REPORT

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The cascade screening in heritable forms of pulmonary arterial hypertension

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Abstract

Heritable pulmonary artery hypertension (HPAH) is an increasingly recognized type of pulmonary arterial hypertension, in both pediatric and adult population. Intrinsic to hereditary disease, screening for genetic mutations within families is an important component of diagnosis and understanding burden of disease. Recently, consensus guidelines are published for genetic screening in PAH. These guidelines include recommendations for screening at diagnosis, noting individuals with presumed PAH due to familial, or idiopathic etiologies. Cascade genetic testing is specifically recommended as a testing paradigm to screen relatives for detection of mutation carriers, who may be asymptomatic. Without targeted genetic testing, familial mutation carriers may only come to attention when pulmonary vascular disease burden is high enough to cause symptoms, suggesting more advanced disease. Here, we present our collective experience with HPAH in five distinct families, specifically to report on the clinical courses of patients who were diagnosed with genetic mutation at diagnosis versus those who were offered genetic screening. In three families, asymptomatic mutation carriers were identified and monitored for clinical worsening. In two families, screening was not done and affected family members presented with advanced disease.

KEYWORDS

BMPR2 mutation, cascade testing, genetics, heritable pulmonary arterial hypertension, screening

INTRODUCTION

Approximately 25%–30% of idiopathic pulmonary arterial hypertension (PAH) is genetically driven, which is known as heritable PAH (HPAH).[1,2] The most frequent mutation causing genetic disease is in the bone morphogenetic protein receptor type 2 (*BMPR2*), a member of the transforming growth factor-β (TGF-β) superfamily, and this accounts for disease in about 70%–80% of families with PAH and 10%–20% of IPAH.[2] Like idiopathic PAH, HPAH progresses from diagnosis and is a life-shortening disorder. It is therefore intuitive that early identification and HPAH diagnosis may lead to improved outcomes for this life-threatening disease by

allowing for earlier treatment initiation.[3–5] However, until recently there have not been definitive guidelines on genetic screening in adults with PAH and therefore, HPAH diagnosis may be delayed or missed altogether.[1] In pediatrics, genetic screening more routinely done during diagnostic work up however recommendations on screening are limited to first-degree relatives.[2,6,7] Genetic testing is further complicated by variable disease penetrance, such as in *BMPR2* carriers.[8] Therefore, when to test, whom to test and how to test remain clinical dilemmas. Finally, a logistic hurdle to expanded screening of relatives is access to subjects for testing and counseling, since patient ages may cross adult and pediatric categories of care, and families may be geographically spread out. Here, we present our combined adult and pediatric experience with HPAH-related screening in five distinct families.

Case 1

A 15-year-old male with no prior medical history presented to a community hospital with presumed exercise-induced asthma exacerbation, coughing fits and syncope associated with activity. Of note, he had a 2-year history of multiple near-syncopal events. Computed tomography (CT) of the chest was read at the referring institution as "consistent with interstitial lung disease." He was transferred to our institution for further work-up and was noted to be tachycardic, dyspneic, and cyanotic with an oxygen saturation of 82% on room air upon presentation. Local review of CT chest appreciated stigma of advanced pulmonary hypertension (PH): enlarged main pulmonary artery, vascular pruning, and parenchymal mosaicism. Echocardiogram measured tricuspid regurgitation velocity of 4.6 m/s, predicting elevated right-sided systolic pressures (100 mmHg), right ventricular (RV) dilation, and significant RV dysfunction. His symptoms were consistent with World Health Organization (WHO) functional class IV. Admission B-type natriuretic peptide (BNP) was >2000 pg/mL. Diagnostic right heart catheterization (RHC) was consistent with PH: right atrial pressure (RAP) 10 mmHg, mean pulmonary artery pressure (mPAP) 61 mmHg, pulmonary capillary wedge pressure (PCWP) 8 mmHg, and indexed pulmonary vascular resistance (PVRi) of 19 Wu·m^2 and vasoreactivity testing with nitric oxide led to flash pulmonary edema. A trial of parenteral epoprostenol was started but aborted within 24 h due to pulmonary edema and bloody tracheal secretions. He was quickly listed for and successfully moved forward with lung transplantation.

Study of his explanted lungs confirmed pulmonary veno-occlusive disease. Given the potential genetic nature of this condition, genetic testing was done. This was positive for compound heterozygous pathogenic variants in the *EIF2AK4* gene, an autosomal recessive form of HPAH that is associated with pulmonary veno-occlusive disease, and a variant of unknown significance in *NOTCH3* gene (Table 1). Genetic screening was offered to the proband's immediate family members. One sister was also found to have biallelic *EIF2AK4* mutations, though she was an asymptomatic mutation carrier (AMC) at screening. She was monitored with scheduled clinical assessments, six-minute walk tests, BNP level, and annual echocardiograms. Six months after her mutation was detected, she was noted to be hypoxemic on a routine six-minute walk test. Echocardiogram suggested elevated right heart pressures and diagnostic RHC confirmed PH: mPAP 35 mmHg, and a PVRi of 8.95 Wu·m.^{2} She was cautiously initiated on sildenafil with good clinical response. However, a year later her symptoms progressed from WHO functional class I to class III. A repeat RHC confirmed worsening disease with mPAP 46 mmHg and PVR of 18.3 Wu·m.^{2} Given the rapid progression, she was cautiously initiated on parenteral epoprostenol and listed for lung transplantation shortly thereafter. Unlike her brother, who was transplanted a little more than 1 month from clinical diagnosis, this patient was transplant-free for 36 months from molecular diagnosis.

TABLE 1 Case 1 illustration.

Case number (relationship)	Type of genetic testing	Genes affected	Outcome
1A (proband)	Symptomatic screening[a]	*EIF2AK4, NOTCH3*	Transplanted at 1 month from diagnosis
1B (sister of 1A)	Cascade screening	*EIF2AK4, compound heterozygote*	Transplanted at 36 months from diagnosis
1C (father of 1A and 1B)	Cascade screening	*EIF2AK4, carrier*	Not applicable
1D (mother of 1A and 1B)	Cascade screening	*EIF2AK4, carrier*	Not applicable
1E (sister of 1A and 1B)	Cascade screening	None	Not applicable

[a]Testing requested and completed after transplantation.

Case 2

A 21-year-old male with no past medical history presented with progressive shortness of breath and exertional dyspnea at an outside healthcare facility. He was noted to be positive for compound heterozygous pathogenic variants in the *EIF2AK4* gene and a variant of uncertain significance in the *BMPR2* gene (Table 2). He successfully underwent bilateral lung transplantation at that age 22 with excellent reported outcomes. The time from diagnosis to transplantation was 5 months. All of this occurred at an outside hospital, and so none of his hemodynamic data pretransplant was available for review. When the family moved to our area, the family contacted our centers for assessment of the asymptomatic 16-year-old male brother. The brother was referred for genetic screening, which confirmed biallelic *EIF2AK4* mutation. His echocardiogram was normal at screening. He was diagnosed as an AMC and is currently being monitored with annual echocardiograms, BNP levels, six-minute walk testing, and PFTs. He has not undergone a cardiac catheterization due to normal screening. He remains transplant-free at the time of this communication (48 months).

Case 3

A 7-year-old female (proband) with a history of allergic rhinitis, asthma, and snoring presented with progressive dyspnea on exertion and recurrent syncopal events. PH was suspected and confirmed by RHC: mPAP 45 mmHg, PCWP 13 mmHg, and PVRi 9.6 Wu·m.^{2} She was quickly initiated on upfront combination therapy. Due to the severity of disease presentation, genetic testing was performed and resulted in a heterozygous pathogenic variant in the *BMPR2* gene. Familial testing was offered but declined, due to various social stressors. Given disease progression, this child underwent lung transplantation 48 months after diagnosis.

Unfortunately, 2 years after diagnosis, the 27-year-old father of the proband was diagnosed with severe PAH at an outside hospital (no data available) and started on epoprostenol for RV failure. A year following this, the 29-year-old paternal uncle of proband was also diagnosed with severe PAH after presenting to the emergency department with symptoms of presyncope. His RHC confirmed PH: mPAP 52 mmHg and PVR of 11.4 wood units. He was started on oral therapies with good response. Genetic testing resulted in the same pathogenic variant in the *BMPR2* gene that had been identified in the proband, his niece (Table 3). Genetic testing was then offered to the uncle's children. His 2-year-old daughter (cousin of the proband) was noted to be a *BMPR2* mutation carrier with normal echocardiogram and normal examination. She continues to be monitored annually as an AMC with echocardiograms and BNP levels, with plans to undergo six-minute walk testing and PFTs when age-appropriate. See Figure 1 for pedigree of this family.

TABLE 2 Case 2 illustration.

Case number (relationship)	Type of genetic testing	Genes affected	Outcome
2A (proband)	Symptomatic screening at diagnosis	*EIF2AK4*, *BMPR2*	Transplanted at 5 months from diagnosis
2B (brother of 2A)	Cascade screening	*EIF2AK4*	AMC, under surveillance monitoring

Abbreviation: AMC, asymptomatic mutation carrier

TABLE 3 Case 3 illustration.

Case number (relationship)	Timing of genetic testing	Genes affected	Outcome
3A (proband)	Symptomatic screening at diagnosis	*BMPR2*	Transplanted 48 months after diagnosis
3B (father)	(Testing declined)	Not known	On combination therapy
3C (uncle of 3A, brother of 3B)	Symptomatic screening at diagnosis	*BMPR2*	On combination therapy
3D (paternal cousin of 3A, daughter of 3C)	Cascade screening	*BMPR2*	AMC, under surveillance
3E (paternal cousin of 3A, son of 3C)	Cascade screening	None	Not applicable
3F (paternal cousin of 3A, daughter of 3C)	Cascade screening	None	Not applicable

Abbreviation: AMC, asymptomatic mutation carrier.

Case 4

A 26-year-old male presented with exertional dyspnea and chest pain for 2 months. Echocardiogram at an outside facility suggested elevated right heart pressures, and a preliminary diagnosis of PH was made. RHC showed mPAP to be elevated to 78 mmHg. His symptoms were consistent with WHO functional class III, so he was quickly started and escalated on combination therapy including parenteral epoprostenol at 6 months after diagnosis. Genetic testing was not performed, and the etiology of his disease remained unknown. However, 5 years following his diagnosis, his 5½-year-old daughter presented with a 2-month history of episodic chest pain with exercise and cyanosis. RHC confirmed PH: RAP 7 mmHg, mPAP 58 mmHg, PCWP 8 mmHg, cardiac index 3.7 L/min/m.^{2} Her symptoms were classified as WHO functional class II. Given the high index of suspicion for hereditary PAH, the daughter underwent genetic testing, which revealed a heterozygous pathogenic variant in the *BMPR2* gene (Table 4). Eventually, her disease progressed and required parenteral prostacyclin 4.5 years later (age 9). Over the course of the next several years, both father and daughter continued to have disease progression, and transplants were sought. The father underwent transplantation 156 months after his initial diagnosis, and the daughter underwent transplantation 90 months after her initial diagnosis.

Case 5

A 25-year-old female (proband, Table 5) with a medical history of sleep apnea and hypothyroidism presented with lower extremity edema, paroxysmal nocturnal dyspnea, and exertional dyspnea. Echocardiogram revealed severely depressed RV systolic failure, and RHC confirmed PH: mPAP 76 mmHg and PVR of 19 wood units. Symptoms were consistent with WHO functional class III. She was consequently started on sildenafil with improvement of her symptoms. However, over the course of 3 years, she required titration of her

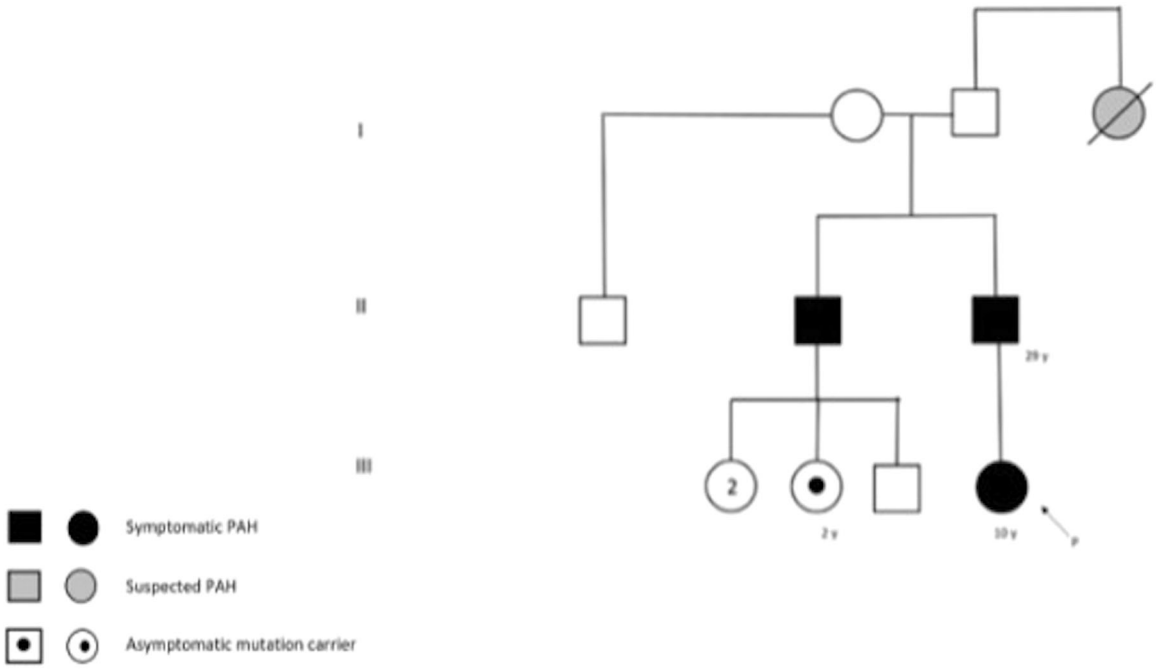

FIGURE 1 Representative family pedigree of Case 3. Pediatric patient (child) diagnosed in 2018; parental testing declined. Cascade testing not done. Father of proband diagnosed in 2020. Cascade testing not done. Brother of father (paternal uncle of proband) diagnosed in 2021. At that time, cascade testing was done and revealed same pathogenic mutation in proband and one child.

TABLE 4 Case 4 illustration.

Case number (relationship)	Type of genetic testing	Genes affected	Outcome
4A (proband)	(Testing not done)	Not known	Transplanted 13 years after diagnosis
4B (daughter of 4A)	Symptomatic screening at diagnosis	*BMPR2*	Transplanted 7.5 years after diagnosis

TABLE 5 Case 5 illustration.

Case number (relationship)	Type of genetic testing	Genes affected	Outcome
5A (proband)	Symptomatic screening for clinical worsening[a]	*BMPR2*	On combination therapy
5B (cousin of proband)	Symptomatic screening at diagnosis	*BMPR2*	On combination therapy

[a]Testing was delayed for 8 years, until after 5B was diagnosed.

regimen to ambrisentan, riociguat, and selexipag. Genetic testing was not done at diagnosis.

At this time, a 10-year-old cousin of the proband was hospitalized for frank right heart failure at an outside healthcare facility, consistent with PH by report (no data available). This cousin underwent genetic testing, which revealed a *BMPR2* mutation.

Eight years after the initial diagnosis of PH, the proband was admitted for cardiogenic shock associated with progressive PH. At that time, she underwent genetic testing and was also found to have *BMPR2* mutation.

DISCUSSION

The importance of screening for genetic mutations in idiopathic PAH is crucial, as this is needed to inform of possible HPAH pathology. And expansion of this testing to asymptomatic relatives through cascade mechanism is vital to support anticipatory guidance and surveillance. It falls to reason that if mutation carriers are not identified by screening, they will only come to attention when pulmonary vascular disease burden is high enough to cause symptoms, risking poorer outcomes.[4,5] This case series highlights a couple of different scenarios. When relatives underwent screening, AMCs were identified before symptomatic disease, allowing for anticipatory and robust management in a methodical manner (Cases 1 and 2). In cases with undiagnosed HPAH or unscreened relatives, affected individuals typically presented with worse initial hemodynamics, requiring aggressive management due to severe disease (Cases 3 and 4). And finally in Case 5, the HPAH diagnosis was missed which resulted in a delay for expectant management and counseling, medication intensification, and significant morbidity (cardiogenic shock for the proband and right heart failure for the younger cousin).

Cascade testing is a screening methodology that is already used in conditions such as familial hypercholesterolemia, Lynch syndrome, and heritable cancers and has now been formally recommended for PAH due to identified pathogenic/likely pathogenic variants.[1,9] In cascade testing, identification of an index case prompts screening of first-degree family members. If these relatives are positive for mutations, the cycle is repeated (cascaded) to their first-degree relatives, and so on, allowing for targeted screening. If no mutation is detected in a relative, no further subsequent first-degree relatives are screened. This approach is especially effective in genetic mutations that are inherited in an autosomal dominant fashion, such as *BMPR2* gene mutation and other HPAH mutations[3,10] However, this screening is not without limitations or obstacles. Identification of AMCs requires medical support for surveillance, especially given age and geographic variability within families. Furthermore, the anxiety associated with generalized genetic testing may be significant, as there are no prevention strategies for AMCs to avoid disease, and positive genetic screening is not definitively associated with disease.[2,7]

There are limitations to our observations. First, we note that screened relatives were younger, as screening is not a common practice for adults; which limits comparability of the groups. It is possible that the index case in each family had a more fulminant course because of presumed increase in severity in different generations and that there may be lead-time bias in assessing our results. However, this argument further supports the need for implementation of genetic screening of adult PAH patients at diagnosis, with hopes to identify pediatric AMCs before they develop clinically significant pulmonary vascular disease.

Second, cascade screening requires positive identification of a mutation(s) in one of the known genes that cause PAH. Although HPAH has been suspected since 1951, and autosomal dominant mode of inheritance since 1984, the current scientific understanding of genetic mutations likely falls short of the myriad of potential mutations that may give rise to HPAH.[1,11] Genetic discovery is advancing rapidly; however, it is possible that genetic testing may be non-diagnostic (i.e., negative) but there is a yet-to-be identified heritable PH mutation. In such instances, cascade testing would not be offered due to the lack of identified mutation, and individuals may not realize or appreciate their at-risk status for HPAH. That would be a missed opportunity for detection of AMC and genetic counseling. Lack of known familial mutation on initial screening should prompt consideration of retesting as mutation panels expand and new

PAH genes are identified. Reconsideration for expanded testing should also be considered in the event of new diagnoses of PH within the family.

Finally, although our two PH centers communicate regularly, they are not part of the same hospital system. We provide care for patients from infancy to elderly, but the care is divided into two distinct care teams and three different hospitals. Patients are referred to our area from a wide geographic region, which can also limit continuity. These team, age and geographic constraints likely limited access to data and family members for more expanded testing. Although this is a limitation in our report, it is also reflective of real-world obstacles to patient care, particularly as it relates to expanded genetic testing.

In conclusion, genetic screening to identify patients with mutations and then to identify affected relatives is an important aspect of PAH care. As noted in previous reports, annual monitoring of these screened, asymptomatic individuals may allow for earlier diagnosis of HPAH and earlier interventions which can potentially affect morbidity and/or mortality.[3] Review of our experience suggests that screening for AMCs may be clinically beneficial and consistent with consensus guidelines, and that cascade testing should be considered as a screening methodology in families with known familial mutation.[7] We encourage larger prospective studies accounting for demographics, risk exposures, and socioeconomic status to better understand the burden of genetic disease and AMCs, particularly in the pediatric population. It is our hope that further study will continue to inform, refine, and apply strategies such as cascade testing and comprehensive genetic testing more broadly in pediatric and adult care settings.

AUTHOR CONTRIBUTIONS

Nidhy P. Varghese: Participated in the study design, interpretation of results, writing, editing and final revisions of this manuscript. Dr. Varghese takes responsibility for her contribution to the final product. **Akhilesh A. Padhye**: Participated in the writing of the research letter, data collection, analysis and the interpretation of the results. Dr. Padhye takes responsibility for his contribution to the research letter. **Pilar L. Magoulas**: Participated in study design, genetic analysis, and pedigree creation and contributed to the genetics discussion. **George B. Mallory**: Participated in planning, research design, data collection, interpretation of results and revision of final manuscript. **Fadel E. Ruiz**: Participated in planning, research design, data collection, interpretation of results and revision of final manuscript. **Sandeep Sahay**: Participated in the design of the study, data collection, interpretation of the results, writing and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted. Dr. Sahay is the guarantor of the paper, taking responsibility for the integrity of the work, from inception to published article.

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CONFLICTS OF INTEREST STATEMENT

Sandeep Sahay: Speaker and advisor for Actelion, Bayer and United Therapeutics. Advisor for Boehringer Ingelheim, Liquidia Technologies, Gossamer Bio and Altavant Sciences. Clinical trial endpoint adjudication committee member for a GSK sponsored RCT, Research grant from ACCP CHEST Foundation, Research grant from United Therapeutics, Consultant for Acceleron Pharmaceuticals. The remaining authors declare no conflict of interest.

ETHICS STATEMENT

Wherever applicable consent has been obtained from the patients. Many patients are deceased now so its impossible to obtain consent. This is IRB exempt as its retrospective case report.

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