Genotypes and Phenotypes: Making Progress Toward a Precision Medicine Approach in Pediatric Pulmonary Hypertension

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Pediatric pulmonary hypertension (PH) is a heterogeneous disease that includes etiologies related to growth and development that are unique to children. Recent pediatric registry studies have characterized diverse phenotypes even within recognized PH subtypes, including PH associated with congenital heart disease and developmental lung disease. Advances in genetics are resulting in increased understanding of the genetic basis for PH, with recent discoveries such as *TBX4* mutations specific for pediatric-onset pulmonary arterial hypertension (PAH) and *SOX17* related to congenital heart disease–associated PAH. In addition to potential genetic underpinnings, PAH risk and clinical presentation in children with congenital heart disease may vary by cardiac condition, such as single-ventricle physiology or transposition of the great arteries. Growth and development of the pulmonary vasculature likely plays a role in all pediatric PH, which is highlighted by the disruption of normal lung growth in patients with PH related to prematurity and developmental lung disease. These diverse pediatric genotypes and phenotypes underscore a need for an individualized approach to diagnose and treat the complex pediatric PH population. Magnetic resonance imaging (MRI) is increasingly being used to improve clinical characterization of PH in children, with recent identification of specific MRI biomarkers associated with PH severity and outcomes. While much progress has been made, additional understanding of the important genetic causes and developmental concepts in pediatric PH is needed to develop a precision medicine approach to diagnosis and treatment of children with PH.

The current World Health Organization (WHO) Nice classification of PH categorizes etiology of PH as: Group 1, PAH; Group 2, PH due to left heart disease; Group 3, PH due to respiratory disease or hypoxia; Group 4, chronic thromboembolic pulmonary hypertension; Group 5, other/multifactorial causes.[1] While this taxonomy includes some pediatric-specific etiologies, classification of the pediatric patient remains challenging. Although similar in many ways to adult disease, there are unique aspects of pediatric PH that may be related to growth and development of the pulmonary vasculature.

PAH presenting in childhood differs in several ways from that in adults, including presenting symptoms (more syncope, less peripheral edema), hemodynamics (more preserved cardiac output, less right atrial pressure elevation), etiology (more idiopathic and congenital heart disease–associated, less connective tissue disease–related PAH), histopathology (more medial hypertrophy, fewer thrombi), and a lack of overwhelming female preponderance.[2] This suggests a need to refine the phenotypes outlined by the current WHO Nice and Pulmonary Vascular Research Institute (PVRI) Panama PH diagnostic classification systems.[1,3]

Recent registry studies have focused on WHO Nice Group 1 PAH, finding idiopathic PAH and PAH associated with congenital heart disease to be most prevalent in children.[4,5] Preliminary data from the Pediatric Pulmonary Hypertension Network (PPHNet) registry of nearly 1500 pediatric subjects in North America indicate that PH secondary to developmental lung disease such as bronchopulmonary dysplasia or congenital diaphragmatic hernia is now the most common PH subtype in children.[6] The recent Spanish registry found significantly higher mortality in children with non-Group 1 PAH, with survival rates at 1 and 3 years of 89% and 85%, respectively, in Group 1 PAH vs. 80% and 74%, respectively, in the entire cohort.[7] This highlights the importance of understanding the role of growth and development in these distinct phenotypes of pediatric pulmonary vascular disease. Additionally, there is increasing recognition of heterogeneity within congenital heart disease–associated PAH. For instance, PAH associated with transposition of the great arteries or single-ventricle physiology have differing causes and outcomes.[3] Finally, there are a number of pediatric patients with mixed etiologies of PH, such as a child with trisomy 21, obstructive sleep apnea, and congenital heart disease[8] or an infant with congenital diaphragmatic

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hernia with related lung hypoplasia and left heart diastolic dysfunction.[9] Therefore, recognition of precise phenotypes is important for assessing prognosis and pursuing targeted therapies for the individual patient using a precision medicine approach.

RECENT ADVANCES IN GENETICS OF PEDIATRIC PAH

Identification of novel gene mutations associated with PAH is revealing heritable underpinnings to what was previously classified as idiopathic PAH. It is estimated that 21% of pediatric PAH has a genetic etiology.[10] In recent years, a number of genes have been implicated in idiopathic and familial PAH. Mutations in bone morphogenetic protein receptor II (*BMPR2*) have been identified as the major cause of heritable PAH (~70%) and idiopathic PAH (~20%). Mutations in *ACVRL1* and *ENG* (associated with hereditary hemorrhagic telangiectasia), *BMPR1B*, *SMAD9*, *CAV1,* and *KCNK3* are also associated with PAH and mutations in *EIF2AK4* have been shown to cause pulmonary capillary hemangiomatosis/pulmonary veno-occlusive disease (PCH/PVOD).[11-13] Also, variants in *SOX17*, a gene encoding a transcription factor involved in vascular development and remodeling, were also recently identified in multiple cohorts of adults and children with heritable, idiopathic, and congenital heart disease–associated PAH.[14-16]

To determine the role of these genetic variants in pediatric- versus adult-onset PAH, Zhu and colleagues recently compared genetic analysis of adults and children with idiopathic and heritable PAH. They observed similar frequencies of pathogenic mutations in *BMPR2* and other known PAH risk genes but found mutations in *TBX4* enriched in pediatric-onset PAH.[10] *TBX4* encodes a transcription factor in the T-box gene family expressed in the heart, limbs, and mesenchyme of the lung and trachea. Mutations or deletions of *TBX4* have been associated with small patella syndrome and skeletal abnormalities of the pelvis and lower limbs as well as PAH, which is predominantly pediatric-onset.[17-19] Zhu et al found *TBX4* mutation carriers had a 20-year earlier mean age of PAH onset compared with *BMPR2* mutation carriers. Within the *TBX4* cohort, PAH severity and associated skeletal abnormalities were variable.[10,18] *TBX4* represents the first genetic diagnosis primarily associated with pediatric-onset PAH and may provide insight into mechanisms of disease development in infants and children.

MULTIPLE PHENOTYPES IN CONGENITAL HEART DISEASE–ASSOCIATED PAH

Multiple phenotypes have been established in the congenital heart disease population. The Nice classification highlights 4 main types of PAH associated with congenital heart disease: Eisenmenger syndrome, PAH with left-to-right shunts, PAH with coincidental congenital heart disease (small defects), and postoperative PAH.[1] Shunt lesions have long been recognized as contributing to development of PAH. Natural history studies show that unrepaired, large post-tricuspid shunts, such as ventricular septal defects, lead to irreversible PAH in the majority of patients by 2 years of age, informing current practice for early repair of such defects.[20,21] Patients with pre-tricuspid shunts, such as atrial septal defects, have a much lower risk of developing PAH that tends to occur later in life (6% in one study).[22] There are also patients with left-to-right shunts who develop PAH despite appropriate surgical repair, which may suggest a genetic predisposition such as the *SOX17* variants described above.[16] Similarly, it is well described that a small number (<1%) of patients with transposition of the great arteries develop PAH despite neonatal surgical repair, again suggesting a potential genetic predisposition to developing PAH, although no genetic target has been identified to date.[23]

Patients with single-ventricle physiology represent another unique subset of children with congenital heart disease who may benefit from a precision medicine approach to diagnosis and treatment. Surgical palliation with cavopulmonary anastomosis (Glenn and Fontan operations) relies on direct drainage of systemic venous return into the pulmonary arteries such that small elevations in pulmonary vascular resistance can impair cardiac output. The pediatric Panama classification recognized that these patients may have pulmonary vascular disease without meeting traditional PAH criteria, and recommended altered criteria of indexed pulmonary vascular resistance >3 indexed Wood units or transpulmonary pressure gradient >6 mm Hg.[3] Pulmonary vasodilator therapy has been described in a number of cohorts of single-ventricle patients with some improvement in exercise tolerance and functional class using sildenafil[24] and bosentan.[25]

Dramatic improvement in pulmonary vascular resistance and oxygen saturation was observed in a cohort of single-ventricle patients who received subcutaneous treprostinil (Figure 1) with dramatic clinical improvement in some cases.[26] Despite these studies suggesting benefit, optimal PH diagnostic criteria for treatment and approach to pulmonary vasodilator therapy in single-ventricle patients remain somewhat unclear.

DEVELOPMENTAL BASIS FOR PH ASSOCIATED WITH PREMATURITY AND LUNG DISEASE

While mechanistically different from WHO Group 1 PAH, PH associated with developmental lung disease (WHO Group 3) also results from a reduction in functional vascular area. Instead of the loss of preexisting vessels observed in PAH, developmental PH results from lack of normal growth of the vasculature. This can be due to interrupted development caused by premature birth and subsequent postnatal lung injury and/or from lung hypoplasia secondary to congenital anomalies, such as congenital diaphragmatic hernia.

Advances in medical care have allowed for survival of premature infants at progressively lower gestational ages. While overall survival of infants born at 22-28 weeks has improved in recent years, the incidence of bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, has increased.[27,28] Retrospective cohort studies estimate the incidence of PH to be 50% to 58% in infants with severe BPD, which contributes to significantly increased

mortality.[29,30] Risk factors for PH in this population include lower gestational age and worse BPD, but prediction remains limited. Additional predisposing conditions include small birth weight for gestational age, oligohydramnios, preeclampsia, and postnatal injuries (ie, prolonged duration of mechanical ventilation and oxygen therapy), suggesting that PH susceptibility is related to a complex interplay of genetic, developmental, and environmental factors (Figure 2).[30,31]

As lung development normally continues until gestation and even beyond, extremely premature infants may be delivered prior to sacculation and alveologenesis, the final phases of lung development that generate functional gas exchange units (alveoli) required for respiration. Understanding of normal lung development is critical both to determine how disruption leads to BPD and PH and to develop therapies directly targeting lung and blood vessel growth. Recent studies have focused specifically on identifying molecular mechanisms driving the saccular and alveolar stages. Generation of normal alveolar structure requires complex interactions between multiple cell lineages, including epithelial, mesenchymal, and endothelial cell types.[32] Studies in rodents have shown an important role for Wnt signaling to promote maturation and expansion of alveolar type 2 cells in alveologenesis.[33]

Studies of BPD pathogenesis demonstrate simplified lung architecture and also immature vasculature, supporting a coordinated codevelopment of the lung airspaces and vasculature.[34,35] This is supported by prospective clinical studies showing that early diagnosis of PH is associated with a high risk of developing BPD at 36 weeks postmenstrual age.[36] Vascular endothelial growth factor (VEGF) is an active angiogenic factor that is also important for distal airspace growth.[37] Recently, inhibition of VEGF with a monoclonal antibody for soluble fms-like tyrosine kinase-1 (sFlt-1) preserved lung structure and function and prevented right ventricular (RV) hypertrophy in an experimental rat model of BPD, suggesting that early targeted therapy may provide a novel strategy for BPD prevention.[38]

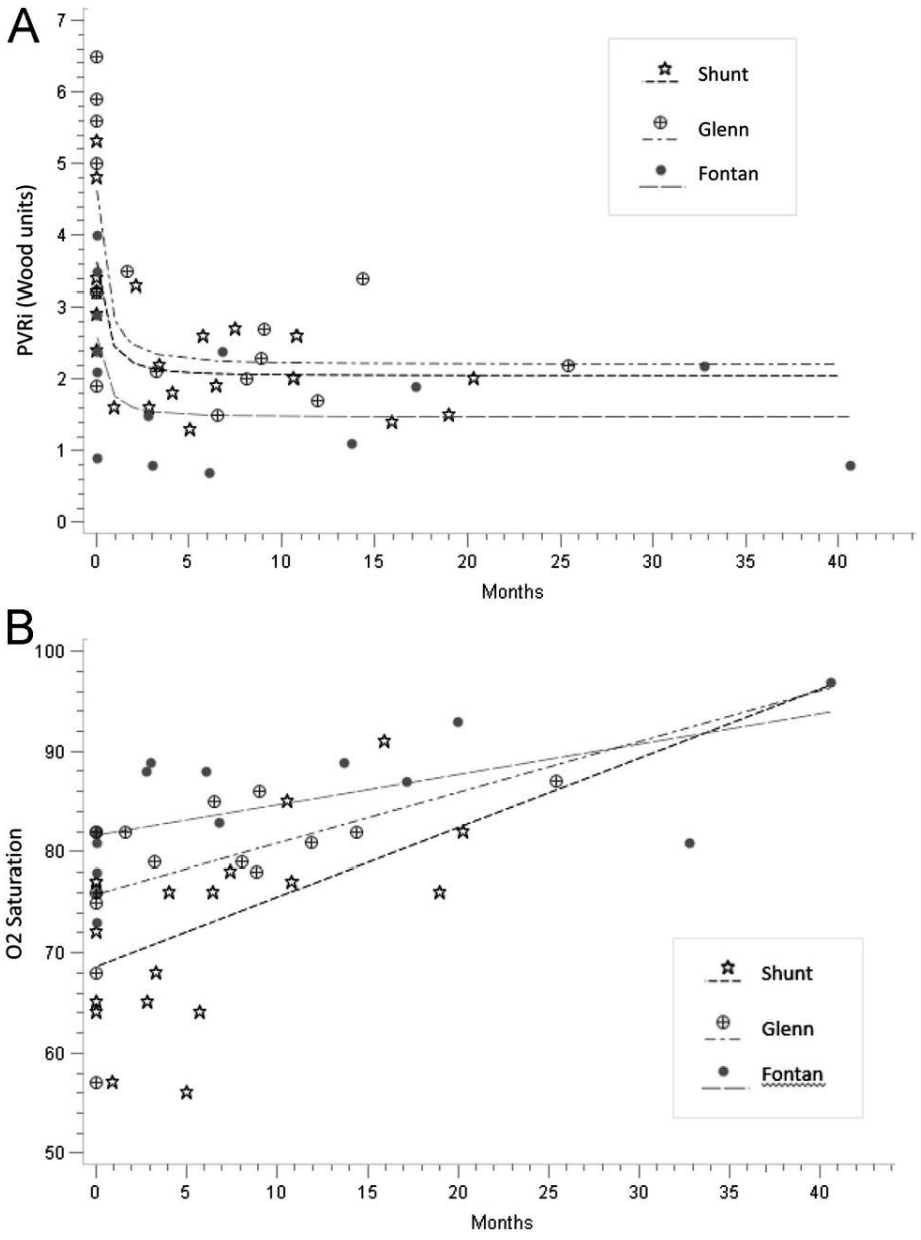

Figure 1: Best polynomial fit for indexed pulmonary vascular resistance (A) and oxygen saturation (B) over time per group in a pediatric cohort of single-ventricle patients treated with treprostinil. Reprinted from Handler SS, Ogawa MT, Hopper RK, Sakarovitch C, Feinstein JA. Subcutaneous treprostinil in pediatric patients with failing single-ventricle physiology. *J Heart Lung Transplant*. 2017 Sep 14, with permission from Elsevier.

Normal vascular development continues postnatally but may be negatively impacted by injury and inflammation from hyperoxia, mechanical ventilation, infection, and aspiration. These insults to the developing lung can decrease alveolar-capillary surface area and worsen PH.[39] Current therapies focus on lung protection and growth by limiting mechanical trauma, optimizing lung expansion, and preventing infection and aspiration.[40] Little is understood about the response of the developing lung to acute injury postnatally and therefore, targeted therapies are not yet available. Studies using a mouse model of regeneration after influenza-induced lung injury reveal a complex process, mediated by lung progenitor cells. Wnt signaling also appears to be important in mediating response to acute lung injury by alveolar epithelial progenitor cells.[41] Detailed understanding of these normal developmental mechanisms and response to injury may be harnessed in the future to develop therapies to promote and restore

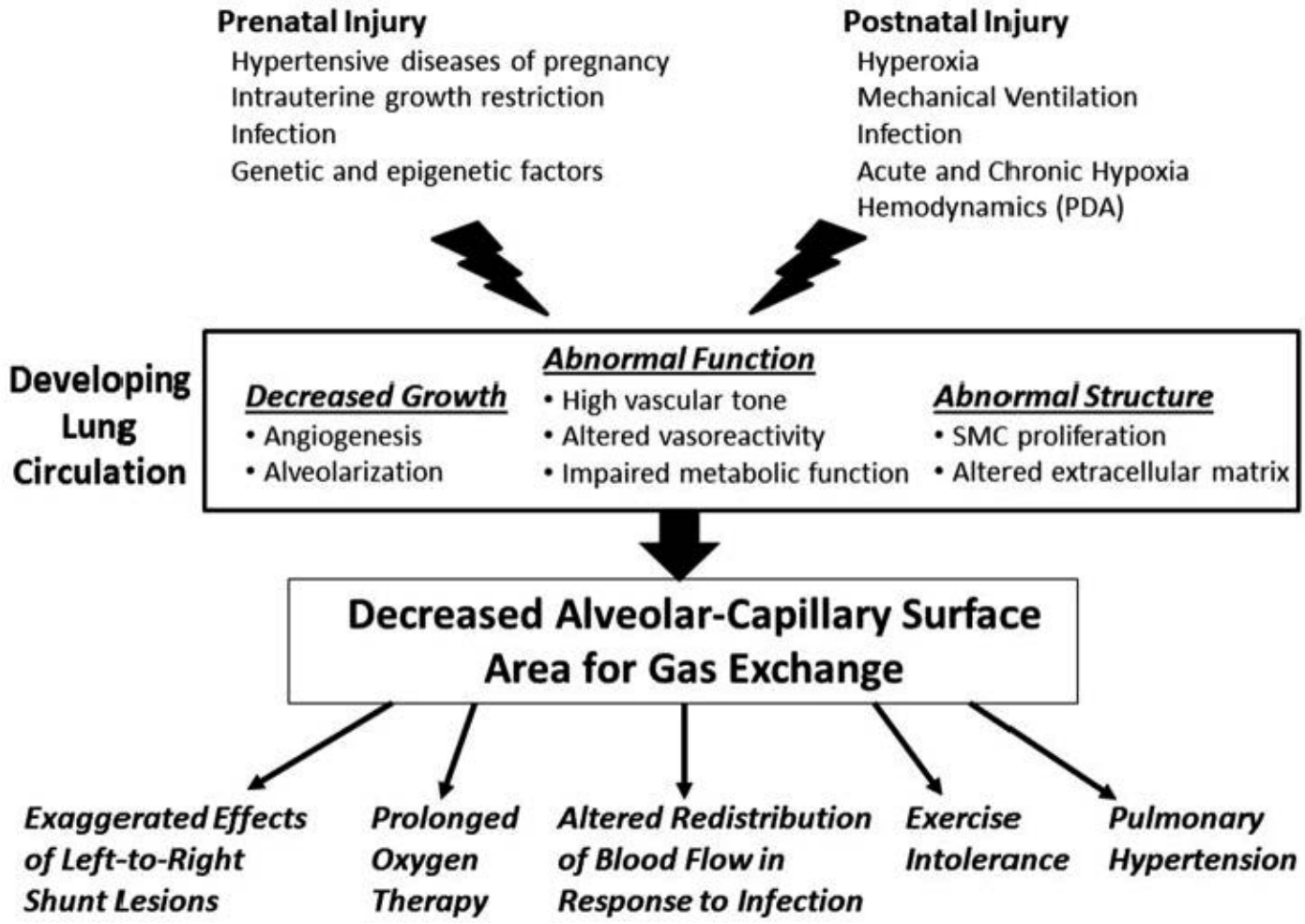

Figure 2: Schematic illustrating the components contributing to pulmonary vascular disease in BPD and the resulting clinical manifestations. Reprinted from Mourani PM, Abman SH. Pulmonary Hypertension and Vascular Abnormalities in Bronchopulmonary Dysplasia. *Clin Perinatol*. 2015;42(4):839-855, with permission from Elsevier.

postnatal alveolar development. Therapies currently being investigated include mesenchymal stem cells and isolated exosomes, which may attenuate lung injury through transfer of VEGF.[42-44]

DIAGNOSIS AND TREATMENT WITH A PRECISION MEDICINE APPROACH

Advances in Phenotyping Using MRI Biomarkers

A personalized medicine approach to the child with PH will need to include an individualized approach to diagnosis and monitoring. While cardiac catheterization remains the gold standard for hemodynamic assessment and PH diagnosis, there is increasing interest in noninvasive assessments by echocardiography and MRI. These modalities not only alleviate risks associated with invasive procedures and anesthesia but also provide additional assessment of RV function not determined by hemodynamics alone. There is growing interest in use of novel MRI biomarkers, including measures of flow dynamics and RV performance, which may add to hemodynamic assessment of PAH severity and inform prognosis. MRI has some distinct advantages compared to other high-resolution imaging modalities, notably the lack of ionizing radiation and high spatial resolution. For some pediatric patients, use may be limited by long image acquisition time that may necessitate anesthesia in younger or developmentally delayed patients and use of metal-containing medication pumps or devices.

Various MRI parameters may provide unique insight into abnormal vascular function and remodeling in PAH patients. MRI assessment of wall shear stress (ie, the stress applied to the vessel endothelium by flowing blood) is decreased in children with PAH relative to controls and may reflect severity of downstream vascular remodeling.[45] Similarly, wave intensity analysis reveals increased pulmonary vascular stiffness in children with PAH relative to controls.[46] Increased main pulmonary artery stiffness and decreased wall stress correlate with metrics of RV performance and predict clinical outcomes in a pediatric PAH cohort.[47]

Much of the focus of recent pediatric studies has been on determining utility of specific MRI biomarkers to predict outcomes. Moledina et al described right ventricular ejection fraction (RVEF) and left ventricular stroke volume index as most strongly associated with survival

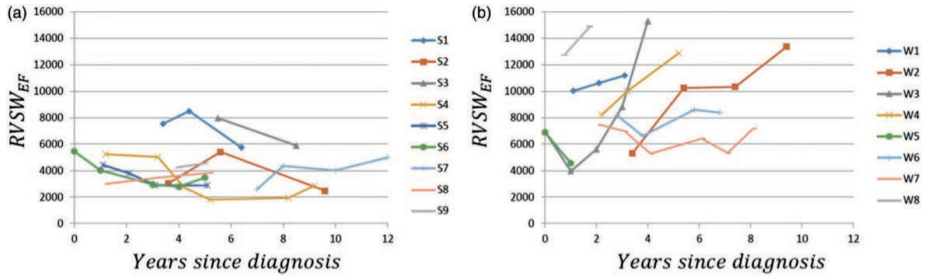

Figure 3: Longitudinal right ventricular stroke work indexed by ejection fraction ($RVSW_{EF}$) by group (stable [a] vs clinical worsening [b]). An upward trend in $RVSW_{EF}$ over time is observed for patients who demonstrated clinical worsening, while the trend for stable patients is downward. From Yang W, Marsden AL, Ogawa MT, et al. Right ventricular stroke work correlates with outcomes in pediatric pulmonary arterial hypertension. *Pulm Circ*. 2018;8(3):2045894018780534. Reprinted by Permission of SAGE Publications, Ltd.

in a cohort of children with PAH but noted that RV volumes were also significant. They identified specific cutoffs of RVEF <44% and left ventricular stroke volume index <34 mL/m^2 correlated with decreased 3-year survival.[48] In addition, Blalock et al found markedly abnormal RV volumes and RVEF in children with PAH despite normal 6-minute walk distances, suggesting that RV changes may precede clinical symptoms of RV failure in children.[49] Novel research measures include MRI assessment of ventricular-vascular coupling (the relationship between vascular and ventricular function) and RV stroke work. The degree of ventricular-vascular decoupling was recently shown to predict RV dysfunction and prognosis.[50] Yang et al used computational modeling to include both hemodynamic and MRI data in a calculation of RV stroke work, which outperformed both pulmonary vascular resistance and RVEF in predicting clinical worsening in a cohort of children with PAH.[51] In addition to the absolute values, the rate of change of RV stroke work may be particularly helpful to predict clinical worsening in the individual patient (Figure 3). While these studies suggest additive value of MRI in routine clinical assessment, there remains significant variability in current clinical practice. Larger pediatric studies are needed to guide optimal use of specific MRI biomarkers in the clinical setting.

Limited Study of PAH Therapeutics in Children

A precision medicine approach to treatment of PH in children remains a challenge. While consensus guidelines have been published, there is still a lack of evidence in the pediatric age group.[52,53] Only one medication (bosentan) is approved by the US Food and Drug Administration for use in children. While several pediatric drug trials are ongoing, most trials are limited to patients with WHO (Nice) Group 1 PAH and therefore do not inform use for the growing population of children with non-Group 1 disease. There is a need for more multicenter trials in well-phenotyped cohorts to guide evidence-based practice with existing therapies and explore novel therapies.

CONCLUSION

There is increasing recognition of unique genotypic and phenotypic subsets in pediatric PH. We are entering an era of individualized and precision approaches to pediatric PH, as we learn more about the genetic factors and developmental contributors to each of these specific syndromes. It is likely that unique subsets of pediatric PH will have distinct outcomes and require distinctive treatments. Reassessing historical approaches to diagnosis, prognosis, and treatment of pediatric PH will be an essential step as care for children with pulmonary vascular disease transitions to more nuanced, tailored, and individualized patterns of disease management.

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