



# Molecular genetics and clinical features of Chinese idiopathic and heritable pulmonary arterial hypertension patients

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**ABSTRACT:** Mutations of the bone morphogenetic protein type II receptor (*BMPR2*) gene predispose to pulmonary arterial hypertension (PAH). 290 idiopathic (I)PAH patients and 15 heritable (H)PAH were screened to determine the spectrum and rate of *BMPR2* mutations in a large Chinese patient group.

Gene sequencing and multiplex ligation-dependent probe amplification (MLPA<sup>®</sup>) were used to detect sequence mutations and large rearrangements (RGTs). Total mutation rate was 14.5% (n=42 out of 290) in Chinese IPAH patients, and 53.3% (n=8 out of 15) in HPAH patients. RGT mutation rate was 3.1% (n=7 out of 229) and represented 14% (n=7 out of 50) of all identified mutations. 25 *BMPR2* mutations were newly identified.

Patients in this study were younger than other reported PAH subjects. *BMPR2* mutation carriers were ~6 yrs younger at diagnosis than noncarriers (p=0.002), but this relationship was significant only in the female group, which was larger. The proportion of females carrying a *BMPR2* mutation was half that of males (12.8% versus 25.3%; p=0.008).

Our results indicate that the overall genetics of Chinese PAH patients is similar to that of other populations, but the clinical picture differs by the precocity of the disease in the whole patient group, and the lower proportion of females found to carry a *BMPR2* mutation.

**KEYWORDS:** *BMPR2* mutation, genetics, pulmonary circulation

**P**ulmonary arterial hypertension (PAH) is a rare and potentially fatal disorder characterised 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 recognised family history, in which the disease segregates as an autosomal dominant trait with incomplete penetrance and an estimated lifetime risk of 10–20% [2]. The disease is more frequent in females, 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 recognised and mutations in the bone morphogenetic protein type II receptor (*BMPR2*) gene (*BMPR2*) have been recognised to cause heritable (H)PAH [5, 6]. Subsequent studies have reported >250 *BMPR2* mutations responsible for 55–70% of heritable

(H)PAH, and 11–40% of sporadic idiopathic (I)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 type II receptor, a member of the transforming growth factor (TGF)- $\beta$  cell signalling 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 mitogen-activated protein kinases (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.

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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 aetiology 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, Shanghai, China, between January 1, 2006 and August 31, 2010. 290 IPAH and 15 HPAH patients were tested for *BMPR2* point mutations and large size rearrangements. The diagnosis of IPAH required the presence of elevated mean pulmonary artery pressure ( $\bar{P}_{pa} > 25$  mmHg, with a pulmonary capillary wedge pressure  $\leq 15$  mmHg measured by right heart catheterisation at rest), and the exclusion of other disorders known to cause pulmonary hypertension by clinical evaluation and objective tests. HPAH was recognised 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 DNA analyser (Applied Biosystems, Carlsbad, CA, USA) was adopted to detect 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'- and 3'-untranslated regions of the *BMPR2* gene (see online supplementary table 1). The results were compared with the reference sequence of the *BMPR2* gene (RefSeq 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 [13]. 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, Amsterdam, The Netherlands), according to the manufacturer's instructions. Samples were analysed on an ABI 377 fluorescent analyser with GeneScan and GenoTyper software (Applied Biosystems, Warrington, UK; www.applied-biosystems.com). RGTs were analysed by the Coffalyser software (MRC-Holland BV). 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 analysed for RGTs ( $n=255$ ). 232 results (229 IPAH and three 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 seven 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 Chi-squared, Fisher's exact t-tests, where appropriate. A p-value of  $<0.05$  was considered to indicate statistical significance. Polyphen-2 software (<http://genetics.bwh.harvard.edu/pph2>) was used for *in silico* analysis of coding variants, and splice site variants were analysed by Splice Site Finder, MaxEntScan, Nnsplise and Genesplicer, all available with Alamut v1.5 software (Interactive Biosoftware, Rouen, France).

## 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); 87 were male. The mean  $\pm$  SD age of the patients was  $33 \pm 16$  (range 1–77) yrs. HPAH patients were  $\sim 14$  yrs younger than those with IPAH ( $20 \pm 10$  versus  $34 \pm 16$  yrs;  $p < 0.001$ ).

### Mutation rate and distribution

Mutation analysis in HPAH and IPAH is summarised in tables 2 and 3. A total of 50 mutations were identified, accounting for eight out of the 15 patients with HPAH (53.3%) and 42 out of the 290 patients with IPAH (14.5%). 25 mutations are reported for the first time.

49 out of the 50 patients had nonsynonymous *BMPR2* variations (table 2). Classification of these variants is shown in table 4.

Four variants were found in at least two unrelated patients. Exon 10 deletion of *BMPR2* was found in two IPAH patients and

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

	All	HPAH	IPAH	p-value <sup>#</sup>
Subjects n	305	15	290	
Age yrs	$33 \pm 16$	$20 \pm 10$	$34 \pm 16$	$<0.001$
Sex male/female n	87/218	6/9	81/209	0.379 <sup>*</sup>
Height cm	$158 \pm 17$	$153 \pm 25$	$158 \pm 17$	0.215
Weight kg	$54 \pm 16$	$48 \pm 20$	$55 \pm 16$	0.125
6MWD m	$381 \pm 113$	$386 \pm 119$	$381 \pm 113$	0.893
$\bar{P}_{pa}$ mmHg	$62 \pm 17$	$71 \pm 15$	$62 \pm 17$	0.053
$\bar{P}_{pcw}$ mmHg	$8 \pm 5$	$9 \pm 4$	$8 \pm 5$	0.870
PVR Wood units	$17 \pm 10$	$23 \pm 12$	$17 \pm 10$	0.042
CO L·min <sup>-1</sup>	$3.8 \pm 1.5$	$3.2 \pm 1.4$	$3.8 \pm 1.5$	0.271
CI L·min <sup>-1</sup> ·m <sup>-2</sup>	$2.5 \pm 1.0$	$2.6 \pm 1.5$	$2.5 \pm 1.0$	0.598

Data are presented as mean  $\pm$  SD, unless otherwise stated. <sup>#</sup>: p-values represent the results of independent unpaired t-tests comparing heritable (H) and idiopathic (I) pulmonary arterial hypertension (PAH) patients, with  $p < 0.05$  chosen as the level of significance; <sup>\*</sup>: Fisher's exact test for the comparison of sex for HPAH and IPAH. 6MWD: 6-min walk distance;  $\bar{P}_{pa}$ : mean pulmonary arterial pressure;  $\bar{P}_{pcw}$ : mean pulmonary capillary wedge pressure; PVR: pulmonary vascular resistance; CO: cardiac output; CI: cardiac index.

**TABLE 2** *BMPR2* mutations in Chinese patients with idiopathic (I) and heritable (H) pulmonary arterial hypertension (PAH)

Identification number	IPAH or HPAH	Location	Domain	Nucleotide change <sup>#</sup>	Amino acid change <sup>†</sup>	Mutation type	[Ref.]
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	[15]
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 <sup>+</sup>	p.Cys60Gly	Missense	This study
1016	IPAH	exon3	ECD	c.251G>T <sup>+</sup>	p.Cys84Phe	Missense	This study
1063	HPAH	exon3	ECD	c.266G>C	p.Gly89Ala	Missense	This study
1001	IPAH	exon3	ECD	c.338A>G <sup>+</sup>	p.Tyr113Cys	Missense	This study
1127	IPAH	exon3	ECD	c.339C>A	p.Tyr113X	Nonsense	[16]
994	IPAH	exon3	ECD	c.349T>C <sup>+</sup>	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	[15]
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, 17]
28	IPAH	exon8	KD	c.1042G>A	p.Val348Ile	Missense	[16, 18]
94	IPAH	exon8	KD	c.1042G>A	p.Val348Ile	Missense	[16, 18]
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, 17]
141	IPAH	exon9	KD	c.1175T>C	p.Val392Ala	Missense	[18]
415	HPAH	exon9	KD	c.1228G>A <sup>+</sup>	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]
432	IPAH	exon12	CD	c.2695C>T	p.Arg899X	Nonsense	[6, 7, 8, 14, 17]
81	HPAH	exon13	CD	c.3117+12G>A	p.?	VUS	This study

<sup>#</sup>: abbreviations are in accord with nomenclature guidelines as recommended by the Human Genome Variation Society [13]; the letter c. indicates coding DNA, where nucleotide 1 is the A of the ATG translation initiation codon; <sup>†</sup>: the letter p. is used to indicate the change at the protein level; <sup>+</sup>: missense mutation predicted to be probably damaging by Polyphen-2 software. UTR: untranslated region; ECD: extracellular domain; KD: kinase domain; CD: cytoplasmic domain; VUS: variant of unknown significance.

has already been reported, but the breakpoints were not determined. The remaining 37 patients had unique *BMPR2* mutations.

These mutations are distributed from the 5'-UTR to exon 13 of *BMPR2*. No mutations in exon 5 or exon 7 were found either in the IPAH or the HPAH cohort.

### Point mutations

15 out of the 43 point mutations were either frameshift (n=6) or nonsense (n=9) mutations that were predicted to result in a truncated *BMPR2* molecule. All six frameshift mutations were newly identified insertions or deletions. Among the 21 missense mutations, eight were novel mutation sites. The effect on protein function was predicted with PolyPhen-2 [21].

**TABLE 3** Pulmonary arterial hypertension (PAH) patients identified with *BMPR2* rearrangements

Identification number	Diagnosis	Rearrangement <sup>#</sup>	[Ref.]
1284	IPAH	Dup <i>BMPR2</i> exon 2-3 c.77-?_c.421+?dup	This study
74	IPAH	Dup <i>BMPR2</i> exon 2-7 c.77-?_c.967+?dup	This study
361	IPAH	Dup <i>BMPR2</i> exon 8-10 c.968-?_c.1413+?dup	This study
294	IPAH	Dup <i>BMPR2</i> exon 10 c.1277-?_c.1413+?dup	This study
860	IPAH	Del <i>BMPR2</i> exon 10 c.1277-?_c.1413+?del	[19]
1247	IPAH	Del <i>BMPR2</i> exon 10 c.1277-?_c.1413+?del	[19]
895	IPAH	Del <i>BMPR2</i> exon 11-13 c.1414-?_c.3117+?del	[20]

<sup>#</sup>: abbreviations are in accord with nomenclature guidelines as recommended by the Human Genome Variation Society [13]. IPAH: idiopathic PAH; Del/del: deletion; Dup/dup: duplication; c.: coding DNA where nucleotide 1 is the A of the ATG translation initiation codon.

Five mutations (table 2) were predicted to be probably damaging, while the remaining three mutations (c.266G>C, c.1117G>C and 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, 17], 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 in 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 analysed by splice-site prediction programs. A possible effect on splicing was predicted for these two variants (see online supplementary table 2). However, further analyses on RNA are needed to draw a conclusion on the deleterious effect of these variants.

### Large rearrangements

255 IPAH patients for whom no mutation was detected by sequencing were analysed by MLPA<sup>®</sup>. Only 229 results were readable among the 255 IPAH patients, and seven RGTs were found. The RGT mutation rate is 3.1% in IPAH patients who had no sequencing mutations (seven out of 229 IPAH patients). Among 15 HPAH patients, eight were found with mutation by sequencing and seven were analysed by MLPA<sup>®</sup>. Only three DNAs from seven HPAH patients could be analysed, and none was found with RGT mutation. Among these seven 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 (online supplementary table 3). Polymorphism c.1-93A>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 analysed 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-2 software.

### Age at diagnosis in mutation carriers and noncarriers

The mean age at diagnosis was compared in carriers and noncarriers of mutations and was found to be significantly lower in carriers (28±11 yrs, n=50) than in noncarriers (34±17 yrs, n=255) (p<0.002). The mean age at onset was found to be significantly lower in females carrying a *BMPR2* mutation (29±12 yrs, n=28) than in those without mutation (35±16 yrs, n=190; p=0.036). In the male group, which was smaller, the age difference between male carriers (27±10 yrs, n=22) and noncarriers (32±19 yrs, 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

**TABLE 4** Categories of *BMPR2* mutations found in Chinese patients with idiopathic (I) and heritable (H) PAH

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

Data are presented as n or n (%). VUS: variant of unknown significance; RGT: rearrangement.



**TABLE 5** Mean age at pulmonary arterial hypertension diagnosis in females and males according to *BMPR2* mutation status

Age yrs	Mutation carrier	Nonmutation carrier	p-value <sup>#</sup>
<b>Female</b>	29 ± 12 (28)	35 ± 16 (190)	0.036
<b>Male</b>	27 ± 10 (22)	32 ± 19 (65)	0.071
<b>Total</b>	28 ± 11 (50)	34 ± 17 (255)	0.002

Data are presented as mean ± SD (n), unless otherwise indicated. <sup>#</sup>: p-values represent the results of independent unpaired t-tests comparing mutation and nonmutation carriers, with p < 0.05 chosen as the level of significance.

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 IPAH [7, 8]. In this study, we found mutation rates in Chinese Han IPAH and HPAH 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.* [18] who reported 12 mutations in 72 Chinese IPAH patients (16.7%) without searching for RGT mutations by MLPA®.

It is worth noting that the proportion of mutations found in groups of PAH patients 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 codons to the *BMPR2* open-reading frame in the present study encompass nonsense (nine out of 50; 18%), frameshift (six out of 50; 12%), splice site (two out of 50; 4%) and gene duplications/deletions (seven out of 50; 14%). The total proportion of mutations found to cause premature termination was 48%, much less than the 68% reported in 2009 by MACHADO *et al.* [16].

We used MLPA® to detect large gene RGTs which underlie a significant proportion of HPAH and IPAH cases [19, 22]. In our study, the proportion of RGTs was 14% of all the mutation categories, and they were present in 3.1% (seven out 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.* [19] who reported a proportion of 12% of RGTs in HPAH (n=58 families) and 5% in IPAH (n=126 patients), but are lower than the proportion reported by COGAN *et al.* [23]: 33% in a smaller series of families (n=12). Among these mutations, the deletion of *BMPR2* exon 10 and exons 11–13 have been previously reported [19, 20], 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 favour of a deleterious effect, based on splice-site software predictions, but the consequences on mRNA maturation were not tested *in vitro* in this study. 21 missense

mutations of *BMPR2* found in Chinese IPAH and HPAH clusters were confined to exons 2, 3, 6, 8 to 9, 11 and 12. Exons 2 and 3 encode the extracellular ligand-binding domain, essential for signalling activity of *BMPR2*, which adopts a precisely folded conformation, exquisitely dependent on the formation of five disulphide bridges by 10 cysteine residues dispersed across exons 2 and 3. Mutations were found on cysteines 60, 84 and 117, affecting three out of the 10 conserved cysteine 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, which is likely to be due to a profound loss of conformational integrity [24]. The kinase region encoded by exons 5–11 harbours 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 compartmentalised into 12 subdomains of variable importance for the processes of adenosine triphosphate binding, substrate recognition and phosphate group transfer [25]. X-ray crystallography revealed that a highly conserved 12 invariant amino acids motif lies within the ~250–300 amino acid core. We found several missense mutations involving key residues, such as Arg 491 and Cys 420, which are essential catalytic residues and their mutations cause a near complete abolition of signalling through the Smad pathway [24].

The variant c.-93A>G in the 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 HPAH and IPAH, as mutations identified are likely to inactivate the receptor [17]. Additional clinical characteristics are of note in the group of patients studied. The first is that the mean age of patients was around 8 yrs younger than the age of patients with IPAH or HPAH in other published series [15, 26], although clinical and haemodynamic 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 geographically 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 ~14 yrs younger than the IPAH patients, a difference in the same range as that found in other studies [15]. Considering that the *BMPR2* mutation rate is 53.3% and 14.5% in HPAH and IPAH, respectively, the inherited risk may be the main factor driving 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.* [27], where the proportion of mutated patients was similar in both sexes and was 30.1%, close to the proportion found in male

**TABLE 6** Sex difference between mutation carriers and noncarriers in the idiopathic and hereditary pulmonary arterial hypertension cohorts

Sex	Mutation carriers	Nonmutation carriers
Female	28 (12.8)**	190 (87.2)
Male	22 (25.3)	65 (74.7)
Total	50 (16.4)	255 (83.6)

Data are presented as n (%). \*\*:  $p < 0.01$  versus male mutation carriers (Chi-squared test).

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 favour such a hypothesis as 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: 1) there is a female-specific aetiological factor for PAH, decreasing the relative proportion of *BMPR2* carriers among female PAH patients, or 2) 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 ~10 yrs earlier in PAH patients with mutations than in those without mutations in the *BMPR2* gene [15], but in a smaller series of patients, AUSTIN *et al.* [26], 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 ~6 yrs younger in mutation carriers than in noncarriers, 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 for this discrepancy.

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 a few characteristics were noteworthy: we found polymorphisms of the *BMPR2* gene that 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 [28] for tumour suppressor genes, which corresponds to a germ-line loss of function for one allele and a somatic loss of the second allele. In the case of PAH, the second event might be, as well as genetic, either environmental as suggested by somatic chromosomal abnormalities found in the lungs of PAH subjects [29], or epigenetic, potentially leading to decreased expression of the normal *BMPR2* allele [30].

## SUPPORT STATEMENT

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## STATEMENT OF INTEREST

A statement of interest for Z-C. Jing can be found at [www.erj.ersjournals.com/site/misc/statements.xhtml](http://www.erj.ersjournals.com/site/misc/statements.xhtml)

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