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Molecular Genetics and Clinical Features of Chinese IPAH and HPAH Patients

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Authorship

Dr Dong Liu and Dr Qian-Qian Liu contributed to the experimental design, operation, data analyzing and manuscript writing. The two first authors contributed equally and are the co-first authors. Dr Wen-Hui Wu, Dr Ping Yuan and Dr Rui Zhang contributed to experiment operation and data analyzing. Prof Florent Soubrier and Dr Melanie Eyries contributed to data analyzing and manuscript revising. Prof Zhi-Cheng Jing contributed to the experimental design and manuscript writing and revising. All authors had full access to all study data and had final responsibility for the decision to submit for publication. All have reviewed the manuscript and approved the final version for submission.

Introduction

Pulmonary arterial hypertension (PAH) is a rare and potentially fatal disorder characterized by plexiform lesions of proliferating endothelial cells and smooth muscle cells in pulmonary arterioles, which lead to elevated pulmonary artery pressures, right ventricular failure, and death [1]. At least 6% of cases have a recognized family history, in which the disease segregates as an autosomal dominant trait with incomplete penetrance and an estimated lifetime risk of 10% to 20% [2]. The disease is more frequent in women, with a female/male ratio of at least 1.7:1 [1, 3, 4]. The average age of onset is in the third decade of life, but there is wide variation within families and childhood onset is common.

Familial cases have long been recognized and mutations in the bone morphogenetic protein type II receptor (BMPR-II) gene (*BMPR2*) have been recognized to cause heritable PAH.[5, 6] Subsequent studies have reported more than 250 *BMPR2* mutations responsible for 55% to 70% of heritable PAH (HPAH), and 11% to 40% of sporadic PAH (idiopathic PAH) cases [7, 8]. After the last world conference on pulmonary hypertension, these forms were grouped under the term heritable [9].

BMPR2 encodes the bone morphogenetic protein receptor II, a member of the transforming growth factor-beta (TGF-β) cell signaling superfamily [5-7]. After ligand binding, type II receptors, which have serine/threonine kinase activity, form heteromeric complexes with membrane-bound type I receptors, initiating phosphorylation of the type I receptor and downstream intracellular Smads or MAPKs [10, 11]. This pathway appears to be critical in both cell differentiation and growth, through transcriptional regulation of target genes.

To improve knowledge of the genetic origin of PAH in Chinese patients with the disorder, we investigated 15 unrelated families with PAH and 290 sporadic PAH patients for *BMPR2* mutations. Direct sequencing to detect mutations in coding regions and flanking splice sites, in addition to

analysis of exon dosage across the entire gene using multiplex ligation-dependent probe amplification (MLPA®) showed a wide *BMPR2* mutation spectrum in Chinese patients with PAH. Our study provides an extensive investigation of the genetic etiology of Chinese heritable and idiopathic PAH which gives insight into the variety of *BMPR2* mutations among different ethnic groups.

Methods

Study patients

All patients belonged to the Chinese Han population and visited Shanghai Pulmonary Hospital in China between January 1, 2008 and August 31, 2010. 290 idiopathic and 15 heritable PAH patients were tested for *BMPR2* point mutations and large size rearrangements. The diagnosis of IPAH required the presence of elevated pulmonary artery pressure (mean PAP >25 mm Hg, with a pulmonary capillary wedge pressure ≤15 mm Hg measured by right heart catheterization at rest), and the exclusion of other disorders known to cause pulmonary hypertension by clinical evaluation and objective tests. HPAH was recognized if there was more than one confirmed case in first- to third-degree relatives in the family [12]. A single proband from each affected pedigree was included in the study (n=15). All participants gave their written informed consent for genetic analyses prior to participation. The study was approved by the Ethics Committee of Shanghai Pulmonary Hospital.

Molecular methods

Direct screening using an ABI 3730 (Applied Biosystems, CA, USA) was adopted to detect the point mutations in the coding regions and intron/exon boundaries of *BMPR2*. Genomic DNA was isolated from peripheral blood leukocytes. 15 pairs of PCR primers were designed to amplify 13 exons and the 5', 3' untranslated region of *BMPR2* gene (see supplementary Table 1). The results were compared with the reference sequence of *BMPR2* gene (accession number NM-001204.5) with the ABI SeqScape software, version 2.5 (Applied Biosystems). The mutation nomenclature followed current guidelines as recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). The mutation numbering employed in this report is based on the cDNA sequence, where +1 designates the A of the ATG initiation codon.

The *BMPR2* gene was screened for large size rearrangements (RGTs) using the SALSA MLPA® P093 HHT probe mix kit (MRC-Holland BV), according to the manufacturer's instructions. Samples were analyzed on an ABI 377 fluorescent analyzer with GeneScan and GenoTyper software (Applied Biosystems, Warrington, UK; http://www.appliedbiosystems.com/). RGTs were analyzed by the Coffalyser software provided by the manufacturer's web site (http://www.mlpa.com). Two DNA samples from unaffected individuals were used as controls in each series of experiments. Electrophoregrams were superimposed on those generated with a control DNA by adjusting to the same levels the peaks obtained for the control amplicons. Only PAH patients for whom no *BMPR2* mutation could be detected by direct sequencing were analyzed for RGTs (n=255). 232 results (229 IPAH and 3 HPAH) were readable because of technical problems on the remaining samples.

The coding sequences and exon-intron boundaries of the *ACVRL1* and *ENG* genes in 7 HPAH patients negative for *BMPR2* mutations were also sequenced.

Statistical analysis and bioinformatic tools

We compared the demographic and basic clinical features of IPAH and HPAH patients with the use of χ^2, fisher exact test or t-tests, where appropriate. A *P* value of less than 0.05 was considered to indicate statistical significance. Polyphen2 was used for *in silico* analysis of coding variants, and splice site variants were analyzed by Splice Site Finder, MaxEntScan, Nnsplice and Genesplicer, all available with Alamut v1.5 software (Biointeractive Software).

Results

Clinical characteristics of the study participants

The study population included 290 patients with sporadic idiopathic PAH and 15 probands from unrelated pedigrees with heritable PAH (15 families; Table 1). Eighty-seven were men. The mean (±SD) age of the patients was 33±16 years (range 1 to 77 years). HPAH patients were about 14 years younger than those with IPAH (20±10 years vs 34±16 years; $P<0.001$).

Mutation rate and distribution

Mutation analysis in heritable and idiopathic PAH is summarized in Tables 2 and 3. A total of 50 mutations were identified, accounting for 8 of the 15 patients with HPAH (53.3%) and 42 of the 290 patients with IPAH (14.5%). Twenty-five mutations are first reported.

Forty-nine of the 50 patients had non synonymous *BMPR2* variations (Table 2). These variants classification was shown in Table 3.

Four variants were found in at least two unrelated patients. Exon 10 deletion of *BMPR2* was found in two IPAH patients and has already been reported, but the breakpoints were not determined. The remaining 37 patients had unique *BMPR2* mutations.

All these mutations are distributed from the 5'UTR to exon 13 of *BMPR2*, except for exon 5 and exon 7 in which no mutation was found either in the IPAH or the HPAH cohort.

Point mutations

Fifteen of the 43 point mutations were either frameshift (n=6) or nonsense (n=9) mutations that were predicted to result in a truncated BMPR-II molecule. All six frameshift mutations were newly identified ins/del. Among the 21 missense mutations eight were novel mutation sites. The effect on protein function was predicted with PolyPhen[13]. Five mutations (Table 2) were predicted to be probably damaging, the remaining three mutations (c.266G>C, c.1117G>C, c.2296A>G) were predicted to be possibly damaging. These three mutations were not found in 200 control chromosomes. Two splice-site mutations were found in IPAH patients. Variant c.1129-3C>G has been reported before [14, 15] whereas the other was identified in this study. Variants of unknown significance found in the 5'UTR (c.-223A>G, c.-310A>G), in intron 7 (c.967+5G>C), and the 3'UTR (c.3117+12G>A) are reported for the first time in this study. Variant c.967+5G>C and the synonymous mutation c.969T>C that affected the second base of exon 8 were analyzed by splice-site predictions programs. A possible effect on splicing was predicted for these two variants (see supplementary Table 2). However, further analyses on RNA are needed to draw a conclusion on the deleterious effect of these variants.

Large rearrangements (RGTs)

255 IPAH patients for whom no mutation was detected by sequencing were analyzed by MLPA. Only 229 results could be readable among the 255 IPAH patients, and 7 RGTs were found. The RGT mutation rate is 3.1% in IPAH patients who has no sequencing mutations (n=7 in 229 IPAH). Among 15 HPAH, 8 were found with mutation by sequencing and 7 were analyzed by

MLPA. Only three DNAs from 7 HPAH could be analyzed, and none was found with RGT mutation.

Among these 7 RGTs, four are newly identified (Table 3).

Overall, RGTs represented 14% of all mutations in the Chinese PAH cohort.

Polymorphisms

Four nucleotide changes were considered to represent polymorphisms, because they were also observed in control individuals (See the supplementary Table 3). Polymorphism c.1-93 A>G was found in eight patients in the 5'UTR and is first reported in this study. 237 normal Chinese Han people were screened and four patients were found to be carrying the same variant. The allele frequency is 0.84% for the G allele.

***ACVRL1* and *ENG* in HPAH**

The coding sequences and intron-exon boundaries of these two genes were analyzed in index cases of PAH families for whom no mutation was found in the *BMPR2* gene. A single missense mutation was found in the *ACVRL1* gene, and is considered as potentially damaging according to Polyphen software.

Age at diagnosis in mutation carriers and non-carriers

The mean age at diagnosis was compared in carriers and non-carriers and was found to be significantly lower in carriers (28±11 years; n=50) than in non-carriers (34±17 years; n=255) (P<0.001). The mean age at onset was found to be significantly lower in females carrying a *BMPR2* mutation (29±12 years; n=28) than those without mutation (35±16 years; n=190) (P=0.036). In the male group, which was smaller, the age difference between male carriers (27±10

years; n=22) and non-carriers (32±19 years; n=65) did not reach statistical significance (*P*=0.071) (Table 5).

Discussion

A total of 298 *BMPR2* mutations have been identified until now in independent PAH patients [14, 16], including those with a known PAH family history or a sporadic onset of the disease. To date, mutations are usually found in around 70% of individuals with HPAH, whereas the mutation detection rate ranges from 10% to 40% in idiopathic cases [7, 8]. In this study, we found a mutation rate in Chinese Han idiopathic and heritable PAH patients of 14.5% and 53.3%, respectively. These mutation detection rates are in a similar range to those reported in the literature and in particular, by Wang et al [17] who reported 12 mutations in 72 Chinese IPAH patients (16.6%) without searching for RGT mutations by MLPA.

It is worth noting that the proportion of mutations found in groups of PAH patient is comparable in all published series, whatever the population of origin. Either these proportions are the consequences of technical limitations in detecting a particular type of mutations, or a relatively constant proportion of HPAH or IPAH among populations is secondary to other causes that remain to be elucidated.

Mutations predicted to introduce premature truncation codon to the *BMPR2* open-reading frame in the present study encompass nonsense (9 of 50, 18%), frameshift (6 of 50, 12%), splice site (2 of 50, 4%), and gene duplications/deletions (7 of 50, 14%). The total proportion of mutations found to cause premature termination was 48%, much less than the 68% reported by Machado et al. in 2009 [16].

We used MLPA® to detect large gene rearrangements (RGTs) which underlie a significant proportion of HPAH and IPAH cases [18, 19]. In our study, the proportion of RGTs was14% of all the mutation categories, and they were present in 3.1% (n=7 of 229) of Chinese Han sporadic IPAH patients negative for *BMPR2* mutation by DNA sequencing. These proportions are close to those observed by Aldred et al. who reported a proportion of 12% of RGTs in HPAH (n=58 families) and 5% in IPAH (n=126 patients), but lower than the proportion reported by Cogan et al. (33%) in a smaller series of families (n=12) [20]. Among these mutations, the deletion of *BMPR2* exon 10 and exon 11 to 13 have been previously reported [19, 21], but the other four RGTs are novel.

We also found two splice-site mutations in this study due to mutations in introns affecting mRNA splicing. One was reported for the first time in our study, and *in silico* prediction was in favor of a deleterious effect, based on splice-site software predictions, but the consequences on mRNA maturation were not tested *in vitro* in this study. Twenty-one missense mutations of *BMPR2* found in Chinese idiopathic and heritable PAH clusters were confined to exons 2, 3, 6, 8 to 9, 11, and 12. Exon 2 and 3 encodes the extracellular ligand-binding domain essential for signaling activity of BMPR-II which adopts a precisely folded conformation, exquisitely dependent on the formation of 5 disulphide bridges by 10 cysteine residues dispersed across exons 2 and 3. Mutations were found on cysteine 60, 84, and 117, affecting 3 of the 10 cysteine-conserved residues. Cysteine 60, 84, and 117 mutations have been reported previously [7, 16], but the substituted amino acids found in our study are different. Cysteine mutant constructs by transient transfection of GFP- or myc-tagged *BMPR2* constructs demonstrated substantial cytosolic retention and significant protein misfolding, likely to be due to a profound loss of conformational integrity [22]. The kinase region encoded by exons 5-11 harbors 13 discrete PAH-related missense mutations seen among 305 probands (Table 2), which constitutes the largest proportion of all the missense variations in this study. The kinase region is compartmentalized into 12 subdomains of

variable importance for the processes of adenosine triphosphate binding, substrate recognition, and phosphate group transfer [23]. X-ray crystallography revealed that a highly conserved 12 invariant amino acids motif lies within the approximately 250-300 amino acid core. We found several missense mutations involving key residues, such as Arg 491 and Cys 420, which are essential catalytical residues and their mutations cause a near complete abolition of signaling through the Smad pathway [22].

The variant c.-93A>G in 5'UTR was frequently observed and was further investigated because it is not present in databases (dbSNP, HapMap, 1000 genome). We genotyped 237 normal subjects, and found this variant at a frequency of 0.84% for the G allele in the different Chinese populations tested in the panel, clearly showing that it is a rare variant.

Our findings suggest haploinsufficiency as the predominant molecular mechanism underlying *BMPR2* predisposition to hereditary and idiopathic PAH, since mutations identified are likely to inactivate the receptor [15]. Additional clinical characteristics are of note in the group of patients studied. The first is that the mean age of patients was around eight years younger than the age of patients with IPAH or HPAH in other published series [24, 25], although clinical and hemodynamic criteria for inclusion in the study were similar. It is difficult to speculate on the reasons for such a difference, since both the environmental and genetic background were different in these studies, but it shows the severity of the disease occurring in the Chinese Han population, and suggests an increased pressure of geographic and population specific factors, in conjunction with known genetic factors when *BMPR2* mutations are present, or unknown in the case of IPAH. In addition, HPAH patients are about 14 years younger than the IPAH patients, a difference in the same range as found in other studies [25]. Considering the *BMPR2* mutation rate is 53.3% and 14.5% in HPAH and IPAH respectively, the inherited risk may be the main factor of earlier detection among HPAH patients. Another striking result is the difference in the proportion of

mutation carriers between males and females, since 25.3% of males were mutation carriers and only 12.8% of females (P=0.008). This proportion contrasts with results reported by Girerd et al [26] where the proportion of mutated patients was similar in both sexes and was 30.1%, close to the proportion found in male Chinese patients. A differential effect on embryo survival was proposed to explain the female/male ratio of PAH, but results in previously published series did not favor such a hypothesis since the proportion of female and male carriers was found to be similar. In our study, the significant difference in the proportion of female carriers and male carriers (Table 6) would suggest two different hypotheses: either there is a female specific etiological factor for PAH, decreasing the relative proportion of *BMPR2* carriers among female PAH patients, or that female embryos carrying the mutation would be disadvantaged either by meiotic drive or later in embryo development, an hypothesis at discrepancy with previously proposed hypotheses.

It has been reported that the age at diagnosis is around 10 years earlier in PAH patients with mutations than in those without mutations in the *BMPR2* gene [25], but in a smaller series of patients, Austin et al [24], found that the younger age at diagnosis in mutation carriers was confined to female patients. In our series of patients, we confirmed that the mean age at diagnosis is around 6 years younger in mutation carriers than in non-carriers, but while the relationship was significant in females, there was only a tendency present in males. Therefore, since the age difference is also present in male carriers, the lack of statistical power due to the smaller size of the male group is the most likely explanation.

In conclusion, our study reports an extensive molecular investigation of the *BMPR2* gene in Chinese patients with PAH. The overall genetics of PAH in Chinese patients was similar to that of other populations already explored for this disease, although few characteristics were noteworthy. We found polymorphisms of the *BMPR2* gene which are important to know in the context of genetic investigation of the disease in Chinese patients. The predisposing effect of the *BMPR2*

gene was similar to that of other populations, and the younger age at diagnosis suggests that the *BMPR2* mutation constitutes the first hit that increases the probability of an early onset of the disease. This observation is similar to hereditary predisposition to cancer, exemplified by the two-hit hypothesis proposed by Knudson for tumor suppressor genes, which corresponds to a germ-line loss of function for one allele and a somatic loss of the second allele [27]. In the case of PAH, the second event might be environmental as well as genetic, as suggested by somatic chromosomal abnormalities found in the lungs of PAH subjects[28], or epigenetic, potentially leading to decreased expression of the normal *BMPR2* allele[29].

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References

1. Rich S, Dantzker DR, Ayres SM, *et al.* Primary pulmonary hypertension. A national prospective study. *Ann Intern Med* 1987; 107: 216-223.
2. Sztrymf B, Yaici A, Girerd B, *et al.* Genes and pulmonary arterial hypertension. *Respiration* 2007; 74: 123-132.
3. Humbert M, Sitbon O, Chaouat A, *et al.* Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med* 2006; 173: 1023-1030.

4. Loyd JE, Butler MG, Foroud TM, *et al.* Genetic anticipation and abnormal gender ratio at birth in familial primary pulmonary hypertension. *Am J Respir Crit Care Med* 1995; 152: 93-97.
5. Deng Z, Morse JH, Slager SL, *et al.* Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 2000; 67: 737-744.
6. Lane KB, Machado RD, Pauciulo MW, *et al.* Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet* 2000; 26: 81-84.
7. Thomson JR, Machado RD, Pauciulo MW, *et al.* 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* 2000; 37: 741-745.
8. Morisaki H, Nakanishi N, Kyotani S, *et al.* BMPR2 mutations found in Japanese patients with familial and sporadic primary pulmonary hypertension. *Hum Mutat* 2004; 23: 632.
9. Simonneau G, Robbins IM, Beghetti M, *et al.* Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2009; 54: S43-S54.
10. Massague J, Chen YG. Controlling TGF-beta signaling. *Genes Dev* 2000; 14: 627-644.
11. Adachi-Yamada T, Nakamura M, Irie K, *et al.* p38 mitogen-activated protein kinase can be involved in transforming growth factor beta superfamily signal transduction in Drosophila wing morphogenesis. *Mol Cell Biol* 1999; 19: 2322-2329.
12. Simonneau G, Galie N, Rubin LJ, *et al.* Clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2004; 43: 5S-12S.
13. Sunyaev S, Ramensky V, Koch I, *et al.* Prediction of deleterious human alleles. *Hum Mol Genet* 2001; 10: 591-597.
14. Machado RD, Aldred MA, James V, *et al.* Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat* 2006; 27: 121-132.
15. Machado RD, Pauciulo MW, Thomson JR, *et al.* BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. *Am J Hum Genet* 2001; 68: 92-102.
16. Machado RD, Eickelberg O, Elliott CG, et al. Genetics and genomics of pulmonary arterial hypertension. *J Am Coll Cardiol* 2009; 54: S32-S42.

17. Wang H, Cui QQ, Sun K, *et al.* Identities and frequencies of BMPR2 mutations in Chinese patients with idiopathic pulmonary arterial hypertension. *Clin Genet* 2010; 77: 189-192.
18. Cogan JD, Vnencak-Jones CL, Phillips JR, *et al.* Gross BMPR2 gene rearrangements constitute a new cause for primary pulmonary hypertension. *Genet Med* 2005; 7: 169-174.
19. Aldred MA, Vijayakrishnan J, James V, *et al.* BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. *Hum Mutat* 2006; 27: 212-213.
20. Cogan JD, Pauciulo MW, Batchman AP, *et al.* High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2006; 174: 590-598.
21. Trembath RC, Thomson JR, Machado RD, *et al.* Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med* 2001; 345: 325-334.
22. Rudarakanchana N, Flanagan JA, Chen H, *et al.* Functional analysis of bone morphogenetic protein type II receptor mutations underlying primary pulmonary hypertension. *Hum Mol Genet* 2002; 11: 1517-1525.
23. Hanks SK, Hunter T. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *Faseb J* 1995; 9: 576-596.
24. Austin ED, Phillips JA, Cogan JD, *et al.* Truncating and missense BMPR2 mutations differentially affect the severity of heritable pulmonary arterial hypertension. *Respir Res* 2009; 28: 87.
25. Sztrymf B, Coulet F, Girerd B, *et al.* Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. *Am J Respir Crit Care Med* 2008; 177: 1377-1383.
26. Girerd B, Montani D, Eyries M, *et al.* Absence of influence of gender and BMPR2 mutation type on clinical phenotypes of pulmonary arterial hypertension. *Respir Res* 2010; 10: 73.
27. Jr Knudson AG. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A* 1971; 68: 820-823.
28. Aldred MA, Comhair SA, Varella-Garcia M, *et al.* Somatic chromosome abnormalities in the lungs of patients with pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2010; 182: 1153-1160.
29. Hamid R, Cogan JD, Hedges LK, *et al.* Penetrance of pulmonary arterial hypertension is modulated by the expression of normal BMPR2 allele. *Hum Mutat* 2009; 30: 649-654.

Tables

TABLE 1. Clinical characteristics of the study participants at the time of diagnosis[*]

	All (n=305)	HPAH (n=15)	IPAH (n=290)	$P^{\dagger}$
Age, years	33±16	20±10	34±16	<0.001
Sex, M/F	87/218	6/9	81/209	0.379[‡]
Height, cm	158±17	153±25	158±17	0.215
Weight, kg	54±16	48±20	55±16	0.125
6MWD, meters	381±113	386±119	381±113	0.893
mPAP, mm Hg	62±17	71±15	62±17	0.053
mPCWP, mm Hg	8±5	9±4	8±5	0.870
PVR, Wood Units	17±10	23±12	17±10	0.042
CO, L/min	3.8±1.5	3.2±1.4	3.8±1.5	0.271
CI, $L \bullet min^{-1} \bullet m^{-2}$	2.5±1.0	2.6±1.5	2.5±1.0	0.598

[*] Values are means±SD.

[†] *P* values represent the results of independent Student t-tests comparing HPAH and IPAH patients, with $P<0.05$ chosen as the level of significance.

[‡] Fisher exact test for the comparison of gender for HPAH and IPAH.

mPAP = mean pulmonary arterial pressure; mPCWP = mean pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; CO = cardiac output; CI = cardiac index.

TABLE 2. *BMPR2* mutations in Chinese patients with idiopathic and heritable PAH

ID	IPAH or HPAH	Location	Domain	Nucleotide Change*	Amino Acid Change†	Mutation Type	Reference
64	IPAH	5'UTR		c.-223A>G	p.?	VUS	This study
63	IPAH	5'UTR		c.-310A>G	p.?	VUS	This study
403	IPAH	exon1		c.21delG	p.Trp9GlyfsX38	Frameshift	This study
375	IPAH	exon1		c.27G>A	p.Trp9X	Nonsense	[14]
406	IPAH	exon1		c.48G>A	p.Trp16X	Nonsense	[25]
344	IPAH	intron1		c.77-36_85del	p.?	Splice defect	This study
307	IPAH	exon2	ECD	c.88C>T	p.Gln30X	Nonsense	This study
336	IPAH	exon2	ECD	c.103delG	p.Ala35ArgfsX12	Frameshift	This study
1143	IPAH	exon2	ECD	c.178T>G‡	p.Cys60Gly	Missense	This study
1016	IPAH	exon3	ECD	c.251G>T‡	p.Cys84Phe	Missense	This study
1063	HPAH	exon3	ECD	c.266G>C	p.Gly89Ala	Missense	This study
1001	IPAH	exon3	ECD	c.338A>G‡	p.Tyr113Cys	Missense	This study
1127	IPAH	exon3	ECD	c.339C>A	p.Tyr113X	Nonsense	[16]
994	IPAH	exon3	ECD	c.349T>C‡	p.Cys117Arg	Missense	This study
265	IPAH	exon3	ECD	c.377A>G	p.Asn126Ser	Missense	[16]
467	HPAH	exon4	ECD	c.439C>T	p.Arg147X	Nonsense	[8]
468	HPAH	exon4	ECD	c.439C>T	p.Arg147X	Nonsense	[8]
913	IPAH	exon6	KD	c.830T>C	p.Leu227Pro	Missense	[25]
498	IPAH	intron7	KD	c.967+5G>C	p.?	VUS	This study
370	IPAH	exon8	KD	c.969T>C	p.Asp323Asp	Synonymous	This study
209	IPAH	exon8	KD	c.994C>T	p.Arg332X	Nonsense	[7, 14],[15]
28	IPAH	exon8	KD	c.1042G>A	p.Val348Ile	Missense	[16],[17]
94	IPAH	exon8	KD	c.1042G>A	p.Val348Ile	Missense	[16],[17]
711	IPAH	exon8	KD	c.1093_1098delinsG	p.Arg365GlyfsX5	Frameshift	This study

534	IPAH	exon8	KD	c.1117G>C	p.Ala373Pro	Missense	This study
326	IPAH	intron8	KD	c.1129-3C>G	p.?	Splice defect	[14],[15]
141	IPAH	exon9	KD	c.1175T>C	p.Val392Ala	Missense	[17]
415	HPAH	exon9	KD	c.1228G>A‡	p.Gly410Arg	Missense	This study
1174	IPAH	exon9	KD	c.1259G>A	p.Cys420Tyr	Missense	[8]
1012	IPAH	exon10	KD	c.1371dup	p.Gln458ThrfsX13	Frameshift	This study
65	IPAH	exon11	KD	c.1471C>T	p.Arg491Trp	Missense	[5]
438	IPAH	exon11	KD	c.1471C>T	p.Arg491Trp	Missense	[5]
295	HPAH	exon11	KD	c.1471C>T	p.Arg491Trp	Missense	[5]
404	HPAH	exon11	KD	c.1471C>T	p.Arg491Trp	Missense	[5]
962	HPAH	exon11	KD	c.1471C>T	p.Arg491Trp	Missense	[5]
869	IPAH	exon11	KD	c.1472G>A	p.Arg491Gln	Missense	[5]
1382	IPAH	exon11	KD	c.1472G>A	p.Arg491Gln	Missense	[5]
379	IPAH	exon12	CD	c.2296A>G	p.Thr766Ala	Missense	This study
853	IPAH	exon12	CD	c.2446_2447dup	p.Asn817Leufsx23	Frameshift	This study
52	IPAH	exon12	CD	c.2503_2506del	p.Thr835ProfsX3	Frameshift	This study
499	IPAH	exon12	CD	c.2617C>T	p.Arg873X	Nonsense	[5],[14] [6],[7],[8],
432	IPAH	exon12	CD	c.2695C>T	p.Arg899X	Nonsense	[14],[15]
81	HPAH	exon13	CD	c.3117+12G>A	p.?	VUS	This study

*Abbreviations are in accord with nomenclature guidelines as recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). The letter c. indicates coding DNA, where nucleotide 1 is the A of the ATG translation initiation codon.

† The letter p. is used to indicate the change at the protein level.

‡ Missense mutation predicted to be probably damaging by polyphen software.

IPAH = idiopathic PAH; HPAH = heritable PAH; UTR = untranslated region; ECD = extracellular domain; KD = kinase domain; CD = cytoplasmic domain; VUS = variant of unknown significance.

TABLE 3. PAH patients identified with *BMPR2* rearrangements

ID	Diagnosis	Rearrangement*	Reference
1284	IPAH	Dup BMPR2 exon 2-3 c.77-?_c.421+?dup	This study
74	IPAH	Dup BMPR2 exon 2-7 c.77-?_c.967+?dup	This study
361	IPAH	Dup BMPR2 exon 8-10 c.968-?_c.1413+?dup	This study
294	IPAH	Dup BMPR2 exon 10 c.1277-?_c.1413+?dup	This study
860	IPAH	Del BMPR2 exon 10 c.1277-?_c.1413+?del	[19]
1247	IPAH	Del BMPR2 exon 10 c.1277-?_c.1413+?del	[19]
895	IPAH	Del BMPR2 exon 11-13 c.1414-?_c.3117+?del	[21]

*Abbreviations are in accord with nomenclature guidelines as recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). Del indicates deletion; Dup indicates duplication; the letter c. indicates coding DNA where nucleotide 1 is the A of the ATG translation initiation codon.

IPAH = idiopathic PAH.

TABLE 4. Categories of *BMPR2* mutations found in Chinese patients with idiopathic and heritable PAH

Patient	Missense	Nonsense	Frameshift	SpliceSite	Synonymous	VUS	RGT	Total
IPAH	16	7	6	2	1	3	7	42
HPAH	5	2	0	0	0	1	0	8
Total	21	9	6	2	1	4	7	50
Percentage	42%	18%	12%	4%	2%	8%	14%	100%

IPAH = idiopathic PAH; HPAH = heritable PAH; VUS = variant of unknown significance.

TABLE 5. Mean age at PAH diagnosis in females and males according to *BMPR2* mutation status*

Gender	Mutation carrier	Non-mutation carrier	$P^{\dagger}$
Females [age, y (n)]	29±12 (28)	35±16 (190)	0.036
Males [age, y (n)]	27±10 (22)	32±19 (65)	0.071
Total [age, y (n)]	28±11 (50)	34±17 (255)	0.002

* Values are means±SD.

† *P* values represent the results of independent Student t-tests comparing mutation carriers and non-mutation carriers, with $P<0.05$ chosen as the level of significance.

TABLE 6. Gender difference between mutation carriers and non-carriers in the IPAH and HPAH cohorts

Gender	Mutation carriers	Non-mutation carriers
Females [n (%)]	28 (12.8%)*	190 (87.2%)
Males [n (%)]	22 (25.3%)	65 (74.7%)
Total [n (%)]	50 (16.4%)	255 (83.6%)

* $P < 0.01$ versus male mutation carriers (χ^2 test).