BMPR2 Mutations Influence Phenotype More Obviously in Male Patients with Pulmonary Arterial Hypertension

Running title: Liu et al.; Genotype-phenotype Relationship in Patients with PAH

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Abstract:

Background - BMPR2 mutations predispose to idiopathic and heritable pulmonary arterial hypertension (IPAH and HPAH). The influence of BMPR2 mutations on clinical outcome is not concordant in different ethnic groups. Although the BMPR2 mutation spectrum and mutation rate in Chinese PAH patients has been reported previously, the influence of genotype on phenotype and whether this influence is associated with sex has not been investigated.

Methods and Results - We analyzed data from 305 PAH patients considered as either idiopathic or heritable who underwent genetic counseling in Shanghai Pulmonary Hospital. The clinical, functional, and hemodynamic characteristics of BMPR2 mutation carriers and noncarriers were compared. The more severe hemodynamic compromise at diagnosis in BMPR2 mutation carriers versus noncarriers is concordant with other ethnic groups. In the Chinese PAH cohort, BMPR2 mutations were associated with a higher risk of mortality after adjustment for age and sex [HR=1.971; 95% CI 1.121-3.466; P = 0.018]. The overall survival difference between mutation carriers and noncarriers was more obvious in male patients, which was reflected by a higher mortality risk of male mutation carriers than that of male noncarriers after adjustment for age at diagnosis [HR = 3.702; 95% CI 1.416-9.679; P = 0.008]. In females, this trend did not reach statistical significance.

Conclusions - BMPR2 mutations influence phenotype more obviously in male PAH patients. The etiology of female PAH patients is more complicated and the influence of BMPR2 mutations may be modified by other unknown factors, making disparities in the prognosis between female mutation carriers and noncarriers less evident.

Key words: genetics; hemodynamics; survival; Pulmonary arterial hypertension
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.\textsuperscript{1,2} Mutations in the bone morphogenetic protein type II receptor (BMPR-II) gene (\textit{BMPR2}) have been recognized to cause heritable PAH (HPAH).\textsuperscript{3,4} Subsequent studies have reported more than 250 \textit{BMPR2} mutations responsible for 55\% to 70\% of heritable PAH, and 11\% to 40\% of idiopathic PAH (IPAH).\textsuperscript{5-9} \textit{BMPR2} encodes the bone morphogenetic protein receptor II, which is a member of the transforming growth factor-beta (TGF-\textbeta) cell signaling superfamily.\textsuperscript{3,4,6} 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).\textsuperscript{10,11} This pathway appears to be critical in both cell differentiation and growth through transcriptional regulation of target genes.

The influence of \textit{BMPR2} mutations on clinical outcomes in patients with PAH have been described recently.\textsuperscript{12-14} \textit{BMPR2} mutation carriers present approximately 10 years earlier than noncarriers, and have more severe hemodynamic compromise at diagnosis.\textsuperscript{14} Austin et al.\textsuperscript{13} reported that an earlier age at diagnosis was only significant in the female mutation carriers, and that patients with missense mutations have more severe disease than those with truncating mutations. In a French cohort, the absence of an influence of sex and \textit{BMPR2} mutation type on clinical phenotypes of PAH was observed.\textsuperscript{12}

Following the demonstration of 50 \textit{BMPR2} mutations, including 25 novel mutation sites, in 305 Chinese IPAH and HPAH patients,\textsuperscript{15} we analyzed their phenotype-genotype relationship.
The aim was to investigate the influence of *BMPR2* mutations on the clinical trait and whether this influence is associated with sex.

**Methods**

**Study Patients**

Patients with IPAH or HPAH were consecutively recruited at the Shanghai Pulmonary Hospital from January 1, 2006 to August 31, 2010. A total of 290 IPAH patients and 15 HPAH patients, all of whom belonged to the Chinese Han population, were recruited and participated in the genetic study. The diagnosis of IPAH required the presence of an elevated mean pulmonary artery pressure >25 mmHg and a pulmonary capillary wedge pressure ≤15 mmHg measured by right heart catheterization at rest, and the exclusion of other disorders known to cause pulmonary hypertension by clinical evaluation and objective tests.16,17 HPAH was recognized before genomic consulting if there was more than one confirmed case in first- to third-degree relatives in the family.18 Among the 305 patients recruited, 50 were found to have *BMPR2* mutations and were termed *BMPR2* mutation carriers, while the remainder were termed *BMPR2* mutation noncarriers. 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.

All patients were treated according to international guidelines.17,19 The start of follow-up was at the time of diagnosis of PAH, and the end of the follow-up was April 30, 2011. 17 patients were lost to follow-up, 4 of whom were *BMPR2* mutation carriers. These 17 patients were included in the analyses and censored at the time of the last follow-up.

**Hemodynamic Measurements and 6-Minute Walk Distance (6MWD) Test**

Hemodynamic evaluation by right heart catheterization was performed at baseline in all patients.
according to previously described protocols.\textsuperscript{20,21} The mean pulmonary artery pressure (mPAP), mean pulmonary capillary wedge pressure (mPCWP), mean right atrial pressure (mRAP), mean right ventricular pressure (mRVP), pulmonary vascular resistance (PVR), and mixed venous oxygen saturation (SvO\textsubscript{2}) were recorded during right heart catheterization. Cardiac output (CO) was measured by the standard thermodilution technique. The cardiac index (CI) was calculated as CO divided by body surface area, and systolic index as CI divided by cardiac frequency. Baseline hemodynamic data and the response to short-term vasodilator administration were determined in all subjects. For vasodilator testing, 5 \textmu g iloprost (Ventavis\textsuperscript{\textregistered}; Bayer-Schering Pharma, Berlin, Germany) was delivered by a PARI LC STAR nebulizer (PARI GmbH, Starnberg, Germany) driven by a PARI TurboBOY-N compressor (PARI GmbH) for 15 minutes.\textsuperscript{22} A positive acute vasodilator response was defined by current consensus guidelines, i.e., as a decrease in mPAP of at least 10 mmHg to a level \leq 40 mmHg with either no change or an increase in cardiac output.\textsuperscript{17,21}

A non-encouraged 6-minute walk distance (6MWD) test, performed according to the American Thoracic Society recommendations,\textsuperscript{23} was undertaken in all patients.

**Molecular Methods**

The genetic study was carried out according to previously reported methods.\textsuperscript{6,14} Patients were screened for \textit{BMPR2} mutation by direct sequencing and large size rearrangements (RGTs) detection.\textsuperscript{15} Direct screening using an ABI 3730 (Applied Biosystems, CA, USA) was adopted to detect the point mutations in the coding regions and the intron/exon boundaries of \textit{BMPR2}. The \textit{BMPR2} gene was screened for RGTs using the SALSA MLPA\textsuperscript{®} P093 HHT probe mix kit (MRC-Holland BV). Samples were analyzed on an ABI 377 fluorescent analyzer with GeneScan and GenoTyper software (Applied Biosystems, Warrington, UK;
RGTs were analyzed by the Coffalyser software provided on the manufacturer’s web site (http://www.mlpa.com).

All mutation detections and nomenclature were completed by two researchers independently. We used 237 normal Chinese Han people as controls to exclude the polymorphism.

**Statistical Analysis and Bioinformatic Tools**

Demographic characteristics (sex, body mass index) and clinical features (age at diagnosis, age at death, 6MWD, hemodynamic parameters, acute vasodilator responsiveness, and survival) were compared between *BMPR2* mutation carriers and noncarriers as appropriate with the use of a Chi-square test or Fisher's exact test, and Student's t-test or Mann-Whitney U-test. Clinical features that were not normally distributed such as age at diagnosis, age at death, mRVP, mPAP, PVR, and CI were described as medians and interquartile range (IQRs), while normally distributed features such as BMI and 6MWD were described as means (± standard deviation). Age at diagnosis, age at death, and survival were compared between male and female patients and among the different mutation classifications. All features were fitted for normal distribution, and homogeneity of variance was analyzed by Student’s t-test or one-way analysis of variance (ANOVA). Otherwise, a Mann-Whitney U-test was used. The 1-, 3-, and 5-year survival rates were estimated using Kaplan-Meier curves. There were 2 steps to the survival analysis. Firstly, a univariable Cox proportional regression analysis was used to estimate the hazard ratios (HRs) and the 95% confidence intervals (CIs) for the association between covariates (age, sex and *BMPR2* mutation status) and outcomes. In the second step of the survival analysis, a multivariable Cox regression model was used to estimate the hazard ratios and the 95% confidence intervals for the association between *BMPR2* mutation status and outcome adjusted for age at diagnosis and sex using noncarriers as the referent group in the total patient cohort or
stratified by sex. Survival between male and female patients and among the different mutation classifications were compared by a log rank test. A $P$ value of less than 0.05 was considered to indicate statistical significance.

**Results**

**Clinical and Functional Characteristics**

The baseline clinical and functional characteristics of the 50 BMPR2 mutation carriers were compared with those of the 255 noncarriers. The sex ratio of females to males was 2.5:1 ($n = 218/87$) in the total population. In BMPR2 mutation carriers, the female/male ratio was 1.3:1 ($n = 28/22$) while in noncarriers, the female/male ratio was 2.9:1 ($n = 190/65$). Mutation carriers had a younger median age at diagnosis (median, 28 yrs; IQR: (24, 47); $n=255$) than noncarriers (median, 32 yrs; IQR: (24, 47); $n=255$) [$P = 0.018$]. There was no significant difference in mutation carriers versus noncarriers ($P = 0.270$). The sex ratio of females to males was 2.5:1 ($n = 190/65$) in the total population. In comparison with noncarriers, BMPR2 mutation carriers were characterized by more severe hemodynamic compromise at diagnosis (Table 1), with a significantly higher mPAP [67 (56, 78) vs 60 (49, 69) mmHg; $P = 0.003$] and PVR [17.1 (13.4, 23.9) vs 14.6 (10.0, 20.3) Wood units; $P = 0.028$]. Although there was a trend for CI to be lower in mutation carriers, the difference versus noncarriers did not reach statistical significance.

Overall, 21 of the 305 patients (6.8%) showed an acute vasodilator response. Two of the 50 patients (4.0%) with a BMPR2 mutation demonstrated vasoreactivity as compared with 19 of 255
(7.5%) without BMPR2 variants. Therefore, while mutation carriers were less likely to exhibit a significant response to acute vasodilator testing than noncarriers, the difference between the groups did not reach statistical significance ($P = 0.546$).

**Survival**

In the total study population ($n = 305$), the 1-, 3-, and 5-year survival rates estimated by the Kaplan-Meier method were 92.4%, 77.5%, and 53.0%, respectively, and the mean survival time was $48.1 \pm 1.5$ months (mean ± standard error). The estimated 1-, 3-, and 5-year survival rates in the 255 BMPR2 noncarriers were 92.5%, 80.6%, and 56.4%, respectively, and the mean survival time was $49.4 \pm 1.6$ months. In the 50 BMPR2 carriers, the estimated 1- and 5-year survival rates were 89.8% and 63%, respectively, and the mean survival time was $39.4 \pm 2.8$ months (Figure 1). Mutation carriers had a younger median age at death (median age at death, 26 yrs; IQR: (20, 30); $n = 17$) than noncarriers (median age at death, 32 yrs; IQR: (26, 48); $n = 46$) [$P = 0.013$]. However, when patients were subgrouped according to sex, the difference in age at death between mutation carriers and noncarriers was only statistically significant in female patients.

In the univariable Cox proportional regression analysis, BMPR2 mutation status was significantly associated with the risk of death [$HR = 2.008; 95\% CI 1.149-3.506; P = 0.014$]. Age was not associated with the risk of death [$HR = 0.991; 95\% CI 0.975-1.008; P = 0.305$], and nor was sex associated with the risk of death [$HR = 0.876; 95\% CI 0.499-1.539; P = 0.646$], which is concordant to the survival analysis between male and female patients by Kaplan-Meier method (Figure 2). In the multivariable Cox regression analysis, BMPR2 mutation carrier status was associated with a higher risk of mortality after adjustment for age and sex [$HR = 1.971; 95\% CI 1.121-3.466; P = 0.018$; Table 2]. Thus, BMPR2 mutation carriers were more likely to die than noncarriers. Although sex was not risk factor of mortality in the univariable Cox
proportional regression analysis, stratification by sex showed that BMPR2 mutations influence clinical traits more clearly. In particular, male BMPR2 carriers were more significantly associated with an increased risk of death after adjustment for age at diagnosis (HR = 3.702; 95% CI 1.416-9.679; P = 0.008]. In females, the trend did not reach statistical significance (HR = 1.349; 95% CI 0.625-2.908; P = 0.446).

**Mutation Location and Categories of BMPR2 Mutations**

BMPR2 gene mutations among all carriers were detected within the extracellular (n = 19), kinase (n = 24), and cytoplasmic (n = 7) functional domains. There was no difference in age at diagnosis or death according to the functional domain location of the BMPR2 mutations. No differences in baseline data were observed, including hemodynamic parameters and 6MWD. Overall survival was also similar among mutation carriers for the different functional domain locations.

BMPR2 mutations found in the patients included missense, frameshift, nonsense, RGT, variant of unknown significance (VUS), and splice site. Frameshift and nonsense mutations are predicted to produce truncated BMPR II proteins and are thought to have damaging effects via a different mechanism from missense mutations.24,25 We therefore compared the baseline data and survival results according to the different mutation categories. The age at diagnosis in patients with missense mutations was younger than that in patients carrying a splice defect or large rearrangement mutation (25 ± 9 vs 33 ± 12 years, respectively; P = 0.02; one-way ANOVA test, least significant difference, LSD). There were no differences among these three groups in age at death (24 ± 7, 22 ± 9, and 27 ± 8 years, respectively; P = 0.585; one-way ANOVA test, LSD). Hemodynamic characteristics (mPAP, PVR, CI) showed no influence of the mutation categories on disease severity. No statistically significant differences were observed in survival and times to
death among the 3 subgroups of BMPR2 mutation carriers (Figure 3).

Discussion

In this study, we analyzed a group of 305 PAH patients with or without germline BMPR2 mutations who were treated according to the standard of care recommended by treatment guidelines\textsuperscript{17,19} in Shanghai Pulmonary Hospital. The median age at diagnosis of the patients was similar to that of the NIH registry study,\textsuperscript{1} but much younger than that of the REVEAL\textsuperscript{26} or French registry\textsuperscript{27} studies. This difference implies that the disease is phenotypically distinct or that genetic or environmental influences are distinct. Since the environmental and genetic backgrounds were different in these studies, it is difficult to speculate on the reasons for such a difference, but it does indicate the severity of the disease in the Chinese Han population. Although our diagnostic and therapeutic techniques strictly followed clinical guidelines, the low awareness of PAH among physicians and the poor general healthcare in China may mean that some older PAH patients in China were not seen. Also, it is possible that the genetic and epidemiologic characteristics of the Chinese Han population may be unique and distinct from those of non-Han (or Western) populations. Thus, comparisons between Han and non-Han patients may give insights into the etiological factors in each population.

Our results indicate significantly greater hemodynamic compromise in BMPR2 mutation carriers at diagnosis in comparison with noncarriers, as demonstrated by higher mPAP, mRVP, and PVR values. The worse hemodynamic parameters at diagnosis in BMPR2 mutation carriers might reflect an accelerated disease process, and this was confirmed by the overall survival data. There were significant differences in overall survival following the diagnosis of PAH with BMPR2 mutation carriers having a significantly shorter time to death, indicating more rapid disease progression in this group. A younger age at death in BMPR2 mutation carriers as
compared with noncarriers further suggests that BMPR2 mutations confer a more severe phenotype. In a study of French patients with PAH, Sztrymf et al.\textsuperscript{14} reported that BMPR2 mutation carriers had a higher mPAP, lower CI, higher PVR, lower SvO\textsubscript{2}, and a younger age at diagnosis and death, but had similar overall survival. In other studies, Girerd et al.\textsuperscript{12} noted that sex did not influence differences in hemodynamic status between BMPR2 mutation carriers and noncarriers in French patients, while Austin et al.\textsuperscript{13} found that there was a statistically significant difference in age at diagnosis between female mutation carriers and noncarriers. In the present study, we confirmed that BMPR2 mutation status influences hemodynamic features and age at death. When the patients were subgrouped into females and males, the difference between mutation carriers and noncarriers was not significant. This is likely due to the small numbers in each subgroup. Elliott et al.\textsuperscript{28} reported that PAH patients with non-synonymous BMPR2 variations are unlikely to demonstrate vasoreactivity. We also found that mutation carriers were less likely to exhibit a significant response to acute vasodilator testing than noncarriers, although the difference did not reach statistical significance \((P = 0.546)\).

Another striking characteristic of our Chinese PAH cohort was that the survival of male mutation carriers was significantly worse than that of male noncarriers, which was reflected in the higher mortality risk of male mutation carriers than that of male noncarriers after adjustment for age at diagnosis [HR = 3.702; 95\% CI 1.416-9.679; \(P = 0.008\)]. In females, the risk of mortality was increased by BMPR2 mutations but this did not reach statistical significance [HR = 1.349; 95\% CI 0.625-2.908; \(P = 0.446\)], even though the female group was much larger than the male group. Girerd et al.\textsuperscript{12} also observed a trend for a worse disease prognosis in males, particularly in male patients carrying a BMPR2 mutation. Although the total number of patients in the study of Girerd et al.\textsuperscript{12} was greater than in ours, their data did not reach statistical
significance, but the trend supported our findings. These findings indicate that genotype influences phenotype differently in the different sexes. In our previous study, we observed that the mutation rate in male patients is 2-fold higher than in females. The worse prognosis plus the higher mutation rate in male patients with PAH suggests that $BMPR2$ mutations influence phenotype more obviously in male patients with pulmonary arterial hypertension. In females, there may be other etiological factors. In this regard, Austin et al. noted a decrease in the ratio of the urinary estrogen metabolites 2-OHE (2-hydroxyestrone) and 16$\alpha$-OHE$_1$ (16$\alpha$-hydroxyestrone) in females with a $BMPR2$ mutation compared with non-affected females, and West et al. found significantly decreased levels of the estrogen metabolizing gene $CYP1B1$ in affected females compared with unaffected females. Their research suggests that altered estrogen metabolism could contribute to the penetrance of PAH in female $BMPR2$ mutation carriers, and that $CYP1B1$ could be a sex-specific modifier gene. Larger numbers (females vs males, $n = 218$ vs 87) and a lower mutation rate of female patients compared with male patients indicate that abnormal estrogen metabolism may be a modifier for $BMPR2$ mutation carrier status, but is more likely to be an independent factor predisposing females to PAH.

In the present study, female Chinese patients with a $BMPR2$ mutation did not exhibit a significantly worse survival than female noncarriers. This phenomenon indicates that the influence of $BMPR2$ mutation is modified by other unknown factors, and that the etiology of PAH in female patients is more complicated. These unknown factors may be responsible for a detrimental effect on survival in $BMPR2$ mutation carriers in females. The unknown non-$BMPR2$ etiologic factors may also be responsible for the female dominance in PAH and for the absolute numbers of female $BMPR2$ mutation carriers and male $BMPR2$ mutation carriers being similar in this study (female vs male, $n = 28$ vs 22). The etiologic factors may be critical molecules in the
**BMPR2** signaling pathway, unknown mutations in which may predispose patients to PAH. It is also possible that the unknown etiologic factors may be important translational regulators that control the level of **BMPR2** expression. Sex hormones are possible candidates and could explain the female dominance in PAH and the lack of a difference in prognosis between female **BMPR2** mutation carriers and noncarriers. However, we cannot exclude limitations of the available technical methods and our knowledge of the types of mutations.

The pathogenic mutations identified within the coding sequence of the **BMPR2** gene are characterized by significant molecular heterogeneity. This heterogeneity includes mutation localization and mutation type. In vitro experiments have demonstrated that disease-associated mutations in **BMPR2** disrupt BMP/Smad signaling by a variety of mechanisms.\(^{24,25}\) We investigated whether the localization of the mutation influences disease phenotype. All 50 mutations in our study were classified into 3 groups according to the extracellular (n = 19), kinase (n = 24), and cytoplasmic (n = 7) functional domains. We did not observe any difference in the hemodynamic characteristics and survival of patients with these three mutation localizations.

In other studies, Austin and colleagues\(^{13}\) showed that the mutation category affects the PAH phenotype. PAH patients with missense mutations in the **BMPR2** gene had more severe disease than patients with truncating mutations, having a significantly younger age at diagnosis and a shorter survival from diagnosis to death or lung transplantation. On the other hand, Girerd et al.\(^{12}\) did not find that the **BMPR2** mutation type had any influence on clinical phenotypes in French patients with PAH. In order to clarify the situation in Chinese patients with PAH, we compared the clinical phenotypes of **BMPR2** missense mutation carriers, **BMPR2** truncating mutation carriers, and patients carrying a splice defect or a large rearrangement in the **BMPR2** gene. We found that the age at diagnosis in missense patients was younger than that in patients carrying a
splice defect or large rearrangement mutation (25 ± 9 vs 33 ± 12 years, respectively; \( P = 0.02 \)). Compared with other mutation types, missense mutations seemed to have a more significant influence on the phenotype of PAH in our Chinese cohort with \( BMPR2 \) mutations. This finding is concordant with the study of Austin et al.\(^{13} \)

There are several limitations of the present study. Firstly, because fresh lymphocytes were not available, we did not carry out an experiment to assess whether nonsense or frameshift mutations predicted to induce premature termination codons (PTCs) and produce unstable mRNA would be degraded by nonsense-mediated decay (NMD), a mRNA surveillance mechanism that detects and degrades mRNA transcripts containing PTCs leaving only wild-type mRNA detectable.\(^{31,32} \)

All truncating mutations should be assessed as NMD active or negative by in vitro experiments to show whether mutant \( BMPR2 \) transcripts are or are not degraded in the absence of puromycin.\(^{32} \) However, \( BMPR2 \) truncating mutations escaping the NMD pathway may only constitute a minority of cases, with 7 of 62 \( BMPR2 \) truncating mutation carriers escaping NMD in the study of Girerd et al.\(^{12} \) and 7 of 96 carriers in the study of Austin et al.\(^{13} \) Even if these patients were excluded from the analysis, no significant differences were seen among \( BMPR2 \) missense mutation carriers, \( BMPR2 \) truncating mutations, and patients carrying a splice defect or a large rearrangement in \( BMPR2 \) gene in French patients. The second limitation of the study is the small number of patients and the smaller numbers with each type of mutation. The power to detect differences in age at diagnosis among the three mutation groups (calculated using SAS Proc Power with a one-way ANOVA statement for one degree of freedom contrasts and the overall F test in one-way ANOVA) was 0.505. The influence of the mutation type on the clinical features or natural history of PAH could be more significant with increased patient numbers.

In summary, our data indicate that \( BMPR2 \) mutation carriers present as a severe phenotype
with more severe hemodynamic compromise, an earlier age at death, and poor overall survival.

The influence of BMPR2 mutation on phenotype is more obvious in male patients which implies that in females, there are other etiologic factors that modify the influence BMPR2 mutations making disparities in the prognosis between female mutation carriers and noncarriers less evident. Patients with missense mutations are younger at diagnosis than patients carrying a splice defect or large rearrangement mutation. However, the mutation location had no influence on the disease pattern or natural history of PAH in patients with BMPR2 mutations.

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**Conflict of Interest Disclosures:** Z-C.J. has relationships with drug companies including Actelion, Bayer Schering, Pfizer and United Therapeutics, in addition to being an investigator in trials sponsored by these companies; relationships include consultancy services and membership of scientific advisory boards.

**References:**


Table 1. Baseline Hemodynamic Characteristics of Male and Female Patients Carrying a BMPR2 Mutation†

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total patients</th>
<th></th>
<th></th>
<th></th>
<th>Female patients</th>
<th></th>
<th></th>
<th></th>
<th>Male patients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Noncarriers (n = 255)</td>
<td>Carriers (n = 50)</td>
<td>P value†</td>
<td>Noncarriers (n = 190)</td>
<td>Carriers (n = 28)</td>
<td>P value†</td>
<td>Noncarriers (n = 65)</td>
<td>Carriers (n = 22)</td>
<td>P value†</td>
<td></td>
<td></td>
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<tr>
<td>Age, years</td>
<td>32 (24, 47)</td>
<td>28 (25, 31)</td>
<td>0.018</td>
<td>34 (25, 48)</td>
<td>28 (21, 38)</td>
<td>0.106</td>
<td>31 (17, 46)</td>
<td>28 (19, 32)</td>
<td>0.231</td>
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<tr>
<td>BMI, kg/m²</td>
<td>21.3 ± 4.1</td>
<td>20.9 ± 3.6</td>
<td>0.519</td>
<td>21.3 ± 4.0</td>
<td>20.6 ± 3.3</td>
<td>0.264</td>
<td>21.3 ± 4.4</td>
<td>21.3 ± 4.0</td>
<td>0.913</td>
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<tr>
<td>6MWD, meters</td>
<td>379 ± 115</td>
<td>399 ± 111</td>
<td>0.270</td>
<td>381 ± 114</td>
<td>368 ± 99</td>
<td>0.538</td>
<td>373 ± 120</td>
<td>439 ± 115</td>
<td>0.033</td>
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<tr>
<td>RAP, mmHg</td>
<td>6 (3, 11)</td>
<td>7 (4, 11)</td>
<td>0.531</td>
<td>6 (3, 10)</td>
<td>5 (4, 11)</td>
<td>0.955</td>
<td>7 (3, 13)</td>
<td>8 (6, 12)</td>
<td>0.659</td>
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<tr>
<td>mPAP, mmHg</td>
<td>60 (49, 69)</td>
<td>67 (56, 78)</td>
<td>0.003</td>
<td>60 (49, 68)</td>
<td>64 (57, 76)</td>
<td>0.031</td>
<td>62 (53, 73)</td>
<td>69 (56, 84)</td>
<td>0.094</td>
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<td></td>
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<td>PVR, Wood units</td>
<td>14.6 (10.0, 20.3)</td>
<td>17.1 (13.4, 23.9)</td>
<td>0.028</td>
<td>14.7 (10.0, 20.1)</td>
<td>17.8 (13.6, 23.5)</td>
<td>0.078</td>
<td>14.2 (10.0, 19.1)</td>
<td>16.7 (12.3, 25.0)</td>
<td>0.178</td>
<td></td>
<td></td>
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<tr>
<td>CI, L•min⁻¹•m⁻²</td>
<td>2.3 (1.9, 3.2)</td>
<td>2.0 (1.7, 2.8)</td>
<td>0.095</td>
<td>2.4 (1.9, 3.2)</td>
<td>2.1 (1.7, 2.8)</td>
<td>0.218</td>
<td>2.2 (1.7, 3.4)</td>
<td>2.0 (1.6, 2.8)</td>
<td>0.319</td>
<td></td>
<td></td>
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<tr>
<td>Acute vasodilator</td>
<td>19</td>
<td>2</td>
<td>0.546†</td>
<td>15</td>
<td>2</td>
<td>1.000</td>
<td>4</td>
<td>0</td>
<td>0.568</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are medians (and interquartile range), means ± standard deviation, or numbers of patients (acute vasodilator responsiveness).
†P values represent the results of a Mann-Whitney U-test or Student's t-test comparing mutation carriers and noncarriers, with P < 0.05 chosen as the level of significance.
‡Fisher’s Exact test for acute vasodilator responsiveness.
BMI = body mass index; CI = cardiac index; 6MWD = 6-minute walk distance; RAP = right atrial pressure; mPAP = mean pulmonary arterial pressure; PVR = pulmonary vascular resistance.
Table 2. Hazard ratios (HRs) of mortality associated with BMPR2 mutation in total, male and female patients.

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>BMPR2 Mutation (HR, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carrier</td>
</tr>
<tr>
<td>Total patients</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>50</td>
</tr>
<tr>
<td>Deaths (n, %)</td>
<td>17 (34%)</td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>1.971(1.121-3.466)</td>
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<tr>
<td>Male patients</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>22</td>
</tr>
<tr>
<td>Deaths (n, %)</td>
<td>9 (40%)</td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>3.702(1.416-9.679)</td>
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<tr>
<td>Female patients</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>28</td>
</tr>
<tr>
<td>Deaths (n, %)</td>
<td>8 (28%)</td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>1.349 (0.625-2.908)</td>
</tr>
</tbody>
</table>

CI = confidence interval; HR = hazard ratio.
Figure Legends:

**Figure 1.** Outcome (cumulative survival) of BMPR2 mutation carriers versus noncarriers with PAH. **A:** total patient cohort. **B:** male patients. **C:** female patients. The survival of mutation carriers and noncarriers was significantly different among males and the total patient cohort (log-rank test).

**Figure 2.** Outcome (cumulative survival) of male vs female patients with PAH. **A:** total patient cohort. **B:** BMPR2 mutation carriers. **C:** BMPR2 mutation noncarriers. The survival of male and female patients was similar in the total patient cohort and in mutation carriers and noncarriers (log-rank test).

**Figure 3.** Influence of BMPR2 mutation types and location on the clinical outcome (cumulative survival) of patients with PAH. No differences in survival among the different BMPR2 mutation types and locations were evident (log-rank test). CD = cytoplasmic domain; ECD = extracellular domain; KD = kinase domain; RGT = large size rearrangements; VUS = variant of unknown significance.
A MUTATION TYPE

- Nonsense + Frame Shift
- Missense
- RGT + VUS + Splice Site

P = 0.595

- Cum Survival (%)
  - Time Year

B MUTATION LOCATION

- KD
- ECD
- CD

P = 0.192

- Cum Survival (%)
  - Time Year

Patients at risk

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<th>2 Year</th>
<th>3 Year</th>
<th>4 Year</th>
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<td>21</td>
<td>17</td>
<td>9</td>
<td>4</td>
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<tr>
<td>Missense</td>
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<td>11</td>
<td>7</td>
<td>5</td>
<td>2</td>
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<tr>
<td>RGT + VUS + Splice Site</td>
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<td>13</td>
<td>9</td>
<td>6</td>
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<table>
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<tr>
<td>KD</td>
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<td>14</td>
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<tr>
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BMPR2 Mutations Influence Phenotype More Obviously in Male Patients with Pulmonary Arterial Hypertension
Dong Liu, Wen-Hui Wu, Yi-Min Mao, Ping Yuan, Rui Zhang, Feng-Ling Ju and Zhi-Cheng Jing

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