Humu-2015-0271

Review

Pulmonary Arterial Hypertension: A Current Perspective on Established and Emerging Molecular Genetic Defects

Rajiv D. Machado[1], Laura Southgate[2,3], Christina A. Eichstaedt[4,5], Micheala A. Aldred[6], Eric D. Austin[7], D. Hunter Best[8,9], Wendy K. Chung[10], Nicola Benjamin[4], C. Gregory Elliott[11], Mélanie Eyries[12,13,14], Christine Fischer[5], Stefan Gräf[15,16], Katrin Hinderhofer[5], Marc Humbert[17,18,19], Steven B. Keiles[20], James E. Loyd[21], Nicholas W. Morrell[15,22], John H. Newman[21], Florent Soubrier[12,13,14], Richard C. Trembath[2], Rebecca Rodríguez Viales[4,5], Ekkehard Grünig[4]

[1]School of Life Sciences, University of Lincoln, Lincoln, United Kingdom

[2]Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

[3]Division of Genetics & Molecular Medicine, King's College London, London, United Kingdom

[4]Centre for Pulmonary Hypertension, Thoraxclinic at the University Hospital Heidelberg, Heidelberg, Germany

[5]Institute of Human Genetics, Heidelberg University, Heidelberg, Germany

[6]Genomic Medicine Institute, Cleveland Clinic, Cleveland, Ohio, USA

[7]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, Tennessee, USA

[8]Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah, USA

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/humu.22904.

[9]ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, Utah, USA

[10]Departments of Pediatrics and Medicine, Columbia University Medical Center, New York, New York, USA

[11]Departments of Medicine, Intermountain Medical Center and the University of Utah School of Medicine, Salt Lake City, Utah, USA

[12]Unité Mixte de Recherche en Santé (UMR_S 1166), Université Pierre and Marie Curie Université Paris 06 (UPMC) and Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France

[13]Genetics Department, Hôpital Pitié-Salpêtrière, Assistance Publique–Hôpitaux de Paris (AP-HP), Paris, France

[14]Institute for Cardiometabolism and Nutrition (ICAN), Paris, France

[15]Department of Medicine, University of Cambridge, United Kingdom

[16]Department of Haematology, University of Cambridge, United Kingdom

[17]Université Paris-Sud, Faculté de Médecine, Le Kremlin Bicêtre, France

[18]Département Hospitalo-Universitaire (DHU) Thorax Innovation (TORINO), Service de Pneumologie, Hôpital Bicêtre, AP-HP, Le Kremlin Bicêtre, France

[19]INSERM UMR_S 999, Laboratoire d'Excellence en Recherche sur le Médicament et l'Innovation Thérapeutique (LERMIT), Centre Chirurgical Marie Lannelongue, Le Plessis Robinson, France

[20]Quest Diagnostics, Action from Insight, 33608 Ortega Highway, San Juan Capistrano, California, USA

[21]Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

[22]Addenbrooke's & Papworth Hospitals, Cambridge, United Kingdom

Corresponding Author:

Dr Rajiv D. Machado

School of Life Sciences, University of Lincoln, Joseph Banks Laboratories, Green Lane, Lincoln, LN6 7DL, United Kingdom

Tel: +44 1522 886887

Email: rmachado@lincoln.ac.uk

Funding Sources:

NIH: PO1 HL108800 (EDA, JEL, JHN); R01 HL098199 (MAA); R01 HL060056 (WKC).

European Union: QLGI-CT-2002-01116 (EG)

Abstract

Pulmonary arterial hypertension (PAH) is an often fatal disorder resulting from several causes including heterogeneous genetic defects. While mutations in the bone morphogenetic protein receptor type II (*BMPR2*) gene are the single most common causal factor for hereditary cases, pathogenic mutations have been observed in approximately 25% of idiopathic PAH patients without a prior family history of disease. Additional defects of the transforming growth factor beta (TGF-β) pathway have been implicated in disease pathogenesis. Specifically, studies have confirmed activin A receptor type II-like 1 (*ACVRL1*), endoglin (*ENG*) and members of the *SMAD* family as contributing to PAH both with and without associated clinical phenotypes. Most recently, next-generation sequencing has identified novel, rare genetic variation implicated in the PAH disease spectrum. Of importance, several identified genetic factors converge on related pathways and provide significant insight into the development, maintenance and pathogenetic transformation of the

pulmonary vascular bed. Together, these analyses represent the largest comprehensive compilation of *BMPR2* and associated genetic risk factors for PAH, comprising known and novel variation. Additionally, with the inclusion of an allelic series of locus-specific variation in *BMPR2*, these data provide a key resource in data interpretation and development of contemporary therapeutic and diagnostic tools.

Key Words: BMPR2; ACVRL1; ENG; SMAD1; SMAD4; SMAD9; CAV1; KCNA5; KCNK3; EIF2AK4; haploinsufficiency; locus heterogeneity

Introduction

Heritable pulmonary arterial hypertension (HPAH) (*PPH1*; MIM# 178600) is a severe, progressive autosomal dominant vascular disorder, predominantly affecting the arterial circulation and, in particular, the pulmonary arterioles [Tuder et al., 2013]. Histopathological investigation reveals abnormal muscularization of these structures, which leads to a chronic elevation of pulmonary arterial pressure, often resulting in right heart failure 2-3 years post-diagnosis in the absence of the contemporary treatment protocols [Vonk-Noordegraaf et al., 2013]. Identification of mutations in the *BMPR2* gene in probands with a family history of disease provided the first insight into the molecular pathogenesis of HPAH [Deng et al., 2000; Lane et al., 2000]. Subsequently, *BMPR2* mutations were identified in a cohort of idiopathic patients (IPAH) [Thomson et al., 2000]. Since, causal variation has been described in nine additional genes, in cases that include PAH associated with other conditions (APAH). Here, we describe molecular genetic analyses of the 10 functionally characterized genes that cause PAH (Figure 1) and provide a compilation of all mutations identified to date. The continuing identification of genetic factors, as explored in this report, provides unique insight

to the genetic mechanisms driving disorders of pulmonary vascular function. Furthermore, these studies offer the foundation for the discovery and delivery of novel therapeutic options.

Key Components of the BMP Signaling Pathway

The *BMPR2* gene (MIM# 600799) encodes a type II receptor of the TGF-β family of signaling molecules. The mature polypeptide is composed of a signal peptide (encoded by exon 1), an extracellular domain (exons 2-3), a single transmembrane domain (exons 4-5), a highly conserved eukaryotic protein kinase region (exons 6-11) and an unusually large cytoplasmic tail (exons 12-13) amongst TGF-β receptors species [Liu et al., 1995]. In the canonical pathway, BMPR-II binds ligand in a heteromeric complex with a type I receptor, which may be activin receptor-like kinase 1 (ALK1), -2 (ALK2), -3 (ALK3/BMPR1A) or -6 (ALK6/BMPR1B), to initiate activation of intracellular partners within a cell-specific context [David et al., 2009; Rigueur et al., 2015]. Phosphorylation of the receptor SMAD proteins (R-SMADs) 1, 5 and 8 leads to their association with the nuclear chaperone SMAD4. This signaling complex translocates to the nucleus, where it acts in combination with transcriptional co-activators and -repressors to effect control of target gene expression (Figure 1). BMPR-II signaling has been established as essential to a multitude of fundamental cellular processes including proliferation, apoptosis, differentiation and migration [Shi and Massague, 2003].

Mutations of *BMPR2* Predispose to the Majority of Hereditary and Idiopathic Forms of PAH

Herein, we describe an additional 370 independent variants of *BMPR2* in patients either previously excluded from or ascertained since the last comprehensive mutation update in 2009 [Machado et al., 2009]. Of these, 108 were identified as part of this study and were

generated by specialist PAH centers based in Germany, France, North America and the UK (Table 1, Table 2). The research was prospectively reviewed and approved by a duly constituted ethics committee for each center. Probands were assessed for point mutations and large gene abnormalities using multiple screening technologies including Southern blotting, denaturing high-performance liquid chromatography, multiplex ligation-dependent probe amplification (MLPA), dye-terminator and next-generation sequencing (NGS). All variants considered to be pathogenic were absent from a control population of at least 200 chromosomes and public variation databases including dbSNP v142 (http://www.ncbi.nlm.nih.gov/SNP) and the 1000 genomes project (http://www.1000genomes.org) and/or have been previously demonstrated to have a functional impact. For a current estimation of population frequencies, we have additionally checked all point mutations reported here against the Broad Institute Exome Aggregation Consortium (ExAC) database v0.3 (http://exac.broadinstitute.org), comprising over 60,000 exomes derived from independent sequencing projects. Mutation nomenclature employs parameters set by the Human Genome Variation Society (http://www.hgvs.org/mutnomen). Taken together with previous reports [Machado et al., 2006; 2009], these data provide evidence of a total of 668 germline variants underlying PAH, thereby consolidating *BMPR2* as the major causal gene for familial cases and subjects previously classified as IPAH. The spectrum and range of *BMPR2* defects in this study comprise the major mutation categories which, in general, correlate with existing data [Machado et al., 2006; 2009]. Namely, we record missense variants leading to amino acid substitution (n=86, 23%), nonsense mutations (n=107, 29%), frameshift defects resulting from small insertions/deletions (n=79, 21%) and splice-site variation (n=33, 9%). However, and by contrast to earlier studies, we identified a significantly higher prevalence of major gene rearrangements (n=61, 16%) and single nucleotide mutations in the 5-prime untranslated region (5′ UTR) (Table 1, Table 2, Supp.

Table S1). This, most likely, is a consequence of screening centers expanding the analysis of gene re-arrangements to include all exons of *BMPR2,* combined with a growing recognition that mutation short-fall within cohorts is potentially explained by defects harbored within non-coding regions of *BMPR2* [Machado et al., 2006]. For example, we report a total of four recurrent 5′ UTR mutations resulting from a guanine to adenine change (c.−669G>A) likely to abolish specificity for an SP3 transcription factor binding site [Wang et al., 2009]. In combination, these genetic findings reinforce haploinsufficiency as the molecular mechanism for this disease [Machado et al., 2001; 2006; 2009]. Moreover, this report provides a comprehensive compilation of distinct variants (n=384) across the *BMPR2* locus since the first identification of the gene (Supp. Table S2). A combination of genetic and functional studies have firmly established a large proportion of these to be likely pathogenic while others, although compelling, remain to be fully elucidated as disease-causing. These data have now been made available in the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar).

Distribution and Biological Significance of *BMPR2* Variation

Although variation has been described across the entire coding structure of the gene, the mutation load differs significantly across exons, indicating both the likely existence of mutation hot-spots and potential regions of key functional importance. Taken together with previously reported findings, our analyses indicate that the majority of amino acid substitutions cluster in exons encoding the ligand-binding domain and key catalytic regions of the kinase domain, namely exons 2-3, 6-9 and 11 respectively. By contrast, exons 1, 4, 10 and 13, encoding receptor regions of uncertain importance to function, have a low frequency of missense mutation (Figure 2A). However, assessment of individual nucleotide defects within the *BMPR2* open reading frame, relative to exon length, illustrates variant load by

exon may be resultant on an abnormally high frequency of recurrence (e.g. exon 12) which corresponds to a low percentage of affected bases (Figure 2B). Conversely, exon 9 whilst harboring a relatively modest 6.7% of all reported coding variants, contains the highest proportion of independent nucleotide defects (n=30) relative to exon size (148 bp). In combination with previous reports, the majority of mutations predict incorporation of a premature termination codon in the mRNA (n=483, 72%) and, as previous functional studies have demonstrated, result in degradation of the message through the nonsense-mediated decay pathway [Aldred et al., 2007; Nasim et al., 2008].

Cysteine substitutions comprise the majority of missense mutations in the extracellular ligand-binding domain and are concentrated on 9 of 10 conserved residues, which are essential for the formation of five disulfide bridges necessary to maintain the integrity of this highly ordered three-dimensional structure (Figure 3) [Greenwald et al., 1999]. Moreover, this analysis has indicated the existence of additional critical residues. Notably, mutation of an asparagine (p.N126S), adjacent to the frequently mutated cysteine residue (p.C123R, p.C123S), is observed on seven independent occasions [Machado et al., 2009] (Table 1, Supp. Table S1), highlighting its putative significance to extracellular domain function. The majority of tested mutations in the kinase domain of BMPR-II abolish catalytic function as determined by *in vitro* BMP/SMAD luciferase reporter gene assays. By contrast, the significance of mutations within the cytoplasmic tail remains enigmatic, as these receptors retain significant capacity for downstream signaling through the SMAD family. Yet, these defects appear to perturb non-canonical pathways which include signaling through the cytoskeleton-associated factors LIMK-1 and Tctex-1 [Foletta et al., 2003; Machado et al., 2003]. In addition, studies have suggested that missense variants present across all the functional domains of BMPR-II trigger constitutive up-regulation of p38MAPK indicating a

perturbation of one or more SMAD-independent pathways yet to be fully investigated [Nishihara et al., 2002; Rudarakanchana et al., 2002].

Clinical Significance

Extracellular Domain Mutation Spectrum

Transient over-expression and subcellular localization of constructs harboring cysteine substitutions, specifically p.C60Y, p.C117Y, p.C118Y, p.C123R and p.C123S, have previously shown intracellular retention of these receptor species combined with a dramatic diminution of SMAD activation [Rudarakanchana et al., 2002]. Here, we report further cysteine substitutions likely to underlie structural variation in BMPR-II (p.C34R, p.C60G/R, p.C66G/R/Y, p.C84F/G/R, p.C94G/R, p.C99F/R/Y, p.C117R/S, p.C118W) (Figure 3). Utilization of these genetic observations with combinatorial functional studies facilitated an exploration of receptor rescue and restoration of signaling. By targeting the p.C118W mutant receptor with chemical chaperones, namely thapsigargin, glycerol or sodium 4-phenylbutyrate, a demonstrable and significant increase in plasma membrane localization of receptor species was observed concomitant with enhanced phosphorylation of SMADs 1 and 5. Rescued trafficking also led to an increase in the density of wild-type BMPR-II at the cell surface [Sobolewski et al., 2008]. This study provides an arresting example of how exploitation of genetic insights may lead to the potential development of future targeted therapeutic options in PAH.

Variants of Unknown Significance

Analyses of amino acid substitutions by PolyPhen, PROVEAN and SIFT bioinformatic tools [Kumar et al., 2009; Adzhubei et al., 2013; Choi and Chan, 2015] lead to ambiguous conclusions of pathogenicity in a proportion of observed missense variants reported herein. In the present dataset, a total of 13 variants are predicted by at least two algorithms to be non-damaging and/or benign (Table 2). In order to achieve greater confidence in assigning pathogenic status, it is important to assess such *in silico* predictions in the context of genomic data derived from substantive populations, for example the ExAC database. Comparison of missense variants against this combined cohort identified five variants with a population allele frequency greater than 0.000015, based on the most conservative measure of PAH prevalence in the literature (15 cases per million) [Archer et al., 2010]. Of interest, the two most commonly observed variants (p.V348I and p.Y589C) had been previously determined as damaging by at least two prediction methods and, therefore, had not been classified as variants of unknown significance (VUS). In the remaining three cases, the population data supported the *in silico* predictions which, taken together, provide further evidence for unclear pathogenicity (Table 2). One additional variant (p.T766A), previously designated VUS status by prediction algorithms, was present in the ExAC database but with a population allele frequency lower than our assigned threshold. Through these combined analytical techniques, we have identified a total of 15 variants that might be considered of uncertain significance from a genetic perspective (Table 2). While employing such analytical tools is of emerging value in mutation data interpretation, these analyses demonstrate that they must be treated with caution as: 1) the two approaches utilized may produce conflicting outputs, leading to ambiguity in interpretation; 2) large cohort datasets may contain study participants for whom the phenotype cannot be definitively assigned. Further, variants with a low population allele frequency in an apparently normal cohort may also be explained by the reduced penetrance of

this condition. Together, this highlights a continuing role for functional studies, as a gold standard, to determine the true impact of observed variation in *BMPR2*, which is of value to both diagnostic and basic science understanding of the physiological role of this receptor. In addition, these observations indicate the likely existence of as yet unexplored pathways underlying pathogenesis.

Uncommon TGF-β Family Variation in PAH

Mutations of Receptor Species and Functional Outcomes

PAH infrequently clinically co-presents with the autosomal dominant vascular disorder hereditary hemorrhagic telangiectasia (HHT), characterized by the presence of mucocutaneous telangiectasia and visceral arteriovenous malformations. PAH-associated HHT is caused by molecular defects in *ACVRL1*, encoding a type I receptor of the TGF-β family, and to a lesser extent by mutations of the *ENG* gene which encodes a type III, or accessory receptor (Figure 1). In rare instances, *ACVRL1* mutations have been identified in PAH patients without HHT but typically in early-onset disease thereby not precluding the development of the latter condition in later life [Harrison et al., 2003; Fujiwara et al., 2008]. Here we have compiled complete data on 66 mutations for both genes (*ACVRL1*, n=57; *ENG*, n=9), including 61 previously reported variants and 5 novel mutations underlying the development of PAH with and without HHT (Table 3). In *ACVRL1*, the majority of this variation occurs within the vital kinase domain of the protein (exons 6-10) resulting in pathogenic amino acid substitutions (n=42, 74%) in marked contrast to the *BMPR2* pattern of predominantly truncating mutations and, indeed, the mutation spectrum in HHT alone. A recent study conducted in a cohort of 43 IPAH patients identified two missense variants in *BMPR1B* (p.S160N and p.F392L). However, contrary to previous reports, these variants

induced SMAD9 signaling with concomitant induction of transcriptional activity [Chida et al., 2012a]. These studies suggest that further functional investigations are required for clarification of the pathogenic impact of these variants.

Mutations within Intracellular Partners of the BMP Signaling Pathway

Conventional functional candidate gene strategies conducted in Asian and European patient panels have subsequently identified independent mutations in the BMP-specific SMAD pathway, namely *SMAD1* (n=1), *SMAD4* (n=2) and *SMAD9* (n=3) [Shintani et al., 2009; Drake et al., 2011; Nasim et al., 2011] (Table 3). Of these, the *SMAD1* and *-4* defects have been described as VUS due to *in vitro* luciferase SMAD responsive elements reporter assays demonstrating an unclear impact on the canonical pathways [Nasim et al., 2011]. However, these analyses did not investigate SMAD-independent pathways, implicated in disease pathogenesis, leaving open the possibility that the identified variants may deleteriously affect other BMP related systems. By contrast, *SMAD9* mutations (*PPH2*; MIM# 615342) lead to a marked reduction of SMAD transcriptional activity and a down-regulation of the BMP target gene *Id1* [Shintani et al., 2009; Nasim et al., 2011]. Of interest, heterozygous *SMAD9* mutations have been observed to perturb non-canonical downstream pathways, in particular, micro-RNA (miRNA) processing. Examination of a human patient with a *SMAD9* nonsense mutation (p.R294*) indicated a modest reduction of *Id1* expression in contrast to a complete abrogation of *miR-21*. *In vitro* restoration of *miR-21* by over-expression led to a reversal of the hyperproliferative mutation-positive phenotype. These data suggest a specific role for SMAD8 in PAH pathogenesis and SMAD4-independent signaling [Drake et al., 2011].

Genotype-Phenotype Correlation in Risk Alleles of the BMP Pathway

In 53-86% of patients with familial aggregation and 14-35% of IPAH patients mutations in the *BMPR2* gene have been identified [Sztrymf et al., 2008; Girerd et al., 2010a; Pfarr et al., 2011; Liu et al., 2012; Kabata et al., 2013]. Patients who carry *BMPR2* mutations differ in several important aspects from IPAH patients who are *BMPR2*-negative [Soubrier et al., 2013]. Investigators have reported that HPAH patients with pathogenic variants in *BMPR2* develop this disorder at a younger age (38.53 ± 12.38 vs. 45.78 ± 11.32 years, p <0.001) [Girerd et al., 2010b; Pfarr et al., 2011], and have a more severe clinical and hemodynamic phenotype at diagnosis [Koehler et al., 2004; Sztrymf et al., 2008; Austin et al., 2009a; Pfarr et al., 2011]. *BMPR2* mutation carriers undergo diagnostic catheterization almost 10 years earlier than patients with no identified *BMPR2* defect. Furthermore, compared with *BMPR2*-negative IPAH patients, *BMPR2*-positive patients have a higher pulmonary vascular resistance measured at diagnostic catheterization, are less likely to demonstrate acute vasoreactivity [Elliott et al., 2006; Rosenzweig et al., 2008], and are more likely to progress to death or lung transplantation [Sztrymf et al., 2008]. These observations all suggest that *BMPR2* mutations lead to a more severe PAH phenotype. Of note, patients with missense variants present with a higher degree of morbidity and mortality than those with truncating defects, suggestive of a more severe impact on the signaling pathway [Austin et al., 2009b]. However, *BMPR2* mutations are not associated with a worse exercise capacity and prognosis. The younger age of *BMPR2* mutation carriers may explain the similar survival and exercise capacity despite worse hemodynamics as compared with *BMPR2*-negative patients. Most recently, Girerd *et al*. indicated that *BMPR2* mutation position may influence clinical phenotype. Specifically, PAH patients with a point mutation within the cytoplasmic tail of *BMPR2* displayed a later age of onset, lower pulmonary vascular resistance and, of note, a higher proportion of acute vasodilator response by contrast to patients harboring mutations

outside of this domain. In addition, *in vitro* assays suggested that cytoplasmic domain mutations tolerated activation of the Smad pathway, which is indicative of a lower degree of penetrance [Girerd et al., 2015].

These findings appear not to be influenced by gender [Girerd et al., 2010b]. However, there is a trend for more severe prognosis of the disease in males, particularly in male *BMPR2* mutation carriers. This observation is consistent with the observation that PAH mortality is most closely associated with male gender [Humbert et al., 2010]. Even though no significant impact of gender was observed on age at diagnosis and outcomes, it should be emphasized that PAH mostly occurs in females, irrespective of *BMPR2* status (sex ratio females:males = 2.4:1 in both *BMPR2* mutation carriers and non-carriers). To explain over-representation of female patients it has been suggested that estrogens and estrogen metabolism might be involved in the pathogenesis of PAH [West et al., 2008a; Austin et al., 2009a; Mair et al., 2015]. These studies support the hypothesis that altered estrogen metabolism could contribute to the penetrance of PAH in women and suggest Cytochrome P450 1B1 (*CYP1B1*) as a sex-specific modifier gene.

Similar findings are observed with *ACVRL1* mutations with a significant number of pediatric cases and a dismal prognosis [Girerd et al., 2010a]. In this study, *ACVRL1* mutation carriers were shown to be characterized by a younger age at PAH diagnosis (21.8 ± 16.7 years) than *BMPR2* mutation carriers and non-carriers (35.7 ± 14.9 and 47.6 ± 16.3 years, respectively; $p < 0.0001$). However, *ACVRL1*-positive patients had better hemodynamic status at diagnosis, but none responded to acute vasodilator challenge. Thus, despite less severe initial hemodynamics and similar management, these patients had a worse prognosis than other patients with PAH, suggesting more rapid disease progression.

Most recently, a 'two-hit' model has been proposed, wherein digenic mutations may account for earlier occurrence, increased severity and more rapid deterioration of PAH patients [Wang et al., 2014].

Expansion of the Genetic Architecture of PAH by Next-Generation Sequence Analysis

Caveolin 1 (*CAV1*)

Whole-exome sequencing was used to study one large family with six PAH cases across three generations with autosomal dominant transmission and without known mutation. Specifically, the exomes of 4 of the 6 PAH patients were evaluated and following bioinformatic analyses 11 rare candidate variants were determined to be shared by all four patients in the family. Genetic analysis of an additional patient in the family supported the conclusion that a rare mutation in the *CAV1* gene (*PPH3*; MIM# 615343) was of pertinence to disease. The observed mutation in exon 3 (c.474delA; p.L159Sfs*22), impacts a highly conserved region and predicts deleterious functional consequences.

An additional 62 independent PAH families and 198 unrelated idiopathic PAH patients, all without detectable TGF-β gene mutations, were screened for *CAV1* mutations by Sanger sequencing. Of 260 patients one early-onset idiopathic patient harbored a *de novo CAV1* mutation in exon 3 of the gene (c.473delC; p.P158Hfs*23) (Table 4). Of note, identified variants were not present in over 1000 ethnically-matched Caucasian controls [Austin et al., 2012].

Potassium Channel, Subfamily K, Member 3 (*KCNK3*)

Most recently, whole-exome sequencing has led to the identification of *KCNK3* as a risk factor for familial and idiopathic disease (*PPH4*; MIM# 615344). By screening three affected subjects from an autosomal dominant family negative for mutation in *BMPR2*, *ACVRL1*,

ENG, *SMAD9* and *CAV1*, Ma *et al*. detected a novel coding variant of *KCNK3* that was shared among all three subjects and predicted to be pathogenic by *in silico* bioinformatic tools. Subsequent Sanger sequencing across the extended family confirmed co-segregation of the disease with the c.608G>A (p.G203D) variant, which was absent from 100 ethnically matched control individuals [Ma et al., 2013]. Analysis of exome sequence from 10 further HPAH probands identified two additional novel heterozygous variants, which also segregated with disease, providing strong evidence for a role of *KCNK3* in PAH pathogenesis. To assess the frequency of *KCNK3* variation in familial and idiopathic disease, an extended cohort of 82 HPAH and 230 IPAH cases were screened for mutation. Three novel heterozygous missense variants were detected in the idiopathic cohort, suggesting that *KCNK3* mutation accounts for 1.3% of IPAH cases and 3.2% of HPAH families [Ma et al., 2013] (Table 4).

***Eukaryotic Translation Initiation Factor 2 Alpha Kinase 4* (*EIF2AK4*)**

Similarly, exome sequencing has revealed several disease causing mutations in the *EIF2AK4* gene in pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH), together classified as group 1′ of PAH in the most recent diagnostic classification [Simonneau et al., 2013]. Both conditions are inherited in an autosomal recessive manner (*PVOD2*; MIM# 234810) and are mainly characterized by proliferation of capillaries in the lung leading to an occlusion of pulmonary vasculature. Eyries *et al*. assessed five families with PVOD and focused on rare variants (minor allele frequency <0.1% in control populations), which were homozygous or compound heterozygous in affected children and heterozygous in unaffected parents. Using this analysis strategy, mutations in *EIF2AK4* were identified in all 13 families studied. The examination of 20 sporadic cases revealed *EIF2AK4* mutations in 5 additional patients [Eyries et al., 2014]. Mutations were distributed throughout the gene and belonged to the major mutation categories (Table 5). The

range of mutations situated upon this locus underlines its functional importance in the development of PVOD.

In parallel, mutations in *EIF2AK4* were independently identified by Best *et al*. in patients with heritable PCH. The exomes of two affected brothers were sequenced and filtered for variants with a minor allele frequency of less than 1% in public repositories of polymorphic data resulting in the identification of two pathogenic variants in the gene. The unaffected parents and sister were confirmed to be heterozygous carriers of one of the two mutations [Best et al., 2014]. Further sequencing of *EIF2AK4* in 10 patients with pathologically verified sporadic PCH and one familial case detected three additional mutations in two sporadic patients (Table 5). The genetic correlation between PVOD and PCH further supports the likelihood that these diseases define different clinical spectra of the same underlying disorder.

Most recently, *EIF2AK4* has also been investigated in an itinerant Iberian population with PAH [Tenorio et al., 2015]. The authors identified a homozygous c.3344C>T (p.P1115L) missense mutation in five patients from five independent families with HPAH. Likely ancestral, this mutation co-segregated with a more severe phenotype than previously reported for other *EIF2AK4* mutations. The majority of affected subjects presented with an early onset, aggressive form of the disease resulting in an abnormally low survival rate post-lung transplantation (1.1 years).

Biological Significance of Mutations in Non-Canonical BMP Pathways

While rare, the biologic plausibility for *CAV1* mutations in PAH is strong. *CAV1* encodes Caveolin-1, a membrane protein required to form the flask-shaped invaginations of the cell membrane known as caveolae, abundant in lung endothelial and mesenchymal cells [Minshall et al., 2003; Xu et al., 2008]. Caveolae are critical to a number of cellular processes and

receptor rich regions of the cell membrane [Nohe et al., 2005; Mercier et al., 2009; Chidlow and Sessa, 2010]. Intriguingly, mice haploinsufficient for *Cav1* display pulmonary vascular disease analogous to PAH [Drab et al., 2001; Zhao et al., 2002; Murata et al., 2007; Maniatis et al., 2008]. Moreover, Caveolin-1 protein staining is reduced in lung endothelial cells from human PAH patients [Zhao and Malik, 2009]. Nevertheless, although under careful scrutiny, the precise mechanism(s) by which *CAV1* mutations promote PAH remain unclear.

The *KCNK3* gene is located on chromosome 2p24 and encodes a pH-sensitive potassium channel with a role in regulation of resting membrane potential in a variety of cell types. Electrophysiological analyses demonstrated that all identified mutations lead to a loss of function as measured by current density. A subset of identified mutations (p.T8K, p.E182K and p.G203D) exhibited a significant increase in potassium-channel current when treated with the phospholipase inhibitor ONO-RS-082. The identification of this gene provides an additional avenue of treatment strategies for PAH.

The *EIF2AK4* gene encodes a kinase, which phosphorylates an initiation factor in protein synthesis that is primarily responsible for the translation of stress response proteins [Donnelly et al., 2013]. Interactions between the kinase and various members of the BMPR-II pathway have also been detected, but the exact link to the clinical manifestations of pulmonary veno-occlusive PH remains to be clarified [Eyries et al., 2014]. The EIF2AK4 protein belongs to a family of kinases that regulate angiogenesis in response to cellular stress. Of interest, these properties are compatible with the angiogenic pathology of PCH, a disorder characterized by uncontrolled proliferation of pulmonary microvessels.

Modifying Predisposition to PAH:

***Cerebellin 2 Precursor* (*CBLN2*)**

PAH demonstrates several complex traits including incomplete penetrance, sex bias and variable age of disease onset both within and across families. This has led to the hypothesis that modifier genes contribute to disease manifestation and/or progression. To examine the role of common variation in PAH predisposition, Germain *et al*. performed a genome-wide association study (GWAS) to identify susceptibility loci in IPAH and HPAH cases without *BMPR2* mutation. The discovery dataset comprised 340 PAH patients and 1,068 healthy controls, genotyped for ~470,000 variants, from which the 384 most significant variants were assessed in an independent replication cohort of 285 cases and 457 controls. This approach, which represents the best powered study to date, identified two variants 52 kb downstream of the *CBLN2* gene that were associated with a two-fold increased risk of disease [Germain et al., 2013].

CBLN2 encodes a secreted neuronal glyocoprotein primarily expressed in the brain. However, real-time PCR studies demonstrated *CBLN2* mRNA expression in the whole lung, significantly higher in explants from PAH patients comparative to histologically normal lung tissue. Similar results were obtained for pulmonary arterial endothelial cells. Furthermore, an inhibition of pulmonary artery smooth muscle cell (PASMC) proliferation was observed when treated with increasing concentrations of CBLN2 peptide [Germain et al., 2013].

***Potassium Channel, Voltage Gated Shaker Related Subfamily A, Member 5* (*KCNA5*)**

Membrane potential is essential for contraction and vasodilation of PASMCs. Expression of potassium channels is regulated by BMP signaling *in vitro* and *in vivo* and knockouts in *Drosophila melanogaster* result in defects that are strikingly similar to phenotypes that result

from disrupted TGF-β/BMP signaling [Young et al., 2006; Dahal et al., 2012]. Variation of the potassium channel *KCNA5* has been identified in IPAH patients [Remillard et al., 2007; Wang et al., 2014] suggesting a potential role in PAH development and penetrance. The potassium channel response in PASMCs of IPAH patients has been proven to be down regulated [Yuan et al., 1998] likely increasing pulmonary vasoconstriction and PASMC proliferation. Moreover, the channel, which is responsive to nitric oxide, is reduced in patients carrying a specific coding missense mutation [Remillard et al., 2007]. Mutations within *KCNA5* have been identified as a so-called 'second hit' in an index patient additional to a *BMPR2* missense mutation leading to an early onset and severe phenotype [Wang et al., 2014]. While these findings may represent a rare case of genetic modification in PAH, replication of this digenic genotype in an independent cohort has not been observed. Further, in the absence of comprehensive functional analysis, interpretation of the significance of this study requires caution.

Animal Models of PAH

Model systems of PAH serve a two-fold purpose in providing 1) a source of relevant, genetically modified cell types for *in vitro* studies, and 2) an *in vivo* platform for the analysis and refinement of direct therapeutic intervention. Several natural and engineered models of disease exist, for example, the fawn-hooded rat and the *Bmpr2* transgenic mouse over-expressing the p.R899* mutant allele, the latter being generated by introduction of a smooth muscle-specific doxycycline-inducible mutant transgene [West et al., 2008b; Ryan et al., 2011]. Further, knock-out models lacking exons 4 and 5 of *Bmpr2* generated by homologous recombination have been developed. These models develop mild to moderate disease phenotypes, often upon the application of environmental insults including exposure to hypoxia and 5-lipoxygenase as previously described [Machado et al., 2006]. Conditional

targeting of mutant alleles to hallmark sites of damage in PAH, in particular the pulmonary artery endothelial or smooth muscle cell layers, has produced a more convincing *in vivo* reflection of the human disease state [West et al., 2004; Hong et al., 2008]. Most recently a heterozygous knock-in mouse model of the p.R899* mutation has been shown to develop age-related disease with close phenotypic relatedness to the human condition [Long et al., 2015]. Additionally a rat model of PAH ($BMPR2^{\Delta 140Ex1/+}$), the first of its kind, has provided support to the hypothesis that endothelial-to-mesenchymal transition represents a pathophysiological process in PAH [Ranchoux et al., 2015]. Of note, mice representative of recently observed defects in previously uncharacterized genes, namely *Cav1* and *Smad9*, support the emerging concept of greater than anticipated genetic heterogeneity in PAH. Homozygous knock-out models of both genes develop spontaneous indications of PAH providing a powerful correlation to the human studies described herein [Zhao et al., 2002; Huang et al., 2009].

Genetic Counseling

Current guidelines recommend offering molecular genetic analysis and genetic counseling for HPAH patients [Badesch et al., 2007; McLaughlin et al., 2009]. Genetic counseling typically offers a combinatorial approach of dealing with putative outcomes and consequences of the analysis, based upon structured protocols determined by specialist centers evaluates the family history, educates patients about the causes of PAH, discusses the risks and benefits of genetic testing and supports patients and families through the process of genetic testing and disclosure of results. In addition to HPAH patients, IPAH patients and their relatives may also benefit from these analyses since up to 25% will have a *BMPR2* mutation; therefore, genetic testing in these populations should be considered [Badesch et al., 2007]. Current findings indicate that in patient populations with PVOD and PCH, autosomal

recessive inheritance of the *EIF2AK4* gene is the most likely mode of transmission. Hence, within the PAH spectrum of disease, based on current classification, genetic counseling should take account of both autosomal dominant and recessive inheritance models, as well as the higher penetrance of bi-allelic mutations in patients carrying this genetic defect.

Following identification of an established molecular defect in the proband, at-risk asymptomatic family members should be offered the option of genetic counseling and targeted mutation analysis within the parameters of full informed consent. Importantly, the data provided here offer an indication of the likely pathogenicity of identified variation. A significant factor to consider is the reduced penetrance of mutant alleles which must be addressed by informed pre-test counseling, comprising robust estimations of risk based on available population-specific epidemiological data and gender [Cogan et al., 2012; Larkin et al., 2012]. Of note, where a mutation is identified, implications for reproductive planning must be considered. In families harboring mutation, early mutational analysis of offspring is essential and should be combined with clinical assessment and initiation of treatment as deemed necessary by specialist centers. Additionally, a possible future avenue of medical care has been explored. Within a *BMPR2*-positive family, pre-implantation genetic analysis of blastomeres following *in vitro* fertilization led to the successful implantation and delivery of a mutation-negative offspring [Frydman et al., 2012]. Although in its earliest stages this strategy is of significant clinical potential in the context of considered, long-term genetic evaluation.

Diagnostic Strategies

Molecular diagnostics for PAH have traditionally focused around dideoxy Sanger sequencing methods to screen the *BMPR2*, *ACVRL1* and *ENG* protein-coding regions for heterozygous mutation. However, based on these and previous studies, for a complete

exploration of deletion and duplication across these three genes, the application of MLPA or targeted comparative genomic hybridization (CGH) array technology is required. The HHT/PPH1 MLPA panel was introduced by MRC-Holland in the mid-2000s, with the current version containing 51 probes across *BMPR2*, *ACVRL1* and *ENG*, and has led to the successful detection of numerous gene rearrangements that would otherwise not be identified by sequence analysis [Aldred et al., 2006; Cogan et al., 2006].

Despite the success of this combined approach to mutation detection in PAH, the recent expansion of candidate disease genes has resulted in traditional sequencing methods becoming more labor-intensive and less cost-effective. Indeed, to comprehensively screen the 10 genes detailed here would involve sequencing approximately 105 coding exons, totaling over 19 kb of DNA sequence. Custom capture and NGS are now becoming chosen methodologies across screening centers globally for the analysis of established candidate genes.

Future Prospects

It is clear that the most significant advance in the identification of risk factors for PAH over recent years has been the advent of exome sequencing, which has led to the rapid identification of multiple novel genes using relatively small sample sets. Whilst exome sequencing is undoubtedly a powerful method for detecting rare, highly penetrant genes in families with multiple affected individuals, there remain important caveats in applying these technologies to the identification of novel genes across cohorts of unrelated subjects and genetically divergent patient groups. In PAH, complexities such as locus heterogeneity, incomplete penetrance, *de novo* mutation and late onset of disease introduce significant challenges to the interpretation of exome sequence data, highlighting the need for large homogeneous patient cohorts to detect pathogenic variation. In terms of technical limitations,

exome sequencing also only focuses on the exonic regions. While these regions represent the most likely sites of functional mutations in PAH, there is increasing evidence supporting pathogenic variants in the promoter and 5′ UTR of *BMPR2*, suggesting this may also be true of other PAH genes. A recent report by Hinderhofer *et al*. has also identified an intronic mutation of *BMPR2* that leads to aberrant splicing due to an insertion of an intronic Alu element 26 bp upstream from exon 6 [Hinderhofer et al., 2014]. This points to a potential role for other intronic variants or regulatory elements such as intra- or intergenic enhancer and repressor motifs in the pathogenesis of PAH.

To address some of these issues, it is likely that future avenues will include the use of more NGS technologies, the pinnacle of which is whole-genome sequencing. Harnessing the complete genetic information of individuals affected with PAH will not only offer opportunities to identify the causative mutation but will also provide an important tool to correlate phenotypic information to individual genotype data, allowing for tailored approaches to the clinical management of disease, or so-called personalized or precision medicine. While this technology remains financially prohibitive to some centers, custom capture of genes with defined causal links to disease offers an alternative, yet powerful, means of analyses. These studies would, ideally, include non-coding sections of the genes to determine the presence of regulatory mutations increasingly implicated in PAH etiology as described herein. The catalogue of mutations described in this report provides an important tool for the determination of deleterious mutation, both within the context of conventional and indeed NGS analyses.

For future gene identification and follow-up GWAS studies, it is of particular importance in PAH, which exhibits traits of complex disease, that phenotype is precisely assigned for this disorder. This degree of rigor is most likely to lead to further advances in both the understanding and treatment of PAH at a significantly accelerated pace.

Acknowledgments

The authors would like to sincerely thank all of the individuals with PAH for their participation in these studies. We also thank our many clinical and scientific collaborators who over a considerable period of time have ascertained patient samples complete with crucial phenotypic information. This work was supported by funding from the National Institutes of Health to EDA, JEL and JHN (PO1 HL108800), MAA (R01 HL098199) and WKC (R01 HL060056). Funding has also been provided within the 5th European Framework "Disposition to PPH" for the examination of patients from the center in Heidelberg.

Conflicts of interest

The authors declare no conflicts of interest.

References

Abdalla SA, Gallione CJ, Barst RJ, Horn EM, Knowles JA, Marchuk DA, Letarte M, Morse JH. 2004. Primary pulmonary hypertension in families with hereditary haemorrhagic telangiectasia. Eur Respir J 23:373-377.

Adzhubei I, Jordan DM, Sunyaev SR. 2013. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet Chapter 7:Unit7.20.

Aldred MA, Vijayakrishnan J, James V, Soubrier F, Gomez-Sanchez MA, Martensson G, Galie N, Manes A, Corris P, Simonneau G, Humbert M, Morrell NW, et al. 2006. BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. Hum Mutat 27:212-213.

Aldred MA, Machado RD, James V, Morrell NW, Trembath RC. 2007. Characterization of the BMPR2 5'-untranslated region and a novel mutation in pulmonary hypertension. Am J Respir Crit Care Med 176:819-824.

Archer SL, Weir EK, Wilkins MR. 2010. Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies. Circulation 121:2045-2066.

Austin ED, Cogan JD, West JD, Hedges LK, Hamid R, Dawson EP, Wheeler LA, Parl FF, Loyd JE, Phillips JA, 3rd. 2009a. Alterations in oestrogen metabolism: implications for higher penetrance of familial pulmonary arterial hypertension in females. Eur Respir J 34:1093-1099.

Austin ED, Phillips JA, Cogan JD, Hamid R, Yu C, Stanton KC, Phillips CA, Wheeler LA, Robbins IM, Newman JH, Loyd JE. 2009b. Truncating and missense BMPR2 mutations differentially affect the severity of heritable pulmonary arterial hypertension. Respir Res 10:87.

Austin ED, Ma L, LeDuc C, Berman Rosenzweig E, Borczuk A, Phillips JA, 3rd, Palomero T, Sumazin P, Kim HR, Talati MH, West J, Loyd JE, et al. 2012. Whole exome sequencing to identify a novel gene (caveolin-1) associated with human pulmonary arterial hypertension. Circ Cardiovasc Genet 5:336-343.

Badesch DB, Abman SH, Simonneau G, Rubin LJ, McLaughlin VV. 2007. Medical therapy for pulmonary arterial hypertension: updated ACCP evidence-based clinical practice guidelines. Chest 131:1917-1928.

Best DH, Vaughn C, McDonald J, Damjanovich K, Runo JR, Chibuk JM, Bayrak-Toydemir P. 2011. Mosaic ACVRL1 and ENG mutations in hereditary haemorrhagic telangiectasia patients. J Med Genet 48:358-360.

Best DH, Sumner KL, Austin ED, Chung WK, Brown LM, Borczuk AC, Rosenzweig EB, Bayrak-Toydemir P, Mao R, Cahill BC, Tazelaar HD, Leslie KO, et al. 2014. EIF2AK4 mutations in pulmonary capillary hemangiomatosis. Chest 145:231-236.

Chaouat A, Coulet F, Favre C, Simonneau G, Weitzenblum E, Soubrier F, Humbert M. 2004. Endoglin germline mutation in a patient with hereditary haemorrhagic telangiectasia and dexfenfluramine associated pulmonary arterial hypertension. Thorax 59:446-448.

Chen YJ, Yang QH, Liu D, Liu QQ, Eyries M, Wen L, Wu WH, Jiang X, Yuan P, Zhang R, Soubrier F, Jing ZC. 2013. Clinical and genetic characteristics of Chinese patients with hereditary haemorrhagic telangiectasia-associated pulmonary hypertension. Eur J Clin Invest 43:1016-1024.

Chida A, Shintani M, Nakayama T, Furutani Y, Hayama E, Inai K, Saji T, Nonoyama S, Nakanishi T. 2012a. Missense mutations of the BMPR1B (ALK6) gene in childhood idiopathic pulmonary arterial hypertension. Circ J 76:1501-1508.

Chida A, Shintani M, Yagi H, Fujiwara M, Kojima Y, Sato H, Imamura S, Yokozawa M, Onodera N, Horigome H, Kobayashi T, Hatai Y, et al. 2012b. Outcomes of childhood pulmonary arterial hypertension in BMPR2 and ALK1 mutation carriers. Am J Cardiol 110:586-593.

Chidlow JH, Jr, Sessa WC. 2010. Caveolae, caveolins, and cavins: complex control of cellular signalling and inflammation. Cardiovasc Res 86:219-225.

Choi Y, Chan AP. 2015. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics 31:2745-2747.

Cogan JD, Pauciulo MW, Batchman AP, Prince MA, Robbins IM, Hedges LK, Stanton KC, Wheeler LA, Phillips JA, 3rd, Loyd JE, Nichols WC. 2006. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. Am J Respir Crit Care Med 174:590-598.

Cogan J, Austin E, Hedges L, Womack B, West J, Loyd J, Hamid R. 2012. Role of BMPR2 alternative splicing in heritable pulmonary arterial hypertension penetrance. Circulation 126:1907-1916.

Dahal GR, Rawson J, Gassaway B, Kwok B, Tong Y, Ptacek LJ, Bates E. 2012. An inwardly rectifying K+ channel is required for patterning. Development 139:3653-3664.

David L, Feige JJ, Bailly S. 2009. Emerging role of bone morphogenetic proteins in angiogenesis. Cytokine Growth Factor Rev 20:203-212.

Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, Kalachikov S, Cayanis E, Fischer SG, Barst RJ, Hodge SE, Knowles JA. 2000. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. Am J Hum Genet 67:737-744.

Donnelly N, Gorman AM, Gupta S, Samali A. 2013. The eIF2alpha kinases: their structures and functions. Cell Mol Life Sci 70:3493-3511.

Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, Menne J, Lindschau C, Mende F, Luft FC, Schedl A, Haller H, et al. 2001. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. Science 293:2449-2452.

Drake KM, Zygmunt D, Mavrakis L, Harbor P, Wang L, Comhair SA, Erzurum SC, Aldred MA. 2011. Altered MicroRNA processing in heritable pulmonary arterial hypertension: an important role for Smad-8. Am J Respir Crit Care Med 184:1400-1408.

Elliott CG, Glissmeyer EW, Havlena GT, Carlquist J, McKinney JT, Rich S, McGoon MD, Scholand MB, Kim M, Jensen RL, Schmidt JW, Ward K. 2006. Relationship of BMPR2 mutations to vasoreactivity in pulmonary arterial hypertension. Circulation 113:2509-2515.

Eyries M, Coulet F, Girerd B, Montani D, Humbert M, Lacombe P, Chinet T, Gouya L, Roume J, Axford MM, Pearson CE, Soubrier F. 2012. ACVRL1 germinal mosaic with two mutant alleles in hereditary hemorrhagic telangiectasia associated with pulmonary arterial hypertension. Clin Genet 82:173-179.

Eyries M, Montani D, Girerd B, Perret C, Leroy A, Lonjou C, Chelghoum N, Coulet F, Bonnet D, Dorfmuller P, Fadel E, Sitbon O, et al. 2014. EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension. Nat Genet 46:65-69.

Feng YX, Liu D, Sun ML, Jiang X, Sun N, Mao YM, Jing ZC. 2014. BMPR2 germline mutation in chronic thromboembolic pulmonary hypertension. Lung 192:625-627.

Foletta VC, Lim MA, Soosairajah J, Kelly AP, Stanley EG, Shannon M, He W, Das S, Massague J, Bernard O. 2003. Direct signaling by the BMP type II receptor via the cytoskeletal regulator LIMK1. J Cell Biol 162:1089-1098.

Frydman N, Steffann J, Girerd B, Frydman R, Munnich A, Simonneau G, Humbert M. 2012. Pre-implantation genetic diagnosis in pulmonary arterial hypertension due to BMPR2 mutation. Eur Respir J 39:1534-1535.

Fujiwara M, Yagi H, Matsuoka R, Akimoto K, Furutani M, Imamura S, Uehara R, Nakayama T, Takao A, Nakazawa M, Saji T. 2008. Implications of mutations of activin receptor-like kinase 1 gene (ALK1) in addition to bone morphogenetic protein receptor II gene (BMPR2) in children with pulmonary arterial hypertension. Circ J 72:127-133.

Germain M, Eyries M, Montani D, Poirier O, Girerd B, Dorfmuller P, Coulet F, Nadaud S, Maugenre S, Guignabert C, Carpentier W, Vonk-Noordegraaf A, et al. 2013. Genome-wide association analysis identifies a susceptibility locus for pulmonary arterial hypertension. Nat Genet 45:518-521.

Girerd B, Montani D, Coulet F, Sztrymf B, Yaici A, Jais X, Tregouet D, Reis A, Drouin-Garraud V, Fraisse A, Sitbon O, O'Callaghan DS, et al. 2010a. Clinical outcomes of pulmonary arterial hypertension in patients carrying an ACVRL1 (ALK1) mutation. Am J Respir Crit Care Med 181:851-861.

Girerd B, Montani D, Eyries M, Yaici A, Sztrymf B, Coulet F, Sitbon O, Simonneau G, Soubrier F, Humbert M. 2010b. Absence of influence of gender and BMPR2 mutation type on clinical phenotypes of pulmonary arterial hypertension. Respir Res 11:73.

Girerd B, Coulet F, Jais X, Eyries M, Van Der Bruggen C, De Man F, Houweling A, Dorfmuller P, Savale L, Sitbon O, Vonk-Noordegraaf A, Soubrier F, et al. 2015. Characteristics of pulmonary arterial hypertension in affected carriers of a mutation

located in the cytoplasmic tail of bone morphogenetic protein receptor type 2. Chest 147:1385-1394.

Greenwald J, Fischer WH, Vale WW, Choe S. 1999. Three-finger toxin fold for the extracellular ligand-binding domain of the type II activin receptor serine kinase. Nat Struct Biol 6:18-22.

Harrison RE, Flanagan JA, Sankelo M, Abdalla SA, Rowell J, Machado RD, Elliott CG, Robbins IM, Olschewski H, McLaughlin V, Gruenig E, Kermeen F, et al. 2003. Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. J Med Genet 40:865-871.

Harrison RE, Berger R, Haworth SG, Tulloh R, Mache CJ, Morrell NW, Aldred MA, Trembath RC. 2005. Transforming growth factor-beta receptor mutations and pulmonary arterial hypertension in childhood. Circulation 111:435-441.

Hinderhofer K, Fischer C, Pfarr N, Szamalek-Hoegel J, Lichtblau M, Nagel C, Egenlauf B, Ehlken N, Grünig E. 2014. Identification of a new intronic BMPR2-mutation and early diagnosis of heritable pulmonary arterial hypertension in a large family with mean clinical follow-up of 12 years. PLoS One 9:e91374.

Hong KH, Lee YJ, Lee E, Park SO, Han C, Beppu H, Li E, Raizada MK, Bloch KD, Oh SP. 2008. Genetic ablation of the BMPR2 gene in pulmonary endothelium is sufficient to predispose to pulmonary arterial hypertension. Circulation 118:722-730.

Huang Z, Wang D, Ihida-Stansbury K, Jones PL, Martin JF. 2009. Defective pulmonary vascular remodeling in Smad8 mutant mice. Hum Mol Genet 18:2791-2801.

Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, et al. 2010. Survival in patients with

idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation 122:156-163.

Ishiwata T, Terada J, Tanabe N, Abe M, Sugiura T, Tsushima K, Tada Y, Sakao S, Kasahara Y, Nakanishi N, Morisaki H, Tatsumi K. 2014. Pulmonary arterial hypertension as the first manifestation in a patient with hereditary hemorrhagic telangiectasia. Intern Med 53:2359-2363.

Jones G, Robertson L, Harrison R, Ridout C, Vasudevan P. 2014. Somatic mosaicism in ACVRL1 with transmission to several offspring affected with severe pulmonary arterial hypertension. Am J Med Genet A 164A:2121-2123.

Kabata H, Satoh T, Kataoka M, Tamura Y, Ono T, Yamamoto M, Huqun, Hagiwara K, Fukuda K, Betsuyaku T, Asano K. 2013. Bone morphogenetic protein receptor type 2 mutations, clinical phenotypes and outcomes of Japanese patients with sporadic or familial pulmonary hypertension. Respirology 18:1076-1082.

Koehler R, Grünig E, Pauciulo MW, Hoeper MM, Olschewski H, Wilkens H, Halank M, Winkler J, Ewert R, Bremer H, Kreuscher S, Janssen B, et al. 2004. Low frequency of BMPR2 mutations in a German cohort of patients with sporadic idiopathic pulmonary arterial hypertension. J Med Genet 41:e127.

Kumar P, Henikoff S, Ng PC. 2009. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4:1073-1081.

Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA, 3rd, Loyd JE, Nichols WC, Trembath RC. 2000. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. Nat Genet 26:81-84.

Larkin EK, Newman JH, Austin ED, Hemnes AR, Wheeler L, Robbins IM, West JD, Phillips JA, 3rd, Hamid R, Loyd JE. 2012. Longitudinal analysis casts doubt on the presence of

genetic anticipation in heritable pulmonary arterial hypertension. Am J Respir Crit Care Med 186:892-896.

Liu D, Liu QQ, Eyries M, Wu WH, Yuan P, Zhang R, Soubrier F, Jing ZC. 2012. Molecular genetics and clinical features of Chinese idiopathic and heritable pulmonary arterial hypertension patients. Eur Respir J 39:597-603.

Liu F, Ventura F, Doody J, Massague J. 1995. Human type II receptor for bone morphogenic proteins (BMPs): extension of the two-kinase receptor model to the BMPs. Mol Cell Biol 15:3479-3486.

Long L, Ormiston ML, Yang X, Southwood M, Graf S, Machado RD, Mueller M, Kinzel B, Yung LM, Wilkinson JM, Moore SD, Drake KM, et al. 2015. Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. Nat Med 21:777-785.

Ma L, Roman-Campos D, Austin ED, Eyries M, Sampson KS, Soubrier F, Germain M, Tregouet DA, Borczuk A, Rosenzweig EB, Girerd B, Montani D, et al. 2013. A novel channelopathy in pulmonary arterial hypertension. N Engl J Med 369:351-361.

Machado RD, Pauciulo MW, Thomson JR, Lane KB, Morgan NV, Wheeler L, Phillips JA, 3rd, Newman J, Williams D, Galie N, Manes A, McNeil K, et al. 2001. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. Am J Hum Genet 68:92-102.

Machado RD, Rudarakanchana N, Atkinson C, Flanagan JA, Harrison R, Morrell NW, Trembath RC. 2003. Functional interaction between BMPR-II and Tctex-1, a light chain of Dynein, is isoform-specific and disrupted by mutations underlying primary pulmonary hypertension. Hum Mol Genet 12:3277-3286.

Machado RD, Aldred MA, James V, Harrison RE, Patel B, Schwalbe EC, Gruenig E, Janssen B, Koehler R, Seeger W, Eickelberg O, Olschewski H, et al. 2006. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. Hum Mutat 27:121-132.

Machado RD, Eickelberg O, Elliott CG, Geraci MW, Hanaoka M, Loyd JE, Newman JH, Phillips JA, 3rd, Soubrier F, Trembath RC, Chung WK. 2009. Genetics and genomics of pulmonary arterial hypertension. J Am Coll Cardiol 54:S32-42.

Mache CJ, Gamillscheg A, Popper HH, Haworth SG. 2008. Early-life pulmonary arterial hypertension with subsequent development of diffuse pulmonary arteriovenous malformations in hereditary haemorrhagic telangiectasia type 1. Thorax 63:85-86.

Mair KM, Yang XD, Long L, White K, Wallace E, Ewart MA, Docherty CK, Morrell NW, MacLean MR. 2015. Sex affects bone morphogenetic protein type II receptor signaling in pulmonary artery smooth muscle cells. Am J Respir Crit Care Med 191:693-703.

Maniatis NA, Shinin V, Schraufnagel DE, Okada S, Vogel SM, Malik AB, Minshall RD. 2008. Increased pulmonary vascular resistance and defective pulmonary artery filling in caveolin-1-/- mice. Am J Physiol Lung Cell Mol Physiol 294:L865-873.

McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, Mathier MA, McGoon MD, Park MH, Rosenson RS, Rubin LJ, Tapson VF, et al. 2009. ACCF/AHA 2009 expert consensus document on pulmonary hypertension a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. J Am Coll Cardiol 53:1573-1619.

Mercier I, Jasmin JF, Pavlides S, Minetti C, Flomenberg N, Pestell RG, Frank PG, Sotgia F, Lisanti MP. 2009. Clinical and translational implications of the caveolin gene family: lessons from mouse models and human genetic disorders. Lab Invest 89:614-623.

Minshall RD, Sessa WC, Stan RV, Anderson RG, Malik AB. 2003. Caveolin regulation of endothelial function. Am J Physiol Lung Cell Mol Physiol 285:L1179-1183.

Möller T, Leren TP, Eiklid KL, Holmstrøm H, Fredriksen PM, Thaulow E. 2010. A novel BMPR2 gene mutation associated with exercise-induced pulmonary hypertension in septal defects. Scand Cardiovasc J 44:331-336.

Murata T, Lin MI, Huang Y, Yu J, Bauer PM, Giordano FJ, Sessa WC. 2007. Reexpression of caveolin-1 in endothelium rescues the vascular, cardiac, and pulmonary defects in global caveolin-1 knockout mice. J Exp Med 204:2373-2382.

Nasim MT, Ghouri A, Patel B, James V, Rudarakanchana N, Morrell NW, Trembath RC. 2008. Stoichiometric imbalance in the receptor complex contributes to dysfunctional BMPR-II mediated signalling in pulmonary arterial hypertension. Hum Mol Genet 17:1683-1694.

Nasim MT, Ogo T, Ahmed M, Randall R, Chowdhury HM, Snape KM, Bradshaw TY, Southgate L, Lee GJ, Jackson I, Lord GM, Gibbs JS, et al. 2011. Molecular genetic characterization of SMAD signaling molecules in pulmonary arterial hypertension. Hum Mutat 32:1385-1389.

Nishihara A, Watabe T, Imamura T, Miyazono K. 2002. Functional heterogeneity of bone morphogenetic protein receptor-II mutants found in patients with primary pulmonary hypertension. Mol Biol Cell 13:3055-3063.

Nohe A, Keating E, Underhill TM, Knaus P, Petersen NO. 2005. Dynamics and interaction of caveolin-1 isoforms with BMP-receptors. J Cell Sci 118:643-650.

Pfarr N, Szamalek-Hoegel J, Fischer C, Hinderhofer K, Nagel C, Ehlken N, Tiede H, Olschewski H, Reichenberger F, Ghofrani AH, Seeger W, Grunig E. 2011. Hemodynamic and clinical onset in patients with hereditary pulmonary arterial hypertension and BMPR2 mutations. Respir Res 12:99.

Pfarr N, Fischer C, Ehlken N, Becker-Grunig T, Lopez-Gonzalez V, Gorenflo M, Hager A, Hinderhofer K, Miera O, Nagel C, Schranz D, Grunig E. 2013. Hemodynamic and genetic analysis in children with idiopathic, heritable, and congenital heart disease associated pulmonary arterial hypertension. Respir Res 14:3.

Ranchoux B, Antigny F, Rucker-Martin C, Hautefort A, Pechoux C, Bogaard HJ, Dorfmuller P, Remy S, Lecerf F, Plante S, Chat S, Fadel E, et al. 2015. Endothelial-to-mesenchymal transition in pulmonary hypertension. Circulation 131:1006-1018.

Remillard CV, Tigno DD, Platoshyn O, Burg ED, Brevnova EE, Conger D, Nicholson A, Rana BK, Channick RN, Rubin LJ, O'Connor DT, Yuan JX. 2007. Function of Kv1.5 channels and genetic variations of KCNA5 in patients with idiopathic pulmonary arterial hypertension. Am J Physiol Cell Physiol 292:C1837-1853.

Rigueur D, Brugger S, Anbarchian T, Kim JK, Lee Y, Lyons KM. 2015. The Type I BMP Receptor ACVR1/ALK2 is Required for Chondrogenesis During Development. J Bone Miner Res 30:733-741.

Rosenzweig EB, Morse JH, Knowles JA, Chada KK, Khan AM, Roberts KE, McElroy JJ, Juskiw NK, Mallory NC, Rich S, Diamond B, Barst RJ. 2008. Clinical implications of determining BMPR2 mutation status in a large cohort of children and adults with pulmonary arterial hypertension. J Heart Lung Transplant 27:668-674.

Rudarakanchana N, Flanagan JA, Chen H, Upton PD, Machado R, Patel D, Trembath RC, Morrell NW. 2002. Functional analysis of bone morphogenetic protein type II receptor mutations underlying primary pulmonary hypertension. Hum Mol Genet 11:1517-1525.

Ryan J, Bloch K, Archer SL. 2011. Rodent models of pulmonary hypertension: harmonisation with the world health organisation's categorisation of human PH. Int J Clin Pract Suppl:15-34.

Shi Y, Massague J. 2003. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113:685-700.

Shintani M, Yagi H, Nakayama T, Saji T, Matsuoka R. 2009. A new nonsense mutation of SMAD8 associated with pulmonary arterial hypertension. J Med Genet 46:331-337.

Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, Gomez Sanchez MA, Krishna Kumar R, Landzberg M, Machado RF, Olschewski H, Robbins IM, et al. 2013. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol 62:D34-41.

Smoot LB, Obler D, McElhinney DB, Boardman K, Wu BL, Lip V, Mullen MP. 2009. Clinical features of pulmonary arterial hypertension in young people with an ALK1 mutation and hereditary haemorrhagic telangiectasia. Arch Dis Child 94:506-511.

Sobolewski A, Rudarakanchana N, Upton PD, Yang J, Crilley TK, Trembath RC, Morrell NW. 2008. Failure of bone morphogenetic protein receptor trafficking in pulmonary arterial hypertension: potential for rescue. Hum Mol Genet 17:3180-3190.

Soubrier F, Chung WK, Machado R, Grunig E, Aldred M, Geraci M, Loyd JE, Elliott CG, Trembath RC, Newman JH, Humbert M. 2013. Genetics and genomics of pulmonary arterial hypertension. J Am Coll Cardiol 62:D13-21.

Sztrymf B, Coulet F, Girerd B, Yaici A, Jais X, Sitbon O, Montani D, Souza R, Simonneau G, Soubrier F, Humbert M. 2008. Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. Am J Respir Crit Care Med 177:1377-1383.

Tenorio J, Navas P, Barrios E, Fernandez L, Nevado J, Quezada CA, Lopez-Meseguer M, Arias P, Mena R, Lobo JL, Alvarez C, Heath K, et al. 2015. A founder EIF2AK4 mutation causes an aggressive form of pulmonary arterial hypertension in Iberian Gypsies. Clin Genet [Epub: 7 Jan 2015] doi:10.1111/cge.12549.

Thomson JR, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, Ward K, Yacoub M, Mikhail G, Rogers P, Newman J, Wheeler L, et al. 2000. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. J Med Genet 37:741-745.

Trembath RC, Thomson JR, Machado RD, Morgan NV, Atkinson C, Winship I, Simonneau G, Galie N, Loyd JE, Humbert M, Nichols WC, Morrell NW, et al. 2001. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. N Engl J Med 345:325-334.

Tuder RM, Archer SL, Dorfmuller P, Erzurum SC, Guignabert C, Michelakis E, Rabinovitch M, Schermuly R, Stenmark KR, Morrell NW. 2013. Relevant issues in the pathology and pathobiology of pulmonary hypertension. J Am Coll Cardiol 62:D4-12.

Vonk-Noordegraaf A, Haddad F, Chin KM, Forfia PR, Kawut SM, Lumens J, Naeije R, Newman J, Oudiz RJ, Provencher S, Torbicki A, Voelkel NF, et al. 2013. Right heart adaptation to pulmonary arterial hypertension: physiology and pathobiology. J Am Coll Cardiol 62:D22-33.

Wang G, Knight L, Ji R, Lawrence P, Kanaan U, Li L, Das A, Cui B, Zou W, Penny DJ, Fan Y. 2014. Early onset severe pulmonary arterial hypertension with 'two-hit' digenic mutations in both BMPR2 and KCNA5 genes. Int J Cardiol 177:e167-169.

Wang H, Li W, Zhang W, Sun K, Song X, Gao S, Zhang C, Hui R, Hu H. 2009. Novel promoter and exon mutations of the BMPR2 gene in Chinese patients with pulmonary arterial hypertension. Eur J Hum Genet 17:1063-1069.

Wang H, Cui QQ, Sun K, Song L, Zou YB, Wang XJ, Jia L, Liu X, Gao S, Zhang CN, Hui RT. 2010. Identities and frequencies of BMPR2 mutations in Chinese patients with idiopathic pulmonary arterial hypertension. Clin Genet 77:189-192.

West J, Fagan K, Steudel W, Fouty B, Lane K, Harral J, Hoedt-Miller M, Tada Y, Ozimek J, Tuder R, Rodman DM. 2004. Pulmonary hypertension in transgenic mice expressing a dominant-negative BMPRII gene in smooth muscle. Circ Res 94:1109-1114.

West J, Cogan J, Geraci M, Robinson L, Newman J, Phillips JA, Lane K, Meyrick B, Loyd J. 2008a. Gene expression in BMPR2 mutation carriers with and without evidence of pulmonary arterial hypertension suggests pathways relevant to disease penetrance. BMC Med Genomics 1:45.

West J, Harral J, Lane K, Deng Y, Ickes B, Crona D, Albu S, Stewart D, Fagan K. 2008b. Mice expressing BMPR2R899X transgene in smooth muscle develop pulmonary vascular lesions. Am J Physiol Lung Cell Mol Physiol 295:L744-755.

Xu Y, Buikema H, van Gilst WH, Henning RH. 2008. Caveolae and endothelial dysfunction: filling the caves in cardiovascular disease. Eur J Pharmacol 585:256-260.

Young KA, Ivester C, West J, Carr M, Rodman DM. 2006. BMP signaling controls PASMC KV channel expression in vitro and in vivo. Am J Physiol Lung Cell Mol Physiol 290:L841-848.

Yuan JX, Aldinger AM, Juhaszova M, Wang J, Conte JV, Jr, Gaine SP, Orens JB, Rubin LJ. 1998. Dysfunctional voltage-gated K+ channels in pulmonary artery smooth muscle cells of patients with primary pulmonary hypertension. Circulation 98:1400-1406.

Zhao YY, Liu Y, Stan RV, Fan L, Gu Y, Dalton N, Chu PH, Peterson K, Ross J, Jr, Chien KR. 2002. Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice. Proc Natl Acad Sci U S A 99:11375-11380.

Zhao YY, Malik AB. 2009. A novel insight into the mechanism of pulmonary hypertension involving caveolin-1 deficiency and endothelial nitric oxide synthase activation. Trends Cardiovasc Med 19:238-242.

Figure 1. Schematic of canonical BMP signaling and additional pathways implicated in PAH pathogenesis by conventional and next-generation sequence analysis. Causal genes are indicated by the asterisks.

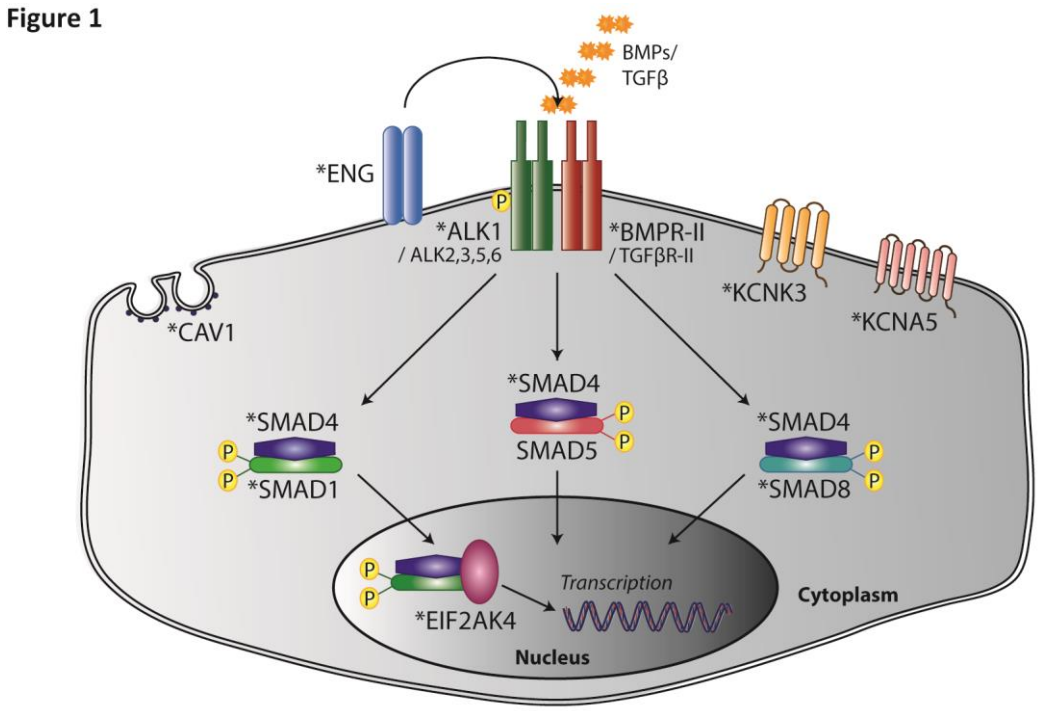

Figure 2. A) Distribution of reported exonic mutations across the *BMPR2* gene. Black bars represent all mutation categories; grey bars indicate missense mutations only. This graph excludes data from non-coding regions and gene rearrangements for which start and/or end points have not been conclusively determined. B) Proportion of distinct point mutations relative to exon size. Multiple and/or recurrent variants at the same nucleotide were counted as a single event. The total number of mutated residues confined to the open-reading frame was calculated as a percentage of exon length in nucleotides.

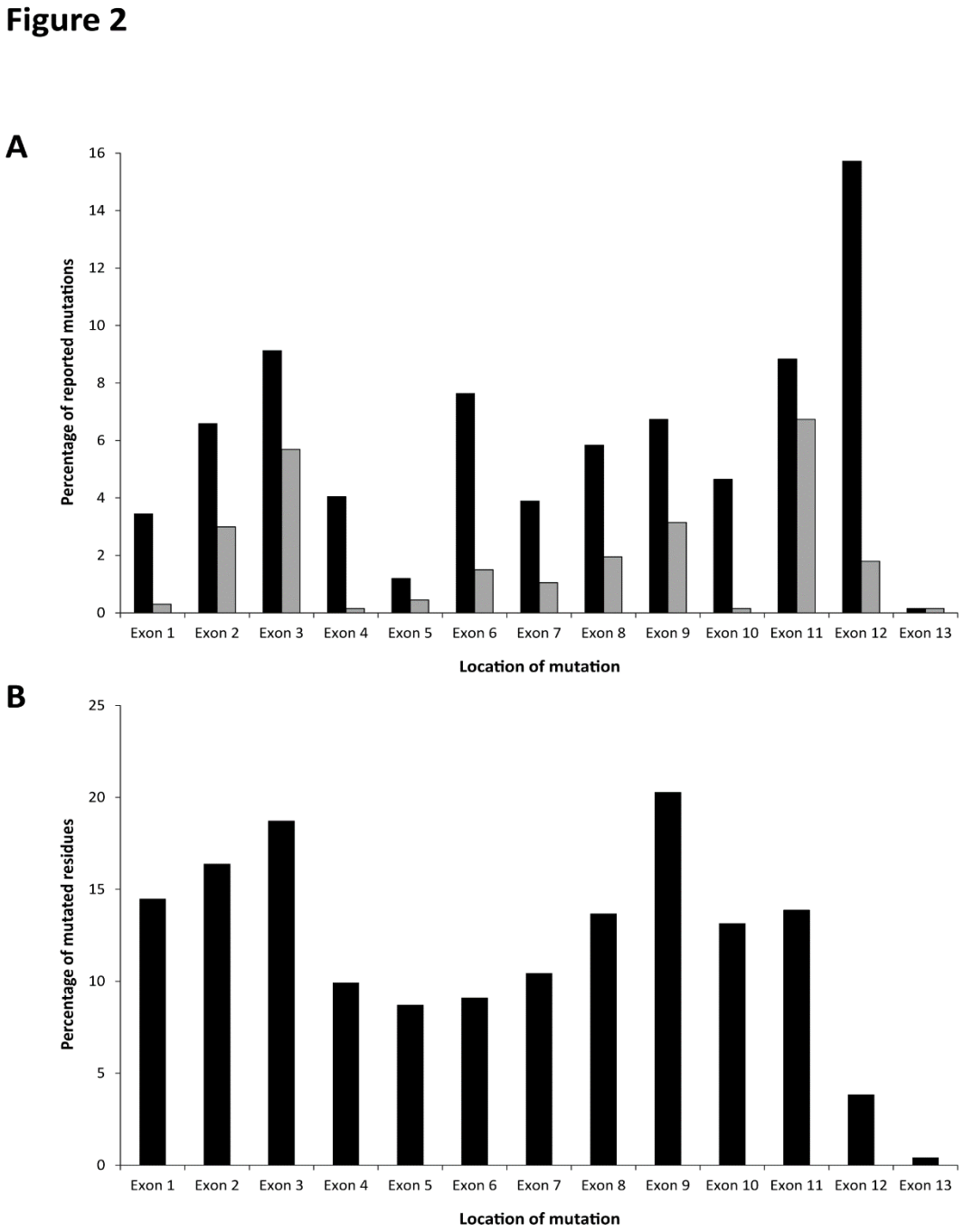

Figure 3. Three-dimensional structure of the BMPR-II extracellular domain highlighting the location of substitutions impacting upon 9 of the 10 key cysteine residues responsible for disulfide bridge formation, indicated in dark blue. Defects in the Cys116 residue have not been identified in PAH thus far. Figure was reproduced from the crystal structure (PDB ID: 2HLQ) and processed using Cn3D v4.3 software.

Figure 3

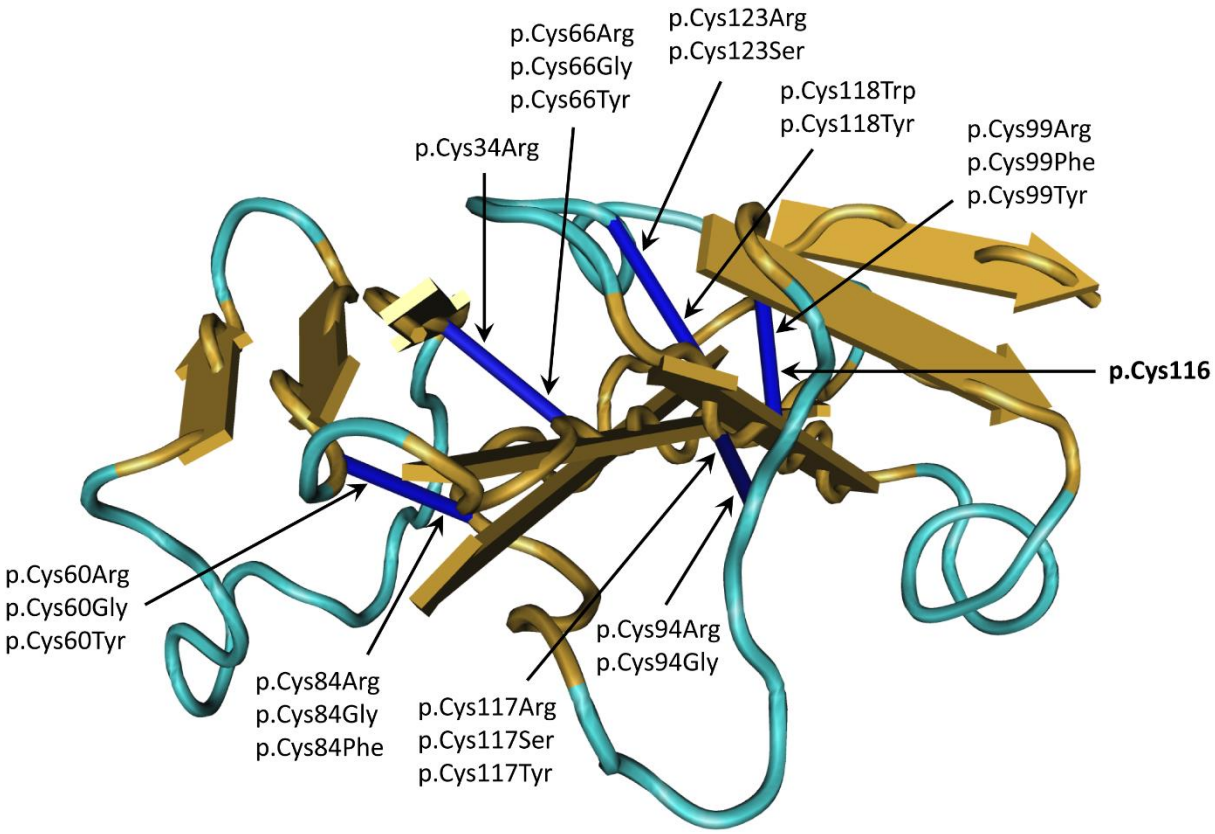

Table 1. Novel pathogenic *BMPR2* mutations identified in this analysis

Location	Mutation category	Nucleotide change[†]	Amino acid change	Frequency in this study[‡]	Clinical classification
5'UTR	Transition	c.-669G>A	p.?	3	I, NK, P
5'UTR to exon 1	Deletion	c.?_-540_76+?del	p.?	1	NK
5'UTR to exon 13	Deletion	c.?_-540_3117+?del	p.?	1	NK
Exon 1	Deletion	c.1-?_76+?del	p.?	[3]	I, NK (n=2)
Exon 1	Frameshift	c.9dupC	p.S4Lfs*34	1	NK
Exon 1	Nonsense	c.16C>T	p.Q6*	2	I, NK
Exon 1	Nonsense	c.38G>A	p.W13*	1	H
Exon 1	Nonsense	c.48G>A	p.W16*	1	I
Intron 1	Splice-site	c.76+1G>T	p.?	1	H
Intron 1	Splice-site	c.76+2T>C	p.?	1	NK
Intron 1	Splice-site	c.77-1G>A	p.A26Efs*9	1	H
Exons 2-5	Deletion	c.77-?_621+?del	p.?	1	H
Exons 2-9	Deletion	c.77-?_1276+?del	p.?	1	NK
Exon 2	Nonsense	c.82C>T	p.Q28*	1	NK
Exon 2	Missense	c.178T>C	p.C60R	1	NK
Exon 2	Missense	c.196T>G	p.C66G	1	NK
Exon 2	Frameshift	c.236_238delinsAAAAGGGGACA	p.L79Qfs*5	1	NK
Exon 2	Frameshift	c.246dupA	p.G83Rfs*15	1	H
Intron 2	Splice-site	c.247+1G>A	p.?	1	NK
Exon 3	Deletion	c.248-?_418+?del	p.?	[4]	I (n=3), NK
Exon 3	Duplication	c.248-?_418+?dup	p.?	1	H
Exon 3	Missense	c.280T>G	p.C94G	1	NK
Exon 3	Frameshift	c.339_340insAA	p.R114Nfs*39	1	H
Exon 3	Frameshift	c.345_346delCT	p.F115Lfs*4	1	NK
Exon 3	Missense	c.350G>A	p.C117Y	1	NK
Exon 3	Frameshift	c.353delG	p.C118Lfs*34	1	NK
Exon 3	Missense	c.354T>G	p.C118W	1	I
Exon 3	Missense	c.377A>G	p.N126S	1	NK
Exon 4	Deletion	c.419-?_529+?del	p.?	1	NK
Exons 4-7	Deletion	c.419-?_967+?del	p.?	[2]	I, NK
Exons 4-10	Deletion	c.419-?_1413+?del	p.?	1	NK
Exon 4	Frameshift	c.435delT	p.F145Lfs*7	1	NK
Exon 4	Nonsense	c.439C>T	p.R147*	1	H
Exon 4	Nonsense	c.482T>A	p.L161*	1	I

Exon 5	Deletion	c.530-?_621+?del	p.?	1	NK
Exon 5	Nonsense	c.541C>T	p.Q181*	1	H
Exon 6	Nonsense	c.637C>T	p.R213*	1	H
Exon 6	Nonsense	c.642T>G	p.Y214*	1	NK
Exon 6	Frameshift	c.673_679delCGTCCAG	p.R225Lfs*3	1	H
Exon 6	Nonsense	c.727G>T	p.E243*	1	I
Exon 6	Frameshift	c.795_796delinsTT	p.E265_L1038delinsD	1	NK
Intron 6	Splice-site	c.853-2A>G	p.?	1	NK
Intron 6	Splice-site	c.853-1G>A	p.?	1	NK
Exon 7	Nonsense	c.860T>A	p.L287*	1	H
Exon 7	Nonsense	c.872T>G	p.L291*	1	NK
Exon 7	Nonsense	c.893G>A	p.W298*	1	NK
Exon 7	Frameshift	c.894_895dupGG	p.V299Gfs*2	1	H
Exon 7	Frameshift	c.961delC	p.R321Efs*14	1	NK
Intron 7	Splice-site	c.967+2T>C	p.?	1	H
Intron 7	Splice-site	c.968-3C>G	p.?	1	NK
Intron 7	Splice-site	c.968-1G>T	p.?	1	NK
Exon 8	Nonsense	c.994C>T	p.R332*	1	NK
Exon 8	Frameshift	c.1011_1015delAAATG	p.R337Sfs*6	1	I
Exon 8	Frameshift	c.1060delC	p.L354Cfs*3	1	NK
Exon 8	Nonsense	c.1126G>T	p.E376*	2	I, NK
Exon 9	Frameshift	c.1129-1_1129dupGG	p.V377Gfs*13	1	I
Exon 9	Frameshift	c.1141dupA	p.R381Kfs*18	1	H
Exon 9	Missense	c.1156G>C	p.E386Q	1	NK
Exon 9	Missense	c.1220A>C	p.Y407S	1	NK
Exon 9	Nonsense	c.1221T>G	p.Y407*	1	H
Exon 9	Frameshift	c.1268dupT	p.F424Lfs*24	1	NK
Exon 9	Missense	c.1276G>C	p.G426R	1	H
Intron 9	Splice-site	c.1277-9A>C	p.?	1	H
Intron 9	Splice-site	c.1277-8A>G	p.?	1	NK
Exon 10	Frameshift	c.1279delG	p.E427Nfs*47	1	NK
Exon 10	Frameshift	c.1285_1286insGGATT	p.V429Gfs*47	2	I, NK
Exon 10	Frameshift	c.1293_1300delGTACCAGA	p.E431Dfs*14	1	NK
Exon 10	Frameshift	c.1371delA	p.K457Nfs*17	1	H
Exon 10	Nonsense	c.1398G>A	p.W466*	1	NK
Exon 10	Nonsense	c.1402G>T	p.E468*	1	H
Exon 11	Frameshift	c.1426_1450del	p.L476Gfs*22	1	NK

Exon 11	Nonsense	c.1441G>T	p.E481*	1	NK
Exon 11	Nonsense	c.1451G>A	p.W484*	1	H
Exon 11	Missense	c.1453G>A	p.D485N	1	P
Exon 11	Nonsense	c.1456C>T	p.Q486*	1	H
Exon 11	Frameshift	c.1477dupA	p.T493Nfs*6	1	NK
Exon 11	Missense	c.1486T>C	p.C496R	1	NK
Intron 11	Splice-site	c.1587-7_1587-4delCTTT	p.?	1	I
Exon 12	Nonsense	c.1629T>G	p.Y543*	1	I
Exon 12	Nonsense	c.1750C>T	p.R584*	1	NK
Exon 12	Nonsense	c.1789C>T	p.R597*	1	NK
Exon 12	Nonsense	c.1969C>T	p.Q657*	2	I, NK
Exon 12	Nonsense	c.1981G>T	p.E661*	1	NK
Exon 12	Frameshift	c.2291dupA	p.N764Kfs*49	1	H
Exon 12	Frameshift	c.2303_2309delAGCCCCG	p.E768Gfs*2	1	NK
Exon 12	Frameshift	c.2308delC	p.R770Gfs*2	2	NK (n=2)
Exon 12	Frameshift	c.2484delG	p.T829Qfs*10	1	H
Exon 12	Nonsense	c.2533G>T	p.E845*	1	I
Exon 12	Nonsense	c.2695C>T	p.R899*	3	H (n=2), NK
Exon 12	Nonsense	c.2730T>A	p.C910*	1	H
Exon 12	Nonsense	c.2737C>T	p.Q913*	1	I

[†]GenBank reference sequence and version number for *BMPR2*: NM_001204.6; numbering is from +1 as A of the ATG initiation codon

[‡]Total number of independent cases. Frequencies in square brackets denote chromosomal rearrangements for which the breakpoints are unknown and may therefore represent distinct mutations

Key to abbreviations: H: heritable pulmonary arterial hypertension; I: idiopathic pulmonary arterial hypertension; NK: not known; P: pediatric pulmonary arterial hypertension

Table 2. *BMPR2* variants of uncertain pathological significance

Location	Mutation category	Nucleotide change†	Amino acid change	Clinical classification	Domain	Reference	Population frequency	a) PolyPhen-2		b) PROVEAN		c) SIFT	
								Prediction	Score	Prediction	Score	Prediction	Score
Exon 3	Missense	c.266G>C	p.G89A	HPAH	ECD	Liu et al., 2012	-	Possibly damaging	0.638	Neutral	-1.785	Tolerated	0.137
Exon 3	Missense	c.276A>C	p.Q92H	HPAH	ECD	Kabata et al., 2013	0.0001071	Benign	0.001	Neutral	-1.388	Tolerated	0.311
Exon 3	Missense	c.292G>A	p.E98K	IPAH	ECD	Wang et al., 2010	-	Possibly damaging	0.515	Neutral	-1.792	Tolerated	0.092
Exon 4	Missense	c.461C>G	p.A154G	IPAH	TM	Pfarr et al., 2011	-	Benign	0.025	Neutral	-1.994	Damaging	0.032
Exon 6	Missense	c.818T>G	p.M273R	IPAH	KD	Pfarr et al., 2011	-	Benign	0.004	Deleterious	-2.716	Tolerated	0.213
Exon 7	Missense	c.901T>C	p.S301P	NK (n=2)	KD	Sztrymf et al., 2008; Girerd et al., 2010b	-	Benign	0.046	Neutral	-2.19	Tolerated	0.086
Exon 7	Missense	c.954A>C	p.E318D	HPAH	KD	This analysis	-	Benign	0.089	Neutral	-0.347	Tolerated	0.273
Exon 8	Missense	c.1042G>A	p.V348I	HPAH; IPAH (n=2)	KD	Wang et al., 2010; Liu et al., 2012	0.0004550	Possibly damaging	0.715	Neutral	-0.45	Damaging	0.023
Exon 8	Missense	c.1066A>T	p.M356L	IPAH	KD	Wang et al., 2010	-	Benign	0.023	Neutral	-0.497	Tolerated	0.417
Exon 8	Missense	c.1117G>C	p.A373P	IPAH	KD	Liu et al., 2012	-	Probably damaging	0.99	Neutral	-2.493	Tolerated	0.087
Exon 11	Missense	c.1516A>G	p.M506V	IPAH	CD	This analysis	0.0000412	Benign	0.008	Neutral	-0.896	Tolerated	0.244
Exon 12	Missense	c.1598A>G	p.H533R	IPAH	CD	Pfarr et al., 2011	-	Benign	0.267	Neutral	-2.049	Damaging	0.021
Exon 12	Missense	c.1766A>G	p.Y589C	CHD-APAH (exercise-induced) (n=2)	CD	Möller et al., 2010	0.0001320	Probably damaging	0.999	Deleterious	-3.436	Damaging	0.001
Exon 12	Missense	c.2296A>G	p.T766A	IPAH; CTEPH	CD	Liu et al., 2012; Feng et al., 2014	0.0000083	Benign	0	Neutral	-0.183	Tolerated	0.424
Exon 12	Missense	c.2618G>A	p.R873Q	NK (n=2)	CD	Sztrymf et al., 2008; Girerd et al., 2010b	0.0001153	Probably damaging	0.966	Neutral	-0.861	Tolerated	0.128

Population frequency data were obtained from the ExAC database (http://exac.broadinstitute.org/gene/ENSG00000204217). The likely pathogenicity of each missense variant was calculated by three *in silico* prediction methods, using the default parameters in each case. Ranges and cut-offs for output scores were as follows: a) PolyPhen-2 v2.2.2 (http://genetics.bwh.harvard.edu/pph2). Range: 0 – 1 [benign: 0 – 0.452; possibly damaging: 0.453 – 0.956; probably damaging: 0.957 – 1]; b) PROVEAN Human Protein v1.1

(http://provean.jcvi.org/protein_batch_submit.php?species=human). Cut-off: -2.5 [deleterious: ≤-2.5; neutral: >-2.5]; c) SIFT Human Protein v1.03 (PROVEAN Protein batch input). Range: 0 – 1 [damaging: ≤0.05; tolerated: >0.05].

[†]GenBank reference sequence and version number for *BMPR2*: NM_001204.6; numbering is from +1 as A of the ATG initiation codon.

Key to abbreviations: CD: cytoplasmic tail domain; CHD-APAH: congenital heart disease-associated pulmonary arterial hypertension; CTEPH: chronic thromboembolic pulmonary hypertension; ECD: extracellular ligand-binding domain; HPAH: heritable pulmonary arterial hypertension; IPAH: idiopathic pulmonary arterial hypertension; KD: kinase domain; NK: not known; TM: transmembrane domain

Table 3. PAH mutations identified in BMP pathway members

Gene name	Location	Mutation category	Nucleotide change[†]	Amino acid change	Frequency in this study[‡]	Clinical classification	Reference(s)
ACVRL1	Exon 2	Frameshift	c.37delC	p.L13Cfs*2	1	PAH-HHT	Trembath et al., 2001; Girerd et al., 2010a
ACVRL1	Exon 3	Missense	c.199C>T	p.R67W	1	PAH-HHT	Chen et al., 2013
ACVRL1	Exon 3	Missense	c.293A>G	p.N98S	1	NK	This analysis
ACVRL1	Exon 4	Nonsense	c.430C>T	p.R144*	1	I	Machado et al., 2009
ACVRL1	Exon 5	Missense	c.536A>C	p.D179A	1	I	Harrison et al., 2003^
ACVRL1	Exon 5	Missense	c.593T>A	p.V198E	1	PAH-HHT	Chen et al., 2013
ACVRL1	Exon 5	Missense	c.602A>G	p.Q201R	1	PAH-HHT	Girerd et al., 2010a
ACVRL1	Exon 6	Missense	c.632G>A	p.G211D	1	PAH-HHT	Harrison et al., 2003
ACVRL1	Exon 6	Missense	c.653_654inv	p.R218P	1	PAH-HHT	Chen et al., 2013
ACVRL1	Exon 6	Deletion	c.760_762delGAC	p.D254del	1	PAH-HHT	Trembath et al., 2001
ACVRL1	Exon 7	Missense	c.788A>G	p.D263G	1	PAH-HHT	This analysis
ACVRL1	Exon 7	Missense	c.818T>C	p.L273P	1	PAH-HHT	Smoot et al., 2009
ACVRL1	Exon 7	Missense	c.853C>T	p.L285F	1	NK	This analysis
ACVRL1	Exon 7	Missense	c.854T>C	p.L285P	1	P	Chida et al., 2012b
ACVRL1	Exon 7	Missense	c.936C>G	p.H312Q	1	P	Fujiwara et al., 2008^
ACVRL1	Exon 7	Missense	c.950T>C	p.I317T	1	P	Pfarr et al., 2013
ACVRL1	Exon 7	Missense	c.955G>C	p.G319R	1	NK	This analysis
ACVRL1	Exon 7	Missense	c.1031G>A	p.C344Y	2	PAH-HHT (n=2)	Harrison et al., 2003
ACVRL1	Exon 8	Missense	c.1055C>A	p.A352D	2	PAH-HHT (n=2)	Smoot et al., 2009
ACVRL1	Exon 8	Missense	c.1120C>T	p.R374W	3	PAH-HHT (n=2), NK	Harrison et al., 2003; Abdalla et al., 2004; This analysis
ACVRL1	Exon 8	Missense	c.1121G>A	p.R374Q	2	PAH-HHT (n=2)	Harrison et al., 2003; Chen et al., 2013
ACVRL1	Exon 8	Missense	c.1124A>G	p.Y375C	1	PAH-HHT	Chen et al., 2013
ACVRL1	Exon 8	Missense	c.1142T>C	p.L381P	1	P	Fujiwara et al., 2008^
ACVRL1	Exon 8	Missense	c.1195T>C	p.W399R	1	PAH-HHT	Chen et al., 2013
ACVRL1	Exon 8	Missense	c.1196G>C	p.W399S	1	PAH-HHT	Harrison et al., 2003
ACVRL1	Exon 8	Missense	c.1196G>T	p.W399L	1	PAH-HHT	Ishiwata et al., 2014
ACVRL1	Exon 8	Missense	c.1231C>T	p.R411W	1	PAH-HHT	Trembath et al., 2001
ACVRL1	Exon 8	Missense	c.1232G>A	p.R411Q	1	PAH-HHT	Harrison et al., 2003
ACVRL1	Exon 9	Missense	c.1270C>A	p.P424T	1	P	Fujiwara et al., 2008^
ACVRL1	Exon 9	Missense	c.1280A>T	p.D427V	1	PAH-HHT	Girerd et al., 2010a
ACVRL1	Exon 9	Missense	c.1324G>A	p.V442M	1	PAH-HHT	Girerd et al., 2010a

ACVRL1	Exon 10	Nonsense	c.1385C>G	p.S462*	1	PAH-HHT	Abdalla et al., 2004
ACVRL1	Exon 10	Frameshift	c.1388delG	p.G463Afs*2	1	PAH-HHT	Girerd et al., 2010a
ACVRL1	Exon 10	Frameshift; Nonsense	c.1388delG; c.1390delC	p.G463Afs*2; p.L464*	1	PAH-HHT (mosaic)	Eyries et al., 2012
ACVRL1	Exon 10	Missense	c.1433C>A	p.A478D	1	P	Chida et al., 2012b
ACVRL1	Exon 10	Nonsense	c.1435C>T	p.R479*	2	PAH-HHT (n=2)	Abdalla et al., 2004; Chen et al., 2013
ACVRL1	Exon 10	Missense	c.1436G>A	p.R479Q	1	P	Fujiwara et al., 2008^
ACVRL1	Exon 10	Missense	c.1436G>C	p.R479P	1	I	Machado et al., 2009
ACVRL1	Exon 10	Missense	c.1450C>G	p.R484G	1	H	Jones et al., 2014
ACVRL1	Exon 10	Missense	c.1450C>T	p.R484W	2	PAH-HHT (n=2)	Trembath et al., 2001; Girerd et al., 2010a
ACVRL1	Exon 10	Frameshift	c.1450delinsTG	p.R484Wfs*10	1	PAH-HHT	Abdalla et al., 2004
ACVRL1	Exon 10	Missense	c.1451G>A	p.R484Q	7	P (n=2); PAH-HHT (n=3; 1 mosaic); NK (n=2)	Harrison et al., 2005^; Fujiwara et al., 2008^; Best et al., 2011; Chen et al., 2013; Pfarr et al., 2013; This analysis
ACVRL1	Exon 10	Missense	c.1460A>C	p.K487T	1	PAH-HHT	Harrison et al., 2003
ACVRL1	Exon 10	Nonsense	c.1468C>T	p.Q490*	1	PAH-HHT	Trembath et al., 2001; Girerd et al., 2010a
ENG	Exon 5	Missense	c.640G>A	p.G214S	1	P	Pfarr et al., 2013
ENG	Exon 5	Frameshift	c.682_686delTCGGC	p.S228Rfs*104	1	PAH-HHT	Harrison et al., 2003^
ENG	Exon 6	Missense	c.788T>A	p.I263N	1	PAH-HHT	Chen et al., 2013
ENG	Exon 11	Frameshift	c.1334delT	p.M445Rfs*46	1	PAH-HHT	Harrison et al., 2003^
ENG	Exon 11	Frameshift	c.1410delG	p.Q471Sfs*20	1	PAH-HHT + dexfenfluramine	Chaouat et al., 2004^
ENG	Exon 12	Missense	c.1633G>A	p.G545S	1	CHD-PAH	Pfarr et al., 2013
ENG	Intron 13	Branch-site	c.1742-22T>C	p.C582_R618del	1	PAH-HHT	Harrison et al., 2005^; Mache et al., 2008^
ENG	Exon 14	Frameshift	c.1804delA	p.I602Sfs*38	1	PAH-HHT	Chen et al., 2013
ENG	Exon 15	Missense	c.1853G>T	p.R618L	1	I	This analysis
SMAD1	Exon 2	Missense	c.8T>C	p.V3A	1	I	Nasim et al., 2011
SMAD4	Exon 2	Missense	c.38A>G	p.N13S	1	I	Nasim et al., 2011
SMAD4	Intron 11	Splice-site	c.1448-6T>C	p.?	1	I	Nasim et al., 2011
SMAD9	Exon 2	Missense	c.127A>G	p.K43E	1	I	Nasim et al., 2011
SMAD9	Exon 3	Nonsense	c.606C>A	p.C202*	1	I	Shintani et al., 2009
SMAD9	Exon 5	Nonsense	c.880C>T	p.R294*	1	H	Drake et al., 2011

†GenBank reference sequence and version number for *ACVRL1*: NM_000020.2; *ENG*: NM_001114753.2; *SMAD1*: NM_005900.2; *SMAD4*: NM_005359.5; *SMAD9*: NM_001127217.2

‡Total number of independent cases

^Previously reported in Machado et al., 2009

Key to abbreviations: H: heritable pulmonary arterial hypertension; I: idiopathic pulmonary arterial hypertension; NK: not known; P: pediatric pulmonary arterial hypertension; PAH-HHT: pulmonary arterial hypertension with hereditary hemorrhagic telangiectasia

Table 4. PAH mutations identified in non-canonical BMP pathways

Gene name	Location	Mutation category	Nucleotide change†	Amino acid change	Frequency in this study‡	Clinical classification	Reference(s)
CAV1	Exon 3	Frameshift	c.473delC	p.P158Hfs*23	1	I	Austin et al., 2012
CAV1	Exon 3	Frameshift	c.474delA	p.L159Sfs*22	1	H	Austin et al., 2012
KCNA5	Exon 1	Missense	c.544G>A	p.G182R	2	I	Remillard et al., 2007
KCNA5	Exon 1	Missense	c.633G>C	p.E211D	2	I	Remillard et al., 2007
KCNA5	Exon 1	Frameshift	c.1448delA	p.Y483Sfs*4	1	H	Wang et al., 2014^
KCNK3	Exon 1	Missense	c.23C>A	p.T8K	1	I	Ma et al., 2013
KCNK3	Exon 2	Missense	c.289G>A	p.G97R	1	H	Ma et al., 2013
KCNK3	Exon 2	Missense	c.544G>A	p.E182K	1	I	Ma et al., 2013
KCNK3	Exon 2	Missense	c.575A>G	p.Y192C	1	I	Ma et al., 2013
KCNK3	Exon 2	Missense	c.608G>A	p.G203D	1	H	Ma et al., 2013
KCNK3	Exon 2	Missense	c.661G>C	p.V221L	1	H	Ma et al., 2013

†GenBank reference sequence and version number for *CAV1*: NM_001753.4; *KCNA5*: NM_002234.3; *KCNK3*: NM_002246.2
‡Total number of independent cases

^Reported as a compound heterozygote with a *BMPR2* c.1471C>T (p.R491W) mutation
Key to abbreviations: H: heritable pulmonary arterial hypertension; I: idiopathic pulmonary arterial hypertension

Table 5. PVOD/PCH mutations identified in *EIF2AK4*

Location	Mutation category	Nucleotide change[†]	Amino acid change	Frequency in this study[‡]	Clinical classification	Reference(s)
Exon 3; Intron 9	Frameshift; Splice-site	c.354_355delTG; c.1554-4C>A	p.C118Wfs*7; p.C519Dfs*17	1	PVOD-F	Eyries et al., 2014
Exon 5	Frameshift	c.560_564delAAGAA; c.560_564delAAGAA	p.K187Rfs*9; p.K187Rfs*9	1	PVOD-S	Eyries et al., 2014
Exon 5	Frameshift	c.567dupG; c.567dupG	p.K190Efs*8; p.K190Efs*8	1	PVOD-F	Eyries et al., 2014
Exon 7; Exon 12	Nonsense; Frameshift	c.745C>T; c.2136_2139dupCACT	p.R249*; p.S714Hfs*21	1	PVOD-F	Eyries et al., 2014
Intron 7; Exon 25	Splice-site; Nonsense	c.860-1G>A; c.3448C>T	p.?; p.R1150*	1	PCH	Best et al., 2014
Exon 9; Exon 28	Frameshift; Nonsense	c.1153dupG; c.3766C>T	p.V385Gfs*30; p.R1256*	1	PCH	Best et al., 2014
Exon 9	Nonsense	c.1387C>T; c.1387C>T	p.R463*; p.R463*	1	PVOD-F	Eyries et al., 2014
Exon 9; Exon 23	Nonsense; Nonsense	c.1387C>T; c.3244C>T	p.R463*; p.Q1082*	1	PVOD-F	Eyries et al., 2014
Exon 9	Frameshift	c.1392delT; c.1392delT	p.R465Vfs*38; p.R465Vfs*38	1	PCH	Best et al., 2014
Exon 9; Exon 28	Frameshift; Nonsense	c.1392delT; c.3802C>T	p.R465Vfs*38; p.Q1268*	1	PVOD-F	Eyries et al., 2014
Exon 11	Missense	c.1754G>A; c.1754G>A	p.R585Q; p.R585Q	1	PVOD-F	Eyries et al., 2014
Exon 12	Missense	c.1928T>G; c.1928T>G	p.L643R; p.L643R	1	PVOD-F	Eyries et al., 2014
Intron 13	Splice-site	c.2319+1G>A; c.2319+1G>A	p.?; p.?	1	PVOD-F	Eyries et al., 2014
Exon 15	Nonsense	c.2458C>T; c.2458C>T	p.R820*; p.R820*	1	PVOD-S	Eyries et al., 2014
Exon 19; Intron 25	Nonsense; Splice-site	c.2857C>T; c.3576+1G>T	p.Q953*; p.?	1	PVOD-S	Eyries et al., 2014
Exon 21	Splice-site	c.3159G>A; c.3159G>A	p.K975_K1053del; p.K975_K1053del	2	PVOD-F, -S	Eyries et al., 2014
Exon 23	Missense	c.3344C>T; c.3344C>T	p.P1115L; p.P1115L	5	H	Tenorio et al., 2014
Exon 24	Nonsense	c.3406C>T; c.3406C>T	p.R1136*; p.R1136*	1	PVOD-F	Eyries et al., 2014
Exon 25; Intron 36	Nonsense; Splice-site	c.3448C>T; c.4728+1_4728+13delinsTTCT	p.R1150*; p.?	1	PVOD-F	Eyries et al., 2014
Intron 29	Splice-site	c.4065+1G>C; c.4065+1G>C	p.?; p.?	1	PVOD-F	Eyries et al., 2014
Exon 31	Frameshift	c.4205dupT; c.4205dupT	p.S1403Kfs*45; p.S1403Kfs*45	1	PVOD-S	Eyries et al., 2014

[†]GenBank reference sequence and version number for *EIF2AK4*: NM_001013703.3

[‡]Total number of independent cases

Key to abbreviations: H: heritable pulmonary arterial hypertension; PCH: pulmonary capillary hemangiomatosis; PVOD: pulmonary veno-occlusive disease (-F: familial; -S: sporadic)

Supp. Table S1. Previously reported pathogenic *BMPR2* mutations identified in pulmonary arterial hypertension

Location	Mutation category	Nucleotide change[†]	Amino acid change	Frequency in this study[‡]	Clinical classification	Reference(s)
5'UTR to intron 3	Deletion	c.-127936_418+7067del	p.?	1	H	Kataoka et al., 2013
5'UTR	Transition	c.-669G>A	p.?	1	H	Wang et al., 2009
5'UTR to exon 1	Deletion	c.?_-540_76+?del	p.?	[2]	H (n=2)	Pfarr et al., 2011
Exon 1	Deletion	c.1-?_76+?del	p.?	1	CHD-APAH (hereditary)	Pfarr et al., 2013
Exons 1-3	Deletion	c.1-?_418+?del	p.?	[2]	H, NK	Girerd et al., 2010; Kabata et al., 2013
Exons 1-13	Deletion	c.1-?_3117+?del	p.?	1	NK	Sztrymf et al., 2008
Exon 1	Nonsense	c.16C>T	p.Q6*	1	I	Kataoka et al., 2013
Exon 1	Frameshift	c.21delG	p.W9Gfs*38	1	I	Liu et al., 2012
Exon 1	Nonsense	c.27G>A	p.W9*	1	I	Liu et al., 2012
Exon 1	Nonsense	c.38G>A	p.W13*	1	P	Chida et al., 2012
Exon 1	Nonsense	c.39G>A	p.W13*	1	H	Hamid et al., 2010
Exon 1	Nonsense	c.47G>A	p.W16*	1	P	Kerstjens-Frederikse et al., 2013
Exon 1	Nonsense	c.48G>A	p.W16*	4	H, I, NK (n=2)	Sztrymf et al., 2008; Pfarr et al., 2011; Liu et al., 2012
Exons 1-6	Deletion	c.51_814del	p.I18Hfs*25	1	I	Machado et al., 2001
Intron 1	Splice-site	c.77-35_86del	p.?	1	I	Liu et al., 2012
Exon 2	Deletion	c.77-?_247+?del	p.?	1	NK	Girerd et al., 2010
Exon 2	Duplication	c.77-?_247+?dup	p.?	1	H	Cogan et al., 2006
Exons 2-3	Deletion	c.77-?_418+?del	p.?	1	H	Pfarr et al., 2011
Exons 2-3	Duplication	c.77-?_418+?dup	p.?	1	I	Liu et al., 2012
Exons 2-7	Duplication	c.77-?_967+?dup	p.?	1	I	Liu et al., 2012
Exons 2-13	Deletion	c.77-?_3117+?del	p.?	1	H	Cogan et al., 2006
Exon 2	Nonsense	c.88C>T	p.Q30*	1	I	Liu et al., 2012
Exon 2	Nonsense	c.91G>T	p.E31*	1	H	Pfarr et al., 2011
Exon 2	Frameshift	c.103delG	p.A35Rfs*12	1	I	Liu et al., 2012
Exon 2	Frameshift	c.116delC	p.P39Rfs*8	1	I	Rosenzweig et al., 2008
Exon 2	Missense	c.178T>G	p.C60G	1	I	Liu et al., 2012
Exon 2	Missense	c.196T>C	p.C66R	1	I	Wang et al., 2009
Exon 2	Missense	c.197G>A	p.C66Y	1	NK	Girerd et al., 2010
Exon 2	Missense	c.200A>G	p.Y67C	2	I, NK	Girerd et al., 2010; Wang et al., 2010
Exon 2	Frameshift	c.200dupA	p.Y67*	1	I	Kataoka et al., 2013
Exon 2	Frameshift	c.237delT	p.V80*	1	H	Kataoka et al., 2013
Exon 2	Frameshift	c.240_241insT	p.K81*	1	H	Johri et al., 2010
Exon 2	Nonsense	c.244C>T	p.Q82*	1	H	Pfarr et al., 2011

Location	Mutation category	Nucleotide change[†]	Amino acid change	Frequency in this study[‡]	Clinical classification	Reference(s)
Intron 2	Splice-site	c.247+2delC	p.G47_Q82del, G63_Q82del	1	H	Cogan et al., 2006
Intron 2	Splice-site	c.247+6T>G	p.G47_Q82del, G63_Q82del	1	H	Cogan et al., 2006
Intron 2 to exon 3	Deletion	c.248-592_413delinsGTAAAGTA	p.?	1	I	Aimi et al., 2013
Intron 2	Splice-site	c.248-3T>G	p.?	1	P	Chida et al., 2012
Exon 3	Deletion	c.248-?_418+?del	p.?	[2]	I (n=2)	Pfarr et al., 2011; Kabata et al., 2013
Exon 3	Missense	c.251G>T	p.C84F	1	I	Liu et al., 2012
Exon 3	Nonsense	c.255G>A	p.W85*	1	H	Kataoka et al., 2013
Exon 3	Nonsense	c.274C>T	p.Q92*	1	I	Kabata et al., 2013
Exon 3	Frameshift	c.277dupG	p.E93Gfs*5	1	I	Cogan et al., 2006
Exon 3	Nonsense	c.292G>T	p.E98*	1	I	Wang et al., 2009
Exon 3	Nonsense	c.320C>G	p.S107*	1	NK	Sztrymf et al., 2008
Exon 3	Missense	c.338A>G	p.Y113C	1	I	Liu et al., 2012
Exon 3	Frameshift	c.338dupA	p.Y113*	2	H, I	Kabata et al., 2013; Kataoka et al., 2013
Exon 3	Nonsense	c.339C>A	p.Y113*	1	I	Liu et al., 2012
Exon 3	Missense	c.349T>C	p.C117R	1	I	Liu et al., 2012
Exon 3	Missense	c.353G>A	p.C118Y	1	I	Pfarr et al., 2011
Exon 3	Missense	c.367T>C	p.C123R	1	NK	Girerd et al., 2010
Exon 3	Frameshift	c.371dupA	p.N124Kfs*6	1	NK	Girerd et al., 2010
Exon 3	Missense	c.377A>G	p.N126S	5	H, I (n=2), NK (n=2)	Girerd et al., 2010; Pfarr et al., 2011; Liu et al., 2012
Exon 3	Frameshift	c.399delT	p.P134Lfs*18	1	P	Kerstjens-Frederikse et al., 2013
Exon 3	Frameshift	c.407_408delCA	p.T136Nfs*10	1	NK	Girerd et al., 2010
Exon 3	Frameshift	c.408_412delAACAC	p.P138Qfs*7	1	NK	Girerd et al., 2010
Intron 3	Splice-site	c.418+3A>T	p.?	2	NK (n=2)	Sztrymf et al., 2008
Intron 3	Splice-site	c.418+5G>A	p.?	1	H	Pfarr et al., 2011
Intron 3	Splice-site	c.419-10T>C	p.?	1	P	Pfarr et al., 2013
Exons 4-5	Deletion	c.419-?_621+?del	p.?	1	P	Pfarr et al., 2013
Exons 4-8	Duplication	c.419-?_1128+?dup	p.?	1	NK	Girerd et al., 2010
Exons 4-13	Deletion	c.419-?_3117+?del	p.?	1	H	Pfarr et al., 2011
Exon 4	Frameshift	c.420_421insG	p.P141Afs*6	1	I	Arbustini et al., 2008
Exon 4	Nonsense	c.439C>T	p.R147*	7	H (n=2), I (n=2), NK (n=3)	Girerd et al., 2010; Wang et al., 2010; Pfarr et al., 2011; Liu et al., 2012; Momose et al., 2015
Exon 4	Frameshift	c.449dupC	p.I151Nfs*30	1	NK	Girerd et al., 2010
Exon 4	Frameshift	c.498delT	p.A167Pfs*9	2	I (n=2)	Kabata et al., 2013; Kataoka et al., 2013
Exon 4	Nonsense	c.507C>A	p.C169*	1	I	Pfarr et al., 2011
Exon 4	Nonsense	c.516C>G	p.Y172*	1	H	Cogan et al., 2006

Location	Mutation category	Nucleotide change[†]	Amino acid change	Frequency in this study[‡]	Clinical classification	Reference(s)
Exon 4	Frameshift	c.528delA	p.G177Efs*10	2	NK (n=2)	Girerd et al., 2010
Intron 4	Splice-site	c.529+2T>C	p.?	1	P	Chida et al., 2012
Exon 5	Frameshift	c.551_573del	p.H184Rfs*8	1	NK	Sztrymf et al., 2008
Exon 5	Frameshift	c.608_609delTG	p.L203Qfs*16	1	I	Wang et al., 2009
Exon 6	Deletion	c.622-?_852+?del	p.?	1	NK	Girerd et al., 2010
Exons 6-7	Deletion	c.622-?_967+?del	p.?	1	I-PAVM	Handa et al., 2014
Exon 6	Nonsense	c.631C>T	p.R211*	4	H (n=2), NK (n=2)	Girerd et al., 2010; Portillo et al., 2010; Pfarr et al., 2011
Exon 6	Nonsense	c.637C>T	p.R213*	1	H-IPF	Raamsteeboers et al., 2014
Exon 6	Frameshift	c.659dupG	p.S221Lfs*4	1	H	Pfarr et al., 2011
Exon 6	Missense	c.690A>T	p.K230N	1	P	Hayes et al., 2014
Exon 6	Missense	c.727G>A	p.E243K	1	I	Wang et al., 2010
Exon 6	Missense	c.727G>C	p.E243Q	1	P	Chida et al., 2012
Exon 6	Frameshift	c.782_783delTA	p.I261Sfs*4	1	NK	Girerd et al., 2010
Exon 6	Frameshift	c.796_799delAGAG	p.R266Sfs*12	1	H	Cogan et al., 2006
Exon 6	Frameshift	c.802dupA	p.T268Nfs*30	1	H	Wang et al., 2010
Exon 6	Frameshift	c.804delT	p.A269Qfs*10	1	H	Cogan et al., 2006
Exon 6	Missense	c.830T>C	p.L277P	2	I, NK	Sztrymf et al., 2008; Liu et al., 2012
Intron 6	Splice-site	c.852+1G>A	p.?	1	NK	Girerd et al., 2010
Intron 6	Splice-site	c.853-2A>G	p.?	1	I	Kataoka et al., 2013
Exon 7	Duplication	c.853-?_967+?dup	p.?	1	H	Aldred et al., 2006
Exon 7	Frameshift	c.872delT	p.L291*	1	H	Cogan et al., 2006
Exon 7	Nonsense	c.961C>T	p.R321*	6	I (n=5), NK	Girerd et al., 2010; Wang et al., 2010; Pfarr et al., 2011
Intron 7	Splice-site	c.967+5G>C	p.?	1	I	Liu et al., 2012
Intron 7	Splice-site	c.967+5G>T	p.?	1	NK	Girerd et al., 2010
Intron 7	Splice-site	c.968-2A>C	p.?	1	I	Wang et al., 2010
Exon 8	Deletion	c.968-?_1128+?del	p.?	[2]	NK (n=2)	Girerd et al., 2010
Exons 8-9	Deletion	c.968-?_1276+?del	p.?	1	I	Aldred et al., 2006
Exons 8-10	Duplication	c.968-?_1413+?dup	p.?	1	I	Liu et al., 2012
Exon 8	Missense	c.992A>G	p.H331R	1	I	Kabata et al., 2013
Exon 8	Nonsense	c.994C>T	p.R332*	3	I (n=2), NK	Girerd et al., 2010; Portillo et al., 2010; Liu et al., 2012
Exon 8	Missense	c.1016T>A	p.V339D	2	H, I	Kabata et al., 2013; Kataoka et al., 2013
Exon 8	Frameshift	c.1044delT	p.I349Lfs*8	1	H	Momose et al., 2015
Exon 8	Frameshift	c.1093_1098delinsG	p.R365Gfs*5	1	I	Liu et al., 2012
Intron 8	Splice-site	c.1128+1G>T	p.?	1	H	Pfarr et al., 2011

Location	Mutation category	Nucleotide change[†]	Amino acid change	Frequency in this study[‡]	Clinical classification	Reference(s)
Intron 8	Splice-site	c.1129-3C>G	p.?	1	I	Liu et al., 2012
Exon 9	Frameshift	c.1141dupA	p.R381Kfs*18	1	H	Hamid et al., 2009
Exon 9	Nonsense	c.1146T>G	p.Y382*	1	I	Rosenzweig et al., 2008
Exon 9	Missense	c.1151C>T	p.A384V	1	I	Kabata et al., 2013
Exon 9	Missense	c.1156G>A	p.E386K	1	I	Rosenzweig et al., 2008
Exon 9	Missense	c.1157A>C	p.E386A	1	I	Kabata et al., 2013
Exon 9	Missense	c.1157A>G	p.E386G	1	I	Pfarr et al., 2011
Exon 9	Missense	c.1175T>C	p.V392A	2	I (n=2)	Wang et al., 2010; Liu et al., 2012
Exon 9	Nonsense	c.1207C>T	p.Q403*	1	H	Kabata et al., 2013
Exon 9	Missense	c.1228G>A	p.G410R	1	H	Liu et al., 2012
Exon 9	Nonsense	c.1243G>T	p.E415*	1	I	Wang et al., 2010
Exon 9	Missense	c.1258T>C	p.C420R	1	H	Pfarr et al., 2011
Exon 9	Missense	c.1259G>A	p.C420Y	2	H, I	Pfarr et al., 2011; Liu et al., 2012
Intron 9	Splice-site	c.1276+3A>T	p.?	1	NK	Girerd et al., 2010
Introns 9-10	Deletion	c.1277-289_1413+4737del	p.?	1	I	Kataoka et al., 2013
Intron 9	Splice-site	c.1277-9A>G	p.?	1	NK	Girerd et al., 2010
Exon 10	Deletion	c.1277-?_1413+?del	p.?	[10]	I (n=3), NK (n=7)	Sztrymf et al., 2008; Girerd et al., 2010; Liu et al., 2012; Kabata et al., 2013
Exon 10	Duplication	c.1277-?_1413+?dup	p.?	1	I	Liu et al., 2012
Exon 10	Nonsense	c.1296C>G	p.Y432*	1	H	Pfarr et al., 2011
Exon 10	Nonsense	c.1297C>T	p.Q433*	2	I, P	Pfarr et al., 2011; Pfarr et al., 2013
Exon 10	Frameshift	c.1313_1316delCAGA	p.T438Rfs*35	1	I	Pfarr et al., 2011
Exon 10	Missense	c.1346T>G	p.M449R	1	H	Cogan et al., 2006
Exon 10	Nonsense	c.1348C>T	p.Q450*	2	I, NK	Girerd et al., 2010; Pfarr et al., 2011
Exon 10	Frameshift	c.1366delinsCA	p.E456Qfs*15	1	NK	Girerd et al., 2010
Exon 10	Frameshift	c.1371dupA	p.Q458Tfs*13	1	I	Liu et al., 2012
Exon 10	Frameshift	c.1387_1388insA	p.P463Hfs*8	1	I	Pfarr et al., 2011
Exon 10	Frameshift	c.1392delA	p.A465Pfs*9	1	NK	Girerd et al., 2010
Exon 10	Nonsense	c.1397G>A	p.W466*	1	I	Pfarr et al., 2011
Exon 10	Frameshift	c.1401delA	p.E468Kfs*6	1	NK	Girerd et al., 2010
Intron 10	Splice-site	c.1413+1G>A	p.?	2	I, NK	Girerd et al., 2010; Pfarr et al., 2011
Intron 10	Splice-site	c.1413+3A>T	p.?	1	NK	Pfarr et al., 2011
Exons 11-12	Deletion	c.1414-?_2866+?del	p.?	[2]	I, NK	Girerd et al., 2010; Pfarr et al., 2011
Exons 11-13	Deletion	c.1414-?_3117+?del	p.?	[3]	I, NK (n=2)	Girerd et al., 2010; Liu et al., 2012
Exon 11	Nonsense	c.1424C>A	p.S475*	1	NK	Girerd et al., 2010
Exon 11	Missense	c.1460A>T	p.D487V	1	H	Pfarr et al., 2011
Exon 11	Missense	c.1471C>T	p.R491W	13	H (n=5), I (n=3),	Girerd et al., 2010; Wang et al., 2010;

Location	Mutation category	Nucleotide change[†]	Amino acid change	Frequency in this study[‡]	Clinical classification	Reference(s)
					NK (n=5)	Pfarr et al., 2011; Liu et al., 2012
Exon 11	Missense	c.1472G>A	p.R491Q	5	H (n=2), I (n=2), P	Portillo et al., 2010; Pfarr et al., 2011; Liu et al., 2012; Pfarr et al., 2013
Exon 11	Nonsense	c.1483C>T	p.Q495*	1	NK	Pfarr et al., 2011
Exon 11	Nonsense	c.1523G>A	p.W508*	1	H	Pfarr et al., 2011
Exon 11	Nonsense	c.1525G>T	p.E509*	1	NK	Girerd et al., 2015
Exon 12	Nonsense	c.1750C>T	p.R584*	1	I	Pfarr et al., 2011
Exon 12	Nonsense	c.1771C>T	p.R591*	1	NK	Sztrymf et al., 2008
Exon 12	Frameshift	c.1968dupA	p.Q657Tfs*18	1	H	Kataoka et al., 2013
Exon 12	Nonsense	c.1978G>T	p.E660*	1	NK	Girerd et al., 2015
Exon 12	Frameshift	c.2009delC	p.P670Qfs*30	1	H	Kabata et al., 2013
Exon 12	Frameshift	c.2128delC	p.L710Sfs*2	2	I (n=2)	Kabata et al., 2013; Kataoka et al., 2013
Exon 12	Frameshift	c.2286delC	p.N764Ifs*8	1	P	Chida et al., 2012
Exon 12	Frameshift	c.2308delC	p.R770Gfs*2	2	H, I	Rosenzweig et al., 2008; Pfarr et al., 2011
Exon 12	Frameshift	c.2413dupA	p.T805Nfs*8	1	I	Girerd et al., 2015
Exon 12	Frameshift	c.2446_2447dupGT	p.N817Lfs*23	1	I	Liu et al., 2012
Exon 12	Frameshift	c.2503_2506delACAA	p.T835Pfs*3	2	I (n=2)	Liu et al., 2012; Kabata et al., 2013
Exon 12	Frameshift	c.2503dupA	p.T835Nfs*8	1	I	Kabata et al., 2013
Exon 12	Frameshift	c.2504delC	p.T835Kfs*4	1	I	Cogan et al., 2006
Exon 12	Frameshift	c.2521_2522dupCA	p.R842Ifs*18	1	NK	Sztrymf et al., 2008
Exon 12	Missense	c.2588G>A	p.S863N	1	H	Wang et al., 2009
Exon 12	Nonsense	c.2617C>T	p.R873*	7	I (n=5), NK (n=2)	Sztrymf et al., 2008; Pfarr et al., 2011; Liu et al., 2012; Kabata et al., 2013
Exon 12	Nonsense	c.2626C>T	p.Q876*	1	H	Pfarr et al., 2011
Exon 12	Frameshift	c.2668delA	p.R890Gfs*6	1	P	Pfarr et al., 2013
Exon 12	Nonsense	c.2695C>T	p.R899*	6	H (n=2), I (n=2), NK, P	Girerd et al., 2010; Wang et al., 2010; Pfarr et al., 2011; Liu et al., 2012; Kerstjens-Frederikse et al., 2013
Exon 12	Nonsense	c.2752C>T	p.Q918*	2	NK (n=2)	Girerd et al., 2015

[†]GenBank reference sequence and version number for *BMPR2*: NM_001204.6; numbering is from +1 as A of the ATG initiation codon

[‡]Total number of independent cases. Frequencies in square brackets denote chromosomal rearrangements for which the breakpoints are unknown and may therefore represent distinct mutations

Key to abbreviations: CHD-APAH: congenital heart disease-associated pulmonary arterial hypertension; H: heritable pulmonary arterial hypertension; I: idiopathic pulmonary arterial hypertension; IPF: idiopathic pulmonary fibrosis; NK: not known; P: pediatric pulmonary arterial hypertension; PAVM: pulmonary arteriovenous malformation

Supp. Table S2. Allelic series of 384 mutations identified across the *BMPR2* locus in pulmonary arterial hypertension

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
5'UTR to intron 3	Deletion	c.-127936_418+7067del	p.?	c.1-128k_418+7kdel
5'UTR	Indel	c.-947_-946delinsAT	p.?	c.*-944/5GC>AT
5'UTR	Transition	c.-669G>A	p.?	G-669A
5'UTR to exon 1	Deletion	c.?_-540_76+?del	p.?	c.?_-540_76_?del
5'UTR to exon 13	Deletion	c.?_-540_3117+?del	p.?	
Exon 1	Deletion	c.1-?_76+?del	p.?	?_IVS1 del
Exons 1-3	Deletion	c.1-?_418+?del	p.?	
Exons 1-4	Deletion	c.1-?_529+?del	p.?	c.1-?_419+?del
Exons 1-8	Deletion	c.1-?_1128+?del	p.?	del exon 1-8
Exons 1-13	Deletion	c.1-?_3117+?del	p.?	
Exon 1	Frameshift	c.9dupC	p.S4Lfs*34	
Exon 1	Nonsense	c.16C>T	p.Q6*	
Exon 1	Frameshift	c.16_20delCAGCG	p.Q6Afs*30	c.15_19delGCAGC
Exon 1	Frameshift	c.21delG	p.W9Gfs*38	
Exon 1	Frameshift	c.21_29delinsA	p.P8Gfs*27	c.21_29delGCCCTGGCGinsA
Exon 1	Nonsense	c.27G>A	p.W9*	
Exon 1	Missense	c.28C>T	p.R10W	p.T10W
Exon 1	Nonsense	c.38G>A	p.W13*	
Exon 1	Nonsense	c.39G>A	p.W13*	
Exon 1	Frameshift	c.44delC	p.P15Hfs*32	del44C
Exon 1	Nonsense	c.47G>A	p.W16*	
Exon 1	Nonsense	c.48G>A	p.W16*	
Exons 1-6	Deletion	c.51_814del	p.I18Hfs*25	51-814 del
Exon 1	Missense	c.71C>A	p.A24E	
Intron 1	Splice-site	c.76+1G>T	p.?	
Intron 1	Splice-site	c.76+2T>C	p.?	
Intron 1	Splice-site	c.76+5G>A	p.?	
Intron 1	Splice-site	c.77-35_86del	p.?	c.77-36_85del
Intron 1	Splice-site	c.77-1G>A	p.A26Efs*9	
Exon 2	Deletion	c.77-?_247+?del	p.A26_Q82del	IVS1_IVS2 del
Exon 2	Duplication	c.77-?_247+?dup	p.?	IVS1_IVS2 dup
Exons 2-3	Deletion	c.77-?_418+?del	p.?	c.76-?_420+?del Del c.77?-c.418?
Exons 2-3	Duplication	c.77-?_418+?dup	p.?	c.77-?_c.421+?dup
Exons 2-5	Deletion	c.77-?_621+?del	p.?	
Exons 2-7	Duplication	c.77-?_967+?dup	p.?	c.77-?_c.967+?dup

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exons 2-9	Deletion	c.77-?_1276+?del	p.?	
Exons 2-13	Deletion	c.77-?_3117+?del	p.?	IVS1_? Del
Exon 2	Nonsense	c.82C>T	p.Q28*	
Exon 2	Nonsense	c.88C>T	p.Q30*	
Exon 2	Nonsense	c.91G>T	p.E31*	
Exon 2	Missense	c.100T>C	p.C34R	
Exon 2	Frameshift	c.103delG	p.A35Rfs*12	
Exon 2	Frameshift	c.116delC	p.P39Rfs*8	
Exon 2	Nonsense	c.120T>G	p.Y40*	
Exon 2	Nonsense	c.124C>T	p.Q42*	
Exon 2	Missense	c.125A>G	p.Q42R	
Exon 2	Missense	c.140G>A	p.G47D	
Exon 2	Frameshift	c.156_157delTC	p.H53*	156-157delTC
Exon 2	Frameshift	c.168delG	p.T57Qfs*21	c.166delG
Exon 2	Missense	c.178T>C	p.C60R	
Exon 2	Missense	c.178T>G	p.C60G	
Exon 2	Missense	c.179G>A	p.C60Y	
Exon 2	Frameshift	c.186_187insTACC	p.G63Yfs*3	c.186insTACC
Exon 2	Frameshift	c.189_207delinsGGAGCATAATCAAA	p.S64Efs*32	c.189_207delins14
Exon 2	Deletion	c.189_209delTAGCACCTGCTATGGCCTTTG	p.S64_W70del	c.188-208delGTAGCACCTGCTATGGCCTTT c.188-208del21 c.189-209del21
Exon 2	Missense	c.196T>C	p.C66R	
Exon 2	Missense	c.196T>G	p.C66G	
Exon 2	Missense	c.197G>A	p.C66Y	
Exon 2	Missense	c.200A>G	p.Y67C	
Exon 2	Frameshift	c.200dupA	p.Y67*	c.201insA
Exon 2	Nonsense	c.201T>G	p.Y67*	
Exon 2	Missense	c.203G>A	p.G68D	
Exon 2	Nonsense	c.218C>G	p.S73*	
Exon 2	Frameshift	c.236_238delinsAAAAGGGGACA	p.L79Qfs*5	
Exon 2	Frameshift	c.237delT	p.V80*	
Exon 2	Frameshift	c.240_241insT	p.K81*	c.241insT
Exon 2	Nonsense	c.244C>T	p.Q82*	
Exon 2	Missense	c.246A>C	p.Q82H	
Exon 2	Frameshift	c.246dupA	p.G83Rfs*15	
Exon 2	Missense	c.247G>A	p.G83R	
Intron 2	Splice-site	c.247+1G>A	p.?	

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Intron 2	Splice-site	c.247+1_247+7delGCAAGTG	p.?	c.247+1delCAAGTG
Intron 2	Splice-site	c.247+2delC	p.G47_Q82del, G63_Q82del	IVS2 247+2delC
Intron 2	Splice-site	c.247+6T>G	p.G47_Q82del, G63_Q82del	IVS2 247+6T>G
Intron 2 to exon 3	Deletion	c.248-592_413delinsGTAAAGTA	p.?	756-bp deletion
Intron 2	Splice-site	c.248-5_248delinsAC	p.?	c.248-5delTATAGGinsAC c.-5-248delTATAGGinsAC
Intron 2	Splice-site	c.248-3T>G	p.?	
Intron 2	Splice-site	c.248-2A>G	p.?	
Intron 2	Splice-site	c.248-1G>A	p.?	c.2481-1G>A
Exon 3	Deletion	c.248-?_418+?del	p.?	IVS2_IVS3 del c.?_248-c.418_?del c.247-?_420+?del
Exon 3	Duplication	c.248-?_418+?dup	p.?	
Exon 3	Missense	c.248G>A	p.G83E	
Exon 3	Missense	c.250T>C	p.C84R	
Exon 3	Missense	c.250T>G	p.C84G	
Exon 3	Missense	c.251G>T	p.C84F	
Exon 3	Nonsense	c.255G>A	p.W85*	
Exon 3	Frameshift	c.260dupA	p.H87Qfs*11	c.261insA
Exon 3	Missense	c.266G>C	p.G89A	
Exon 3	Nonsense	c.274C>T	p.Q92*	
Exon 3	Missense	c.276A>C	p.Q92H	
Exon 3	Frameshift	c.277dupG	p.E93Gfs*5	277insG
Exon 3	Missense	c.280T>C	p.C94R	
Exon 3	Missense	c.280T>G	p.C94G	
Exon 3	Missense	c.292G>A	p.E98K	
Exon 3	Nonsense	c.292G>T	p.E98*	
Exon 3	Missense	c.295T>C	p.C99R	
Exon 3	Missense	c.296G>A	p.C99Y	
Exon 3	Missense	c.296G>T	p.C99F	
Exon 3	Missense	c.304A>G	p.T102A	
Exon 3	Missense	c.319T>C	p.S107P	
Exon 3	Nonsense	c.320C>G	p.S107*	
Exon 3	Missense	c.338A>G	p.Y113C	
Exon 3	Frameshift	c.338dupA	p.Y113*	c.399insA
Exon 3	Nonsense	c.339C>A	p.Y113*	
Exon 3	Nonsense	c.339C>G	p.Y113*	
Exon 3	Frameshift	c.339_340insAA	p.R114Nfs*39	

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exon 3	Frameshift	c.345_346delCT	p.F115Lfs*4	
Exon 3	Missense	c.349T>C	p.C117R	
Exon 3	Missense	c.350G>A	p.C117Y	
Exon 3	Missense	c.350G>C	p.C117S	
Exon 3	Missense	c.353G>A	p.C118Y	c.353C>T
Exon 3	Frameshift	c.353delG	p.C118Lfs*34	
Exon 3	Missense	c.354T>G	p.C118W	
Exon 3	Frameshift	c.354_355delinsAG	p.C118*	c.354-355TA>AG
Exon 3	Frameshift	c.355delA	p.S119Afs*33	
Exon 3	Frameshift	c.359_360delCA	p.T120Rfs*4	
Exon 3	Missense	c.367T>A	p.C123S	
Exon 3	Missense	c.367T>C	p.C123R	
Exon 3	Missense	c.370A>G	p.N124D	
Exon 3	Frameshift	c.371dupA	p.N124Kfs*6	c.371dup
Exon 3	Missense	c.377A>G	p.N126S	
Exon 3	Frameshift	c.399delT	p.P134Lfs*18	
Exon 3	Frameshift	c.407_408delCA	p.T136Nfs*10	c.407_408del
Exon 3	Frameshift	c.408_412delAACAC	p.P138Qfs*7	
Intron 3	Splice-site	c.418+1G>C	p.?	
Intron 3	Splice-site	c.418+2_418+4delinsGAG	p.?	c.418+2_418+4TAA>GAG
Intron 3	Splice-site	c.418+3A>T	p.?	
Intron 3	Splice-site	c.418+5G>A	p.?	
Intron 3	Splice-site	c.418+5_418+8delGTAA	p.C84_S140del	c.247+1_+4delGTAA
Intron 3	Splice-site	c.419-10T>C	p.?	
Exon 4	Deletion	c.419-?_529+?del	p.?	
Exons 4-5	Deletion	c.419-?_621+?del	p.?	IVS3_IVS5 del del exon 4-5
Exons 4-7	Deletion	c.419-?_967+?del	p.?	
Exons 4-8	Duplication	c.419-?_1128+?dup	p.?	
Exons 4-10	Deletion	c.419-?_1413+?del	p.?	
Exons 4-13	Deletion	c.419-?_3117+?del	p.?	Del c.419?-c.3017?
Exon 4	Frameshift	c.420_421insG	p.P141Afs*6	
Exon 4	Frameshift	c.435delT	p.F145Lfs*7	
Exon 4	Nonsense	c.439C>T	p.R147*	
Exon 4	Frameshift	c.449dupC	p.I151Nfs*30	c.449dup
Exon 4	Missense	c.461C>G	p.A154G	
Exon 4	Nonsense	c.482T>A	p.L161*	
Exon 4	Frameshift	c.498delT	p.A167Pfs*9	c.497delT

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exon 4	Frameshift	c.503dupT	p.L168Ffs*13	504insT c.504_505insT
Exon 4	Nonsense	c.507C>A	p.C169*	
Exon 4	Frameshift	c.507_510delinsAAA	p.C169*	c.507-510delCTTTinsAAA c.507_510delCTTTinsAAA
Exon 4	Nonsense	c.516C>G	p.Y172*	
Exon 4	Frameshift	c.528delA	p.G177Efs*10	
Intron 4	Splice-site	c.529+2T>C	p.?	
Exon 5	Deletion	c.530-?_621+?del	p.?	
Exons 5-7	Deletion	c.530-?_967+?del	p.?	del exon 5/6/7
Exon 5	Nonsense	c.541C>T	p.Q181*	
Exon 5	Missense	c.545G>A	p.G182D	
Exon 5	Frameshift	c.551_573delACAGTATGAACATGATGGAGGCA	p.H184Rfs*8	c.551_573del
Exon 5	Missense	c.556A>G	p.M186V	
Exon 5	Nonsense	c.583G>T	p.E195*	
Exon 5	Missense	c.604A>T	p.N202Y	
Exon 5	Frameshift	c.608_609delTG	p.L203Qfs*16	c.608-609delTG
Exon 5	Frameshift	c.612delA	p.K204Nfs*5	
Exon 6	Deletion	c.622-?_852+?del	p.?	
Exons 6-7	Deletion	c.622-?_967+?del	p.?	
Exon 6	Nonsense	c.631C>T	p.R211*	c.631G>A c.633C>T
Exon 6	Nonsense	c.637C>T	p.R213*	
Exon 6	Nonsense	c.642T>G	p.Y214*	
Exon 6	Frameshift	c.659dupG	p.S221Lfs*4	c.660insG
Exon 6	Frameshift	c.664_665delinsAAGG	p.L222Kfs*9	c.664_665delTTinsAAGG
Exon 6	Frameshift	c.673_679delCGTCCAG	p.R225Lfs*3	
Exon 6	Frameshift	c.689_690delAA	p.K230Sfs*25	c.689-690del c.689_690del c.689-690delAA
Exon 6	Missense	c.690A>T	p.K230N	
Exon 6	Frameshift	c.690_691delinsT	p.K230Nfs*22	c.690_691delAGinsT
Exon 6	Missense	c.727G>A	p.E243K	
Exon 6	Missense	c.727G>C	p.E243Q	
Exon 6	Nonsense	c.727G>T	p.E243*	
Exon 6	Frameshift	c.775delC	p.R259Afs*3	
Exon 6	Frameshift	c.782_783delTA	p.I261Sfs*4	c.782_783del
Exon 6	Frameshift	c.786dupT	p.G263Wfs*3	787insT
Exon 6	Frameshift	c.790delG	p.D264Mfs*15	

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exon 6	Missense	c.794A>G	p.E265G	
Exon 6	Frameshift	c.795_796delinsTT	p.E265_L1038delinsD	
Exon 6	Frameshift	c.796_799delAGAG	p.R266Sfs*12	796-799delAGAG
Exon 6	Missense	c.797G>C	p.R266T	
Exon 6	Frameshift	c.802dupA	p.T268Nfs*30	c.802-803insA
Exon 6	Frameshift	c.804delT	p.A269Qfs*10	
Exon 6	Missense	c.818T>G	p.M273R	
Exon 6	Missense	c.830T>C	p.L277P	
Intron 6	Splice-site	c.852+1G>A	p.?	
Intron 6	Splice-site	c.852+1G>C	p.?	
Intron 6	Splice-site	c.853-2A>G	p.?	
Intron 6	Splice-site	c.853-1G>A	p.?	
Intron 6	Splice-site	c.853-1G>C	p.?	
Exon 7	Duplication	c.853-?_967+?dup	p.?	
Exon 7	Frameshift	c.855delA	p.S286Lfs*6	
Exon 7	Nonsense	c.860T>A	p.L287*	
Exon 7	Nonsense	c.872T>G	p.L291*	
Exon 7	Frameshift	c.872delT	p.L291*	
Exon 7	Nonsense	c.893G>A	p.W298*	
Exon 7	Frameshift	c.894_895dupGG	p.V299Gfs*2	
Exon 7	Missense	c.901T>C	p.S301P	
Exon 7	Missense	c.908G>A	p.R303H	
Exon 7	Nonsense	c.928A>T	p.R310*	
Exon 7	Missense	c.932G>A	p.G311E	
Exon 7	Missense	c.937G>C	p.A313P	
Exon 7	Missense	c.954A>C	p.E318D	
Exon 7	Nonsense	c.961C>T	p.R321*	
Exon 7	Frameshift	c.961delC	p.R321Efs*14	
Intron 7	Splice-site	c.967+2T>C	p.?	
Intron 7	Splice-site	c.967+4delA	p.G285Ifs*12	IVS7 958+3delT c.968+3delA
Intron 7	Splice-site	c.967+5G>C	p.?	
Intron 7	Splice-site	c.967+5G>T	p.?	
Intron 7	Splice-site	c.968-5A>G	p.?	
Intron 7	Splice-site	c.968-3C>G	p.?	
Intron 7	Splice-site	c.968-2A>C	p.?	c.965-2A>C
Intron 7	Splice-site	c.968-1G>T	p.?	
Exon 8	Deletion	c.968-?_1128+?del	p.?	c.968-?_1129+?del

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exons 8-9	Deletion	c.968-?_1276+?del	p.?	c.967-?_1275+?del
Exons 8-10	Duplication	c.968-?_1413+?dup	p.?	c.968-?_c.1413+?dup
Exon 8	Frameshift	c.969dupT	p.H324Sfs*3	c.967_968insA c.968_969insT
Exon 8	Frameshift	c.980delC	p.P327Lfs*8	
Exon 8	Missense	c.992A>G	p.H331R	
Exon 8	Nonsense	c.994C>T	p.R332*	
Exon 8	Nonsense	c.1001T>G	p.L334*	
Exon 8	Frameshift	c.1011_1015delAAATG	p.R337Sfs*6	
Exon 8	Missense	c.1016T>A	p.V339D	
Exon 8	Missense	c.1019T>C	p.L340P	
Exon 8	Missense	c.1039T>C	p.C347R	
Exon 8	Missense	c.1040G>A	p.C347Y	1042G>A
Exon 8	Missense	c.1042G>A	p.V348I	
Exon 8	Frameshift	c.1044delT	p.I349Lfs*8	
Exon 8	Frameshift	c.1060delC	p.L354Cfs*3	
Exon 8	Missense	c.1066A>T	p.M356L	
Exon 8	Frameshift	c.1076delC	p.T359Mfs*16	
Exon 8	Frameshift	c.1093_1098delinsG	p.R365Gfs*5	
Exon 8	Frameshift	c.1097delC	p.P366Qfs*9	c.1095delC
Exon 8	Frameshift	c.1101_1105delGGAGG	p.E368Rfs*2	c.1099_1103del c.1099-1103delGGGGA c.1099_1103delGGGGA
Exon 8	Frameshift	c.1113dupT	p.A372Cfs*27	c.1113delT
Exon 8	Missense	c.1117G>C	p.A373P	
Exon 8	Frameshift	c.1120delA	p.I374*	
Exon 8	Nonsense	c.1126G>T	p.E376*	
Intron 8	Splice-site	c.1128+1G>A	p.?	
Intron 8	Splice-site	c.1128+1G>T	p.?	IVS8 +1G>T IVS8 1128+1G>T
Intron 8	Splice-site	c.1129-3C>G	p.?	IVS8 1129-3C>G
Exon 9	Frameshift	c.1129-1_1129dupGG	p.V377Gfs*13	
Exon 9	Frameshift	c.1141dupA	p.R381Kfs*18	c.1141_1142insA
Exon 9	Nonsense	c.1146T>G	p.Y382*	
Exon 9	Missense	c.1151C>T	p.A384V	
Exon 9	Missense	c.1156G>A	p.E386K	
Exon 9	Missense	c.1156G>C	p.E386Q	
Exon 9	Missense	c.1157A>C	p.E386A	

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exon 9	Missense	c.1157A>G	p.E386G	
Exon 9	Missense	c.1157A>T	p.E386V	
Exon 9	Missense	c.1171G>A	p.A391T	
Exon 9	Missense	c.1175T>C	p.V392A	
Exon 9	Frameshift	c.1191_1192delTG	p.C397*	1191/1192delTG c.1189-1190delTG
Exon 9	Nonsense	c.1196C>G	p.S399*	
Exon 9	Missense	c.1202T>C	p.L401S	
Exon 9	Nonsense	c.1207C>T	p.Q403*	C1207T
Exon 9	Frameshift	c.1214delA	p.D405Afs*7	
Exon 9	Missense	c.1220A>C	p.Y407S	
Exon 9	Nonsense	c.1221T>G	p.Y407*	
Exon 9	Missense	c.1228G>A	p.G410R	
Exon 9	Nonsense	c.1241G>A	p.W414*	
Exon 9	Nonsense	c.1243G>T	p.E415*	
Exon 9	Frameshift	c.1245_1246dupGA	p.I416Rfs*4	c.1246dupGA 1247/8insGA
Exon 9	Frameshift	c.1246_1247insG	p.I416Sfs*32	c.1246dupG
Exon 9	Frameshift	c.1247_1250delinsGA	p.I416Rfs*31	c.1246_1247dupGA; 1250_1253delTTAT
Exon 9	Frameshift	c.1248delA	p.F417Lfs*2	
Exon 9	Frameshift	c.1250_1253delTTAT	p.F417*	c.1248-1251delATTT
Exon 9	Missense	c.1257A>T	p.R419S	
Exon 9	Missense	c.1258T>C	p.C420R	
Exon 9	Missense	c.1259G>A	p.C420Y	
Exon 9	Frameshift	c.1268dupT	p.F424Lfs*24	
Exon 9	Frameshift	c.1271_1276delinsCGGAGA	p.F424_G426delinsSER	c.1271delTCCCAGinsCGGAGA
Exon 9	Frameshift	c.1274dupC	p.G426Rfs*22	c.1272insC c.1274dup
Exon 9	Missense	c.1276G>C	p.G426R	
Intron 9	Splice-site	c.1276+1G>A	p.?	
Intron 9	Splice-site	c.1276+3A>G	p.?	
Intron 9	Splice-site	c.1276+3A>T	p.?	
Intron 9	Splice-site	c.1276+4A>G	p.?	
Introns 9-10	Deletion	c.1277-289_1413+4737del	p.?	c.1277-291_1413+4735del
Intron 9	Splice-site	c.1277-9A>C	p.?	
Intron 9	Splice-site	c.1277-9A>G	p.?	
Intron 9	Splice-site	c.1277-8A>G	p.?	
Exon 10	Deletion	c.1277-?_1413+?del	p.?	c.1277-?_c.1413+?del

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exon 10	Duplication	c.1277-?_1413+?dup	p.?	IVS9_IVS10 dup c.1277-?.1413+?dup c.1277-?_c.1413+?dup
Exon 10	Frameshift	c.1279delG	p.E427Nfs*47	
Exon 10	Frameshift	c.1285_1286insGGATT	p.V429Gfs*47	
Exon 10	Frameshift	c.1293_1300delGTACCAGA	p.E431Dfs*14	
Exon 10	Nonsense	c.1296C>G	p.Y432*	
Exon 10	Nonsense	c.1297C>T	p.Q433*	
Exon 10	Frameshift	c.1313_1316delCAGA	p.T438Rfs*35	c.1313-1316delCAGA
Exon 10	Missense	c.1346T>G	p.M449R	
Exon 10	Nonsense	c.1348C>T	p.Q450*	
Exon 10	Frameshift	c.1366delinsCA	p.E456Qfs*15	
Exon 10	Frameshift	c.1371delA	p.K457Nfs*17	
Exon 10	Frameshift	c.1371dupA	p.Q458Tfs*13	c.1371dup
Exon 10	Frameshift	c.1376_1377delGA	p.R459Tfs*11	c.1375_1376delAG
Exon 10	Frameshift	c.1387_1388insA	p.P463Hfs*8	c.1388insA
Exon 10	Frameshift	c.1389dupA	p.E464Rfs*7	c.1388-1389insA c.1388_1389insA
Exon 10	Frameshift	c.1392delA	p.A465Pfs*9	
Exon 10	Nonsense	c.1397G>A	p.W466*	
Exon 10	Nonsense	c.1398G>A	p.W466*	
Exon 10	Frameshift	c.1401delA	p.E468Kfs*6	c.1399delA c.1401del
Exon 10	Nonsense	c.1402G>T	p.E468*	
Intron 10	Splice-site	c.1413+1G>A	p.?	
Intron 10	Splice-site	c.1413+3A>T	p.?	IVS10 +3A>T
Intron 10	Splice-site	c.1414-2A>T	p.?	
Exons 11-12	Deletion	c.1414-?_2866+?del	p.?	Del c.1414-?_2866+?
Exons 11-13	Deletion	c.1414-?_3117+?del	p.?	c.1414-?_3114+?del c.1414-?_c.3117+?del
Exon 11	Nonsense	c.1424C>A	p.S475*	
Exon 11	Frameshift	c.1426_1450delCTCAAGGAGACAATCGAAGACTGTT	p.L476Gfs*22	c.1426_1450del
Exon 11	Frameshift	c.1427delT	p.L476Pfs*30	c.1426delT
Exon 11	Nonsense	c.1441G>T	p.E481*	
Exon 11	Missense	c.1447T>C	p.C483R	
Exon 11	Nonsense	c.1451G>A	p.W484*	
Exon 11	Missense	c.1453G>A	p.D485N	
Exon 11	Missense	c.1454A>G	p.D485G	
Exon 11	Nonsense	c.1456C>T	p.Q486*	

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exon 11	Missense	c.1460A>T	p.D487V	
Exon 11	Missense	c.1469C>T	p.A490V	
Exon 11	Missense	c.1471C>T	p.R491W	
Exon 11	Missense	c.1472G>A	p.R491Q	
Exon 11	Frameshift	c.1477dupA	p.T493Nfs*6	
Exon 11	Nonsense	c.1483C>T	p.Q495*	
Exon 11	Missense	c.1486T>C	p.C496R	
Exon 11	Missense	c.1487G>A	p.C496Y	
Exon 11	Missense	c.1509A>C	p.E503D	
Exon 11	Missense	c.1516A>G	p.M506V	
Exon 11	Nonsense	c.1523G>A	p.W508*	
Exon 11	Nonsense	c.1525G>T	p.E509*	
Exon 11	Missense	c.1535A>C	p.K512T	
Exon 11	Missense	c.1557T>A	p.N519K	
Exon 11	Frameshift	c.1585delC	p.R529Afs*35	
Intron 11	Splice-site	c.1587-7_1587-4delCTTT	p.?	
Exon 12	Missense	c.1598A>G	p.H533R	
Exon 12	Nonsense	c.1629T>G	p.Y543*	
Exon 12	Missense	c.1687G>A	p.V563M	
Exon 12	Nonsense	c.1750C>T	p.R584*	
Exon 12	Missense	c.1766A>G	p.Y589C	
Exon 12	Nonsense	c.1771C>T	p.R591*	
Exon 12	Nonsense	c.1789C>T	p.R597*	
Exon 12	Frameshift	c.1954_1955dupAC	p.V654Lfs*6	c.1954_1955dup c.1954dupA c.1956insAC c.1956_1957insAC
Exon 12	Frameshift	c.1968dupA	p.Q657Tfs*18	c.1969insA c.1969_1970insA
Exon 12	Nonsense	c.1969C>T	p.Q657*	
Exon 12	Nonsense	c.1978G>T	p.E660*	
Exon 12	Nonsense	c.1981G>T	p.E661*	
Exon 12	Frameshift	c.2009delC	p.P670Qfs*30	
Exon 12	Nonsense	c.2124C>G	p.Y708*	
Exon 12	Frameshift	c.2128delC	p.L710Sfs*2	
Exon 12	Frameshift	c.2286delC	p.N764Ifs*8	c.2289delC
Exon 12	Frameshift	c.2291dupA	p.N764Kfs*49	2292insA
Exon 12	Missense	c.2296A>G	p.T766A	

Location	Mutation category	Nucleotide change[†]	Amino acid change	Alternative published nomenclature
Exon 12	Frameshift	c.2297delC	p.T766Kfs*6	delC2705
Exon 12	Frameshift	c.2303_2309delAGCCCCG	p.E768Gfs*2	
Exon 12	Frameshift	c.2308delC	p.R770Gfs*2	c.2305delC
Exon 12	Frameshift	c.2386delG	p.A796Qfs*7	
Exon 12	Frameshift	c.2407_2408insTG	p.T803Mfs*5	2408insTG c.2408_2409insTG
Exon 12	Frameshift	c.2410_2413delGTCA	p.V804Pfs*2	c.2410-2413delGTCA
Exon 12	Frameshift	c.2413dupA	p.T805Nfs*8	
Exon 12	Frameshift	c.2442_2443delCA	p.H814Qfs*3	c.2441-2442delAC
Exon 12	Frameshift	c.2446_2447dupGT	p.N817Lfs*23	c.2446_2447dup
Exon 12	Frameshift	c.2484delG	p.T829Qfs*10	
Exon 12	Frameshift	c.2503dupA	p.T835Nfs*8	c.2504insA
Exon 12	Frameshift	c.2503_2506delACAA	p.T835Pfs*3	c.2500delCAAA c.2503_2506del
Exon 12	Frameshift	c.2504delC	p.T835Kfs*4	
Exon 12	Frameshift	c.2506_2522delACCAACATAGTGACACA	p.T836*	c.2506_2522del17
Exon 12	Frameshift	c.2521_2522dupCA	p.R842Ifs*18	
Exon 12	Frameshift	c.2527delG	p.A843Pfs*16	
Exon 12	Nonsense	c.2533G>T	p.E845*	
Exon 12	Frameshift	c.2580delT	p.N861Ifs*11	c.2579delT 2579-2580 delT
Exon 12	Missense	c.2588G>A	p.S863N	
Exon 12	Frameshift	c.2609delT	p.L870Yfs*2	
Exon 12	Nonsense	c.2617C>T	p.R873*	
Exon 12	Missense	c.2618G>A	p.R873Q	
Exon 12	Nonsense	c.2620G>T	p.E874*	
Exon 12	Nonsense	c.2626C>T	p.Q876*	
Exon 12	Frameshift	c.2668delA	p.R890Gfs*6	
Exon 12	Nonsense	c.2695C>T	p.R899*	
Exon 12	Missense	c.2696G>C	p.R899P	
Exon 12	Missense	c.2708A>G	p.N903S	
Exon 12	Nonsense	c.2730T>A	p.C910*	
Exon 12	Nonsense	c.2737C>T	p.Q913*	
Exon 12	Nonsense	c.2752C>T	p.Q918*	
Exon 12	Nonsense	c.2789C>G	p.S930*	
Exon 13	Missense	c.2945A>G	p.K982R	

[†]GenBank reference sequence and version number for *BMPR2*: NM_001204.6; numbering is calculated from +1 as A of the ATG initiation codon. All nomenclature has been updated according to the most recent HGVS guidelines: http://www.hgvs.org/mutnomen.

Supp. References

Aimi Y, Hirayama T, Kataoka M, Momose Y, Nishimaki S, Matsushita K, Yoshino H, Satoh T, Gamou S. 2013. A novel break point of the BMPR2 gene exonic deletion in a patient with pulmonary arterial hypertension. J Hum Genet 58:815-818.

Aldred MA, Vijayakrishnan J, James V, Soubrier F, Gomez-Sanchez MA, Martensson G, Galie N, Manes A, Corris P, Simonneau G, Humbert M, Morrell NW, et al. 2006. BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. Hum Mutat 27:212-213.

Arbustini E, Marziliano N, Porcu E, Pasotti M, Grasso M, Tagliani M, Disabella E, Diegoli M, Pilotto A, Ghio S, Campana C, D'Armini A, Vigano M. 2008. Gene symbol: BMPR2. Disease: Pulmonary hypertension, primary. Hum Genet 123:112-113.

Chida A, Shintani M, Yagi H, Fujiwara M, Kojima Y, Sato H, Imamura S, Yokozawa M, Onodera N, Horigome H, Kobayashi T, Hatai Y, et al. 2012. Outcomes of childhood pulmonary arterial hypertension in BMPR2 and ALK1 mutation carriers. Am J Cardiol 110:586-593.

Cogan JD, Pauciulo MW, Batchman AP, Prince MA, Robbins IM, Hedges LK, Stanton KC, Wheeler LA, Phillips JA, 3rd, Loyd JE, Nichols WC. 2006. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. Am J Respir Crit Care Med 174:590-598.

Girerd B, Montani D, Eyries M, Yaici A, Sztrymf B, Coulet F, Sitbon O, Simonneau G, Soubrier F, Humbert M. 2010. Absence of influence of gender and BMPR2 mutation type on clinical phenotypes of pulmonary arterial hypertension. Respir Res 11:73.

Girerd B, Coulet F, Jais X, Eyries M, Van Der Bruggen C, De Man F, Houweling A, Dorfmuller P, Savale L, Sitbon O, Vonk-Noordegraaf A, Soubrier F, et al. 2015. Characteristics of pulmonary arterial hypertension in affected carriers of a mutation located in the cytoplasmic tail of bone morphogenetic protein receptor type 2. Chest 147:1385-1394.

Hamid R, Cogan JD, Hedges LK, Austin E, Phillips JA, 3rd, Newman JH, Loyd JE. 2009. Penetrance of pulmonary arterial hypertension is modulated by the expression of normal BMPR2 allele. Hum Mutat 30:649-654.

Hamid R, Hedges LK, Austin E, Phillips JA, 3rd, Loyd JE, Cogan JD. 2010. Transcripts from a novel BMPR2 termination mutation escape nonsense mediated decay by downstream translation re-initiation: implications for treating pulmonary hypertension. Clin Genet 77:280-286.

Handa T, Okano Y, Nakanishi N, Morisaki T, Morisaki H, Mishima M. 2014. BMPR2 gene mutation in pulmonary arteriovenous malformation and pulmonary hypertension: a case report. Respir Investig 52:195-198.

Hayes D, Jr., Ramanathan C, Kopp BT. 2014. The BMPR2 missense mutation p.K230N and pulmonary arterial hypertension. Pediatr Pulmonol 49:E5-6.

Johri S, Dunnington GH, Vnencak-Jones CL. 2010. A novel BMPR2 mutation associated with pulmonary arterial hypertension in an octogenarian. Lung 188:349-352.

Kabata H, Satoh T, Kataoka M, Tamura Y, Ono T, Yamamoto M, Huqun, Hagiwara K, Fukuda K, Betsuyaku T, Asano K. 2013. Bone morphogenetic protein receptor type 2 mutations, clinical phenotypes and outcomes of Japanese patients with sporadic or familial pulmonary hypertension. Respirology 18:1076-1082.

Kataoka M, Aimi Y, Yanagisawa R, Ono M, Oka A, Fukuda K, Yoshino H, Satoh T, Gamou S. 2013. Alu-mediated nonallelic homologous and nonhomologous recombination in the BMPR2 gene in heritable pulmonary arterial hypertension. Genet Med 15:941-947.

Kerstjens-Frederikse WS, Bongers EM, Roofthooft MT, Leter EM, Douwes JM, Van Dijk A, Vonk-Noordegraaf A, Dijk-Bos KK, Hoefsloot LH, Hoendermis ES, Gille JJ, Sikkema-Raddatz B, et al. 2013. TBX4 mutations (small patella syndrome) are associated with childhood-onset pulmonary arterial hypertension. J Med Genet 50:500-506.

Liu D, Liu QQ, Eyries M, Wu WH, Yuan P, Zhang R, Soubrier F, Jing ZC. 2012. Molecular genetics and clinical features of Chinese idiopathic and heritable pulmonary arterial hypertension patients. Eur Respir J 39:597-603.

Machado RD, Pauciulo MW, Thomson JR, Lane KB, Morgan NV, Wheeler L, Phillips JA, 3rd, Newman J, Williams D, Galie N, Manes A, McNeil K, et al. 2001. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. Am J Hum Genet 68:92-102.

Momose Y, Aimi Y, Hirayama T, Kataoka M, Ono M, Yoshino H, Satoh T, Gamou S. 2015. De novo mutations in the BMPR2 gene in patients with heritable pulmonary arterial hypertension. Ann Hum Genet 79:85-91.

Pfarr N, Szamalek-Hoegel J, Fischer C, Hinderhofer K, Nagel C, Ehlken N, Tiede H, Olschewski H, Reichenberger F, Ghofrani AH, Seeger W, Grunig E. 2011. Hemodynamic and clinical onset in patients with hereditary pulmonary arterial hypertension and BMPR2 mutations. Respir Res 12:99.

Pfarr N, Fischer C, Ehlken N, Becker-Grunig T, Lopez-Gonzalez V, Gorenflo M, Hager A, Hinderhofer K, Miera O, Nagel C, Schranz D, Grunig E. 2013. Hemodynamic and genetic analysis in children with idiopathic, heritable, and congenital heart disease associated pulmonary arterial hypertension. Respir Res 14:3.

Portillo K, Santos S, Madrigal I, Blanco I, Pare C, Borderias L, Peinado VI, Roca J, Mila M, Barbera JA. 2010. Study of the BMPR2 gene in patients with pulmonary arterial hypertension. Arch Bronconeumol 46:129-134.

Raamsteeboers AJ, Bogaard HJ, Vonk Noordegraaf A. 2014. Pulmonary arterial hypertension preceding idiopathic pulmonary fibrosis in a BMPR2 mutation positive patient. Eur Respir Rev 23:147-149.

Rosenzweig EB, Morse JH, Knowles JA, Chada KK, Khan AM, Roberts KE, McElroy JJ, Juskiw NK, Mallory NC, Rich S, Diamond B, Barst RJ. 2008. Clinical implications of determining BMPR2 mutation status in a large cohort of children and adults with pulmonary arterial hypertension. J Heart Lung Transplant 27:668-674.

Sztrymf B, Coulet F, Girerd B, Yaici A, Jais X, Sitbon O, Montani D, Souza R, Simonneau G, Soubrier F, Humbert M. 2008. Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. Am J Respir Crit Care Med 177:1377-1383.

Wang H, Li W, Zhang W, Sun K, Song X, Gao S, Zhang C, Hui R, Hu H. 2009. Novel promoter and exon mutations of the BMPR2 gene in Chinese patients with pulmonary arterial hypertension. Eur J Hum Genet 17:1063-1069.

Wang H, Cui QQ, Sun K, Song L, Zou YB, Wang XJ, Jia L, Liu X, Gao S, Zhang CN, Hui RT. 2010. Identities and frequencies of BMPR2 mutations in Chinese patients with idiopathic pulmonary arterial hypertension. Clin Genet 77:189-192.