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Genes that Drive the Pathobiology of Pediatric Pulmonary Arterial Hypertension

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Abstract

Emerging data from studies of pediatric-onset pulmonary arterial hypertension (PAH) indicate that the genomics of pediatric PAH is different than that of adults. There is a greater genetic burden in children, with rare genetic factors contributing to at least 35% of pediatric-onset idiopathic pulmonary arterial hypertension (IPAH) compared to ~11% of adult-onset IPAH. *De novo* variants are the most frequent genetic cause of PAH in children, likely contributing to ~15% of all cases. Rare deleterious variants in bone morphogenetic protein receptor 2 (*BMPR2*) contribute to pediatric-onset familial PAH and IPAH with similar frequency as adult-onset. While likely gene disrupting (LGD) variants in *BMPR2* contribute across the lifespan, damaging missense variants are more frequent in early-onset PAH. Rare deleterious variants in T-box 4 containing protein (*TBX4*) are more common in pediatric-compared to adult-onset PAH, explaining ~8% of pediatric IPAH. PAH associated with congenital heart disease (APAH-CHD) and other developmental disorders account for a large proportion of pediatric PAH. SRY-related HMG box transcription factor (*SOX17*) was recently identified as an APAH-CHD risk gene, contributing less frequently to IPAH, with greater prevalence of rare deleterious variants in children compared to adults. The differences in genetic burden and genes underlying pediatric- vs adult-onset PAH indicate that genetic information relevant to pediatric PAH cannot be extrapolated from adult studies. Large cohorts of pediatric-onset PAH are necessary to identify the unique etiological differences of PAH in children, as well as the natural history and response to therapy.

Keywords

genomics; lung disease

Introduction

Pulmonary hypertension (PH) is a diverse group of pulmonary vascular diseases sharing a common pathophysiological endpoint. Endothelial dysfunction, aberrant cell proliferation

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and vasoconstriction give rise to increased pulmonary vascular pressures, increased vascular resistance, heart failure and premature death. The disease is caused by genetic, epigenetic and environmental factors, as well as gene x environment interactions wherein genetic contributions to disease risk are modified by environmental exposures. Most of our understanding of PH etiology is based upon studies in adults. Here, we highlight current knowledge of the genetic causes for World Symposium on Pulmonary Hypertension (WSPH) Group I PH, or pulmonary arterial hypertension (PAH), for pediatric-onset disease and highlight the need for dedicated studies of children with PAH since etiologies differ by age.

PAH usually manifests in early to mid-life with an estimated prevalence of 4.8–8.1 cases/million for pediatric-onset[1] and 15–50 cases/million for adult-onset disease[2]. Pediatric PAH differs from adult-onset disease in several important aspects including gender bias, disease etiology, clinical presentation, and response to therapy [3–5]. The 3–4-fold higher disease prevalence among females compared to males in adult-onset PAH is not observed in pediatric-onset disease, suggesting less dependence on sex-specific interacting factors in children[6–8]. Etiologically, pediatric-onset PAH has a higher proportion of idiopathic PAH (IPAH), PAH associated with congenital heart disease (APAH-CHD) and developmental lung diseases ((including persistent pulmonary hypertension of the newborn (PPHN)) compared to adult-onset disease[3, 4, 6, 8]. Data from the National Biological Sample and Data Repository for PAH (aka PAH Biobank, n=2572 cases) indicate that children present with slightly higher mean pulmonary arterial pressure, decreased cardiac output and increased pulmonary vascular resistance compared to adults at diagnosis[8] (Table 1). Due to the lack of pediatric clinical trial data, fewer therapeutic options are indicated for use in children with PAH. In practice, therapeutic regimens are based on experiences of individual centers [9] or recent statements of consensus guidelines [10, 11]. The management of pediatric PAH remains challenging due to the paucity of data regarding the natural history, mechanisms of disease and treatment response of PAH molecular subtypes in children.

Knowledge of genetic differences between pediatric- and adult-onset PAH are starting to emerge. Most of the data to date is from IPAH or APAH-CHD patients. As has been described for other pediatric developmental diseases including CHD [12–14] and congenital diaphragmatic hernia [15–17], *de novo* genetic variants contribute to a significant proportion of pediatric IPAH[7] (Figure 1). Furthermore, rare heritable variants in at least three developmental pathways and/or transcription factors have been implicated in pediatric PAH: *BMPR2* (encoding bone morphogenetic protein receptor 2), *TBX4* (T-box 4 containing protein) and *SOX17* (SRY-related HMG box transcription factor) (Figures 1 and 2). Other known PAH risk genes such as *ACVRL1*, *ENG*, *KCNK3* and *SMAD9* rarely contribute to pediatric PAH [7] and in the aggregate account for ~3% of IPAH cases (Figure 1). No effect of sex on risk allele frequencies has been identified to date.

The PAH genetics community has introduced the term hereditary PAH (HPAH) to include familial PAH (PAH that occurs in two or more family members) as well as PAH without a family history of PAH when there is a clear genetic diagnosis. Thus, patients classified as IPAH at the time of diagnosis should be classified as HPAH when a causal genetic variant is identified.

Role of *de novo* variants

De novo variants have emerged as an important class of genetic factors underlying rare diseases, especially early-onset severe or lethal conditions[13, 17–19], due to strong negative selection decreasing reproductive fitness[20]. Using a cohort of 34 pediatric-onset idiopathic PAH (IPAH) cases for which we had samples from unaffected parents, we demonstrated a 2-fold enrichment of *de novo* variants in cases compared to an estimated background mutation rate [7]. The variants included both missense variants that change an amino acid with strong predictions of deleterious protein function (D-Mis) and likely gene damaging variants (LGD: frameshift, stop-gain, canonical splice site) often causing protein truncation and nonsense-mediated decay leading to a state of haploinsufficiency (loss of one functional copy of the gene). Among genes highly-expressed in developing heart and lung, the enrichment was increased 4-fold. All six of the LGD variants were identified in patients with an age-of-onset less than 5 years [7]. We now have additional data confirming the relative contribution of *de novo* variants in an expanded cohort of 124 trios with pediatric PAH probands, including both IPAH and APAH (primarily APAH-CHD) (manuscript in preparation). The majority of the cohort was recruited and consented at Columbia University Medical Center (CUMC) with whole exome sequencing and variant identification as described[7]. Whole genome sequencing data for ten trios was shared by the UK NIHR BioResource – Rare Diseases PAH Study[21] and analyzed together with the CUMC cohort data. The estimated fraction of pediatric PAH explained by *de novo* variants is ~15%. Some of the *de novo* variants occur in known risk genes (3 *TBX4*, 2 *BMPR2*, 1 *ACVRL1*, 1 *ABCC8*). However, the others occur in genes not previously implicated in PAH. Notably, some of the novel *de novo* variants occur in candidate genes with known or plausible roles in lung/vascular development. For example, *AMOT* (angiomotin) encodes an angiostatin-binding protein involved in embryonic endothelial cell migration and tube formation as well as endothelial cell tight junctions and angiogenesis [22–24]. *KEAP1* (Kelch-like ECH associated protein 1) regulates oxidative stress and apoptosis through interactions with NRF2 in murine vascular cells[25], and endothelial-specific deletion of *NRF2* reduces endothelial cell sprouting *in viv*o [26]. *MAPK6* encodes ERK3 (extracellular signal-regulated kinase 3), and mice carrying null alleles exhibit intrauterine pulmonary hypoplasia and early neonatal death [27]. Due to the rarity of *de novo* mutations in the general population[28], statistical evidence of a candidate risk gene is effectively equivalent to multiplicity (≥2 occurrences) of rare deleterious variants among cases. While our data implicate a role for *de novo* variants in ~15% of pediatric PAH cases, larger trio cohorts will be required to confirm the role of individual genes with replication and develop a comprehensive list of PAH genes.

BMPR2

BMPR2 is a member of the TGFβ superfamily including TGFβ /BMP ligands, receptors, accessory proteins, activins, and downstream signaling mediators (including SMADs and *NOTCH3*).

Rare deleterious variants in *BMPR2* underlie ~70% of FPAH and 10–20% of IPAH cases, with similar frequencies of *BMPR2* variants in pediatric- vs adult-onset disease for both

FPAH and IPAH [7, 8]. Overall, *BMPR2* variant carriers have younger mean age-of-onset and more severe PAH compared to non-carrier patients, at least in part due to impaired response to oxidative stress [29]. However, *BMPR2* variants are less frequent causes of APAH-CHD and have not been observed in PPHN. In a cohort of 258 APAH-CHD cases, only 7 (2.7%) cases carried rare deleterious *BMPR2* variants [30]. In the PAH Biobank - comprised of 4% FPAH, 43% IPAH, 48% APAH and 5% other PAH - only 12/119 (10%) of all *BMPR2* carriers had PAH diagnoses other than FPAH/IPAH [8]. Among 88 infants with PPHN, no rare deleterious variants in *BMPR2* were identified [31]. The wide distribution of deleterious variant locations across the *BMPR2* gene has limited genotype-phenotype analyses in the sample sizes studied to date due to small numbers of individuals carrying any one variant. However, an analysis of mutation type – truncating or missense – showed earlier age-of-onset and decreased survival among carriers of missense compared to truncating variants [32]. These data suggest that the presence of mutant/dysfunctional BMPR2 proteins arising from missense variants are more deleterious than haploinsufficiency of normal BMPR2 protein. Similarly, mice carrying a *Bmpr2* extracellular domain missense mutation developed more severe PH in response to hypoxia or hypoxia with vascular endothelial growth factor inhibition than mice heterozygous for a *Bmpr2* null allele [33]. In our cohort of 412 pediatric- and adult-onset PAH patients, there was a significant enrichment of deleterious missense variants in *BMPR2* in patients with younger age-of-onset compared to LGD variant carriers[7] (Figure 3), providing independent confirmation of the importance of missense variants in early-onset disease. Thus, in pediatric PAH, rare deleterious *BMPR2* variants contribute primarily to FPAH/IPAH with deleterious missense variants associated with increased disease severity.

TBX4

TBX4 is not part of the TGFB pathway. *TBX4* encodes a transcription factor in the T-box gene family expressed in the developing atrium of the heart, limb buds, and mesenchyme of lung and trachea, with important roles in limb development as well as lung growth and branching [34]. Rare deleterious *TBX4* variants have been associated with small patella syndrome [35] and PAH [7, 36]. *TBX4* was first suggested as a candidate PAH risk gene because of its location on chromosome 17q23.1–23.2, where microdeletions were associated with severe neurodevelopmental delays and pulmonary hypertension [37, 38]. Kerstjens-Frederikse and colleagues [36] sequenced *TBX2* and *TBX4*, both located within the 17q23.1–23.2 deletion, and identified three rare *TBX4* variants among 49 adults with PAH and no rare variants in *TBX2*. In a small European cohort of 66 pediatric PAH cases, 3/40 FPAH/IPAH cases carried rare deleterious *TBX4* variants[39] compared to 3/136 adult carriers in a Spanish PAH cohort [40]. In our larger cohort of 412 pediatric and adult onset FPAH/IPAH cases, we reported rare deleterious *TBX4* variants in 13 cases with a significant enrichment of variants among pediatric (12/155) compared to adult onset (1/257) patients [7]. Furthermore, the mean age of onset was 20 years younger for *TBX4* variant carriers compared to *BMPR2* carriers. In the PAH Biobank, variants in *TBX4* were the second most common genetic cause of PAH and accounted for ~1% of cases [8]. While 13/23 *TBX4* variant carriers had a diagnosis of IPAH, the other diagnoses included APAH-CHD, PAH associated with connective tissue disease, FPAH and PAH due to dietary toxin exposure. Age-of-onset for the *TBX4* carriers exhibited a bi-modal distribution with significant

enrichment of variants among pediatric-onset cases (Figure 2). Interestingly, recent clinical and histological analysis of 19 children carrying rare deleterious *TBX4* variants revealed a high frequency of severe developmental defects of the lung, skeleton and heart [41]. Ten of the infants presented with PPHN which resolved; however, the children were subsequently diagnosed with PAH later in infancy or childhood. This evidence indicates that *TBX4* is especially important in pediatric-onset PAH, with rare deleterious variants causing a variety of disease subclasses and predicting disease recurrence in young patients initially diagnosed with PPHN.

SOX17

SOX17 also works outside of the TBFB pathway. *SOX17* is a highly-constrained gene encoding a transcription factor involved in Wnt/β-catenin and Notch signaling during development [42]. Genetic studies in mice show that *Sox17* is required for correct development and function of the pulmonary vascular tree. Endothelial-specific inactivation of *Sox17* leads to impaired arterial specification and embryonic death or, with conditional postnatal inactivation, arterial-venous malformations [43]. Deletion in mesenchymal progenitor cells causes abnormal pulmonary vascular morphogenesis resulting in postnatal cardiopulmonary dysfunction and juvenile death [44]. Moreover, in an elegant endothelial lineage tracing study in mice, Liu and colleagues recently demonstrated that transcriptional activation of *Sox17* via hypoxia-induced factor 1α, leads to upregulation of cyclin-E1 and endothelial regeneration in response to lung injury [45]. Thus, there are multiple mechanisms through which defective or deficient SOX17 could result in developmental cardiopulmonary defects or impaired response to hypoxic injury.

We identified *SOX17* as a candidate risk gene for PAH using exome sequencing data in a cohort of 256 APAH-CHD patients [30]. Using a case-control gene-based association test, *SOX17* was the only gene, out of ~18,000 genes, to reach genome-wide significance. Three of the top associated genes (*BZW2*, *FTSJ3*, *BAZ1B*) were putative SOX17 transcriptional targets[46], and enrichment analysis of a gene-set including 1947 putative SOX17 target genes revealed enrichment of rare missense variants in the patient cohort, suggesting that multiple genes in the SOX17 pathway way be important in APAH-CHD. The majority of these genes are expressed in pulmonary arterial endothelial cells or developing heart, and 28% (42/149) are expressed in the top quartile in both tissues/cell types. Pathway enrichment analysis showed that the SOX17 target genes with deleterious variants are overrepresented in developmental processes, transmembrane transport of small molecules, ion homeostasis and extracellular matrix interactions. The association signal for *SOX17* was due to LGD and deleterious missense variants carried by 10 APAH-CHD patients, and 7/10 of these patients had pediatric-onset disease. Screening of our separate cohort of 412 FPAH/IPAH patients identified an additional three carriers (2 pediatric, 1 adult)[30]. Rare deleterious variants have been identified in two additional IPAH cohorts, comprised mostly of adults. Genome sequencing data from the UK NIHR BioResource – Rare Diseases PAH Study identified 9/1038 IPAH *SOX17* variant carriers[21] and candidate gene analysis of a Japanese cohort found 4 (3 unrelated)/140 FPAH/IPAH *SOX17* variant carriers[47]. In the PAH Biobank, rare deleterious *SOX17* variants were identified in 10/2572 patients (6 IPAH, 2 APAH-CHD, 1 PAH associated with portopulmonary disease, and 1 PAH due to dietary

toxin exposure)[8]. The mean age-of-onset for carriers in the PAH Biobank was 26 years, markedly younger than the overall cohort (48 years) or of *BMPR2* variant carriers (38 years) (Figure 2). Notably, 15/18 rare missense variants carried by patients from all five cohorts are located in the high mobility group (HMG, DNA binding) domain of the SOX17 protein (Figure 4). The HMG domain is evolutionally conserved, down to unicellular yeast species, and is essential for target-specific transcriptional control[42, 48]. Based on these data, we estimate that rare deleterious variants in *SOX17* contribute to ~7% (19/273) of pediatric-onset PAH, especially APAH-CHD, compared to ~0.4% (13/3455) of adult-onset PAH. In addition, common SNPs in a putative endothelial-acting enhancer region of *SOX17* have been associated with PAH [49], suggesting that variation in *SOX17* gene expression may increase risk for developing PAH or other vascular endothelium-related diseases more commonly.

Genetic ancestry

The contribution of individual genes in PAH is likely heterogeneous across different genetic ancestries. The results of genetic studies predominantly in individuals of European ancestry may not be generalizable to all other populations. A pediatric study of an Asian cohort revealed a higher carrier frequency of *ALK1*/*ACVRLI* variants (7/54, 12.9%) [50] compared to studies of predominantly Europeans. A recent association analysis involving 331 IPAH cases and 10,508 controls of Asian ancestry identified *BMP9/GDF2* as a significant risk gene in this population, second in frequency to *BMPR2* [51]. Among 22 carriers of rare deleterious *GDF2* variants, three had pediatric-onset disease accounting for 5.2% of the 57 pediatric cases. In a case study of a five-year-old boy of Hispanic ancestry, a homozygous loss of function *BMP9* variant, c.76C>T;p.Gln26Ter, was identified [52]. The gnomAD population database (gnomad.broadinstitute.org) contains only two heterozygous counts of this allele, both of Latino ancestry, suggesting that this might be an ancestry-specific allele. Clearly, larger studies of children with greater diversity are needed to define ancestral-specific genetic factors and their overall role in pediatric-onset PAH.

Genetic testing for PAH

Clinical genetic testing panels for PAH-associated genes are widely available. Typical panels include *BMPR2*, *ACVRL1*, *CAV1*, *EIF2AK4*, *ENG*, *KCNK3*, and *SMAD9* with varied inclusion of additional genes. For children, targeted sequencing of *BMPR2*, *TBX4* and *SOX17* should be prioritized for sequence variants, as well as *ACVRL1* and *GDF2* for Asian patients. In addition, deletion/duplication analysis for *BMPR2* should be performed. If the results of these tests are not diagnostic, then reflexive exome sequencing of family trios (affected child and unaffected biological parents) should be performed to identify *de novo* or inherited rare variants. Genetic diagnoses can inform management of PAH as well as risk stratification for relatives of patients. However, the low penetrance associated with PAH genes complicates risk prediction, and genetic counseling should be offered to families before genetic testing. Although there is no current means to prevent PAH, screening by echocardiogram to enable early diagnosis and treatment may improve outcomes.

Summary –

Genetic information relevant to pediatric PAH cannot be extrapolated from adults. Pediatric-onset PAH differs from adult-onset in many important aspects including the genetic burden and specific genes involved. Rare genetic factors contribute to ~35% of pediatric-onset IPAH compared to ~11% of adult-onset IPAH. *De novo* variants and rare deleterious variants in *BMPR2*, *TBX4*, and *SOX17* currently explain most of the known genetic burden in pediatric PAH. However, ancestry-specific factors likely play a role as well, with *ACVRL1* and *GDF2* variants likely contributing in Asian patients. Large cohorts of pediatric-onset PAH are necessary to identify the unique etiological genes for PAH in children, as well as the natural history and response to therapy. Furthermore, genetic information is immediately clinically relevant and used by families with pediatric onset PAH to make reproductive decisions and screen family members. Identification of new causal genes will illuminate underlying disease pathophysiology and mechanisms that may identify therapeutic targets for adults and children. Given the increased burden of genetic etiologies in children, genomic studies in children with a trio design to identify inherited and *de novo* variants will yield more new targets than similarly powered studies in adults.

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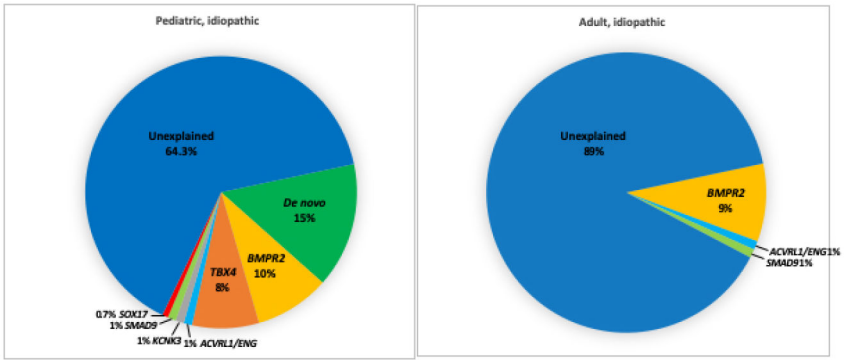

Figure 1.

Relative contributions of de novo mutations and 11 established PAH risk genes in idiopathic pediatric- and adult-onset PAH in a cohort of 412 cases (130 pediatric IPAH, 178 adult IPAH). Risk genes included BMPR2, ACVRL1, BMPR1A, BMPR1B, CAV1, EIF2AK4, ENG, KCNK3, SMAD4, SMAD9, and TBX4.

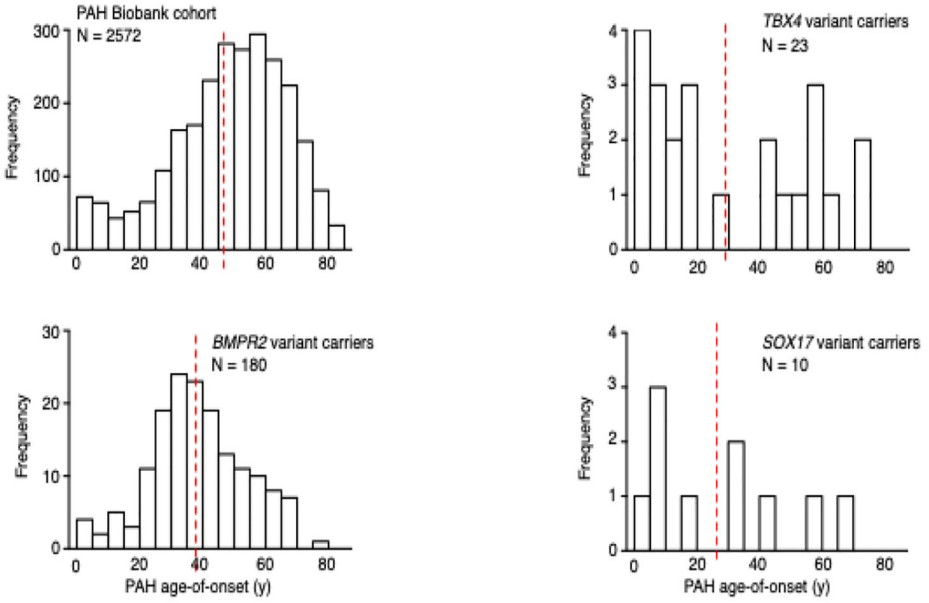

Figure 2.

Age distributions for PAH cases from the National Biological Sample and Data Repository for PAH (n=2572). BMPR2, TBX4 and SOX17 variant carriers have younger mean age-of-onset compared to the whole cohort with significant enrichment of pediatric-onset cases among TBX4 variant carriers compared to the whole cohort (Zhu et al 2019 #8). Red vertical lines indicate the group means.

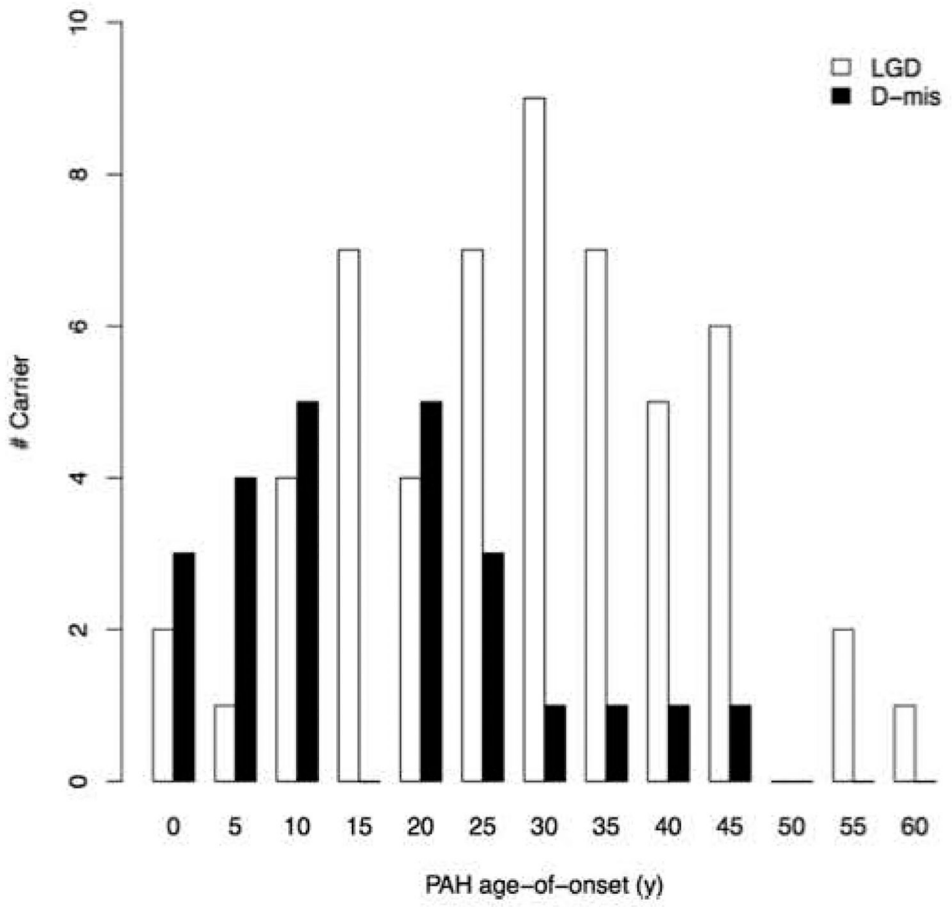

Figure 3.

Age distributions of BMPR2 likely gene damaging (LGD) or predicted damaging missense (D-mis) variant carriers from a cohort of 412 pediatric- and adult-onset PAH patients. There was significant enrichment of D-mis variants among patients with younger age-of-onset compared to LGD variant carriers (n=83 total variant carriers)

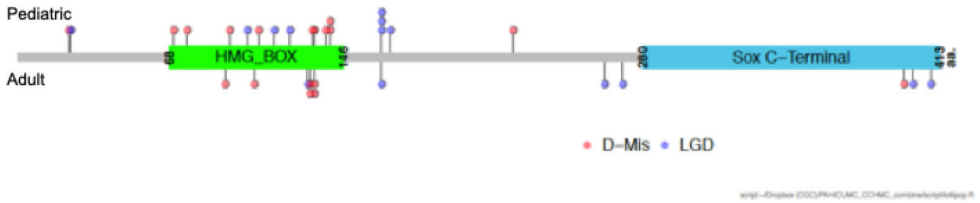

Figure 4.

Locations of SOX17 likely gene damaging (LGD) and rare predicted deleterious (D-Mis) variants carried by PAH patients from five cohorts from the US, UK and Japan. Variants carried by pediatric patients (n=19) are shown above the protein schematic and variants carried by adult patients (n=13) below the schematic. The combined datasets include 273 pediatric and 3455 adult patients (Zhu et al 2018 #7; Zhu et al 2019 #8; Graf et al 2018 #21; Zhu et al 2018 #30; Hiraide et al 2018 #47).

Table 1.

Clinical characteristics of child- vs adult-onset PAH cases at diagnosis.

Data from the National Biological Sample and Data Repository for PAH (n=2572 cases) (Zhu et al 2019 #8). Child-onset, <18 years of age at diagnosis. MPAP, mean pulmonary artery pressure; CO, cardiac output; PVR, pulmonary vascular resistance; dx, diagnosis.

Group	Age at dx (y)	MPAP (mmHg)	CO, Fick (L/min)	PVR (Woods units)
Child (n=226)	8 ± 6 (226)	55 ± 18 (225)	3.3 ± 1.6 (168)	17.7 ± 11.6 (164)
Adult (n=2345)	52 ± 19 (2345)	50 ± 14 (2293)	4.6 ± 1.7 (1630)	10.0 ± 5.9 (1579)
P-value	<0.0001	<0.0001	<0.0001	<0.0001