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

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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 have 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 (hazard ratio, 1.971; 95% confidence interval, 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 (hazard ratio, 3.702; 95% confidence interval, 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 pathogenesis 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. (Circ Cardiovasc Genet. 2012;5:511-518.)

Key Words: genetics ■ hemodynamics ■ survival ■ pulmonary arterial hypertension

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.1,2 Mutations in the bone morphogenetic protein type II receptor (BMPR-II) gene (BMPR2) have been recognized to cause heritable PAH (HPAH).3,4 Subsequent studies have reported >250 BMPR2 mutations responsible for 55% to 70% of heritable PAH, and 11% to 40% of idiopathic PAH (IPAH).5-9 BMPR2 encodes the bone morphogenetic protein receptor II, which is a member of the transforming growth factor-β cell signaling superfamily.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.10,11 This pathway appears to be critical in both cell differentiation and growth through transcriptional regulation of target genes.

The influence of BMPR2 mutations on clinical outcomes in patients with PAH have been described recently.12-14 BMPR2 mutation carriers present ≈10 years earlier than noncarriers, and have more severe hemodynamic compromise at diagnosis.14 Austin et al15 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 BMPR2 mutation type on clinical phenotypes of PAH was observed.12

Clinical Perspective on p 518

Following the demonstration of 50 BMPR2 mutations, including 25 novel mutation sites, in 305 Chinese IPAH and HPAH patients,5 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 mm Hg and 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.16,17 IPAH was recognized before genomic consultation if there was >1 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, whereas 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. Seventeen 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.20,21 The mean pulmonary artery pressure (mPAP), mean pulmonary capillary wedge pressure (mPcWP), mean right atrial pressure, mean right ventricular pressure (mRVP), pulmonary vascular resistance (PVR), and mixed venous oxygen saturation (Svo2) were recorded during right heart catheterization. Cardiac output (CO) was measured by the standard thermodilution technique. The cardiac index (CI) was calculated as cardiac output 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 μg iloprost (Ventavis; 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.22 A positive acute vasodilator response was defined by current consensus guidelines, ie, as a decrease in mPAP of at least 10 mm Hg to a level ≤40 mm Hg with either no change or an increase in cardiac output.20,21 A nonencouraged 6-minute walk distance (6MWD) test, performed according to the American Thoracic Society recommendations,23 was undertaken in all patients.

Molecular Methods

The genetic study was carried out according to previously reported methods.6,14 Paired fragments for BMPR2 mutation screening were screened by direct sequencing and large size rearrangements (RGTs) detection.15 Direct screening using an ABI 3730 (Applied Biosystems, CA) was adopted to detect the point mutations in the coding regions and the intron/exon boundaries of BMPR2. The BMPR2 gene was screened for RGTs using the SALSA MLPA P093 HHT probe mix kit (MRCL-Holland BV). 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 on the manufacturer’s website (http://www.mlpa.com).

All mutation detections and nomenclature were completed by 2 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 y2 test or Fisher exact test, and Student t-test or Mann–Whitney U-test. Clinical features that were not normally distributed, such as 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 t-test or 1-way 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. First, a univariable Cox proportional regression analysis was used to estimate the hazard ratios (HRs) and the 95% confidence intervals 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 HRs 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 was compared by a log rank test. A P value of <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), whereas in noncarriers, the female/male ratio was 2.9:1 (n=190/65).

Mutation carriers had a younger median age at diagnosis (median, 28 years; IQR, 25–31; n=50) than noncarriers (median, 32 years; IQR, 24–47; n=255; P=0.018).

The 6-minute walk distance (6MWD) at diagnosis was 399±111 m in BMPR2 mutation carriers versus 379±115 m in noncarriers (P=0.270). There was no significant difference in 6MWD between carriers and noncarriers (Table 1).

Hemodynamic Parameters

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] versus 60 [49, 69] mm Hg; P=0.003) and PVR (17.1 [13.4, 23.9] versus 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 compared with 19 of 255 (7.5%) without BMPR2 variants. Therefore, although 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).
In the total study population (n=305), the 1-, 3-, and 5-year survival rates estimated by the Kaplan–Meier method were 92.4%, 89.8%, and 87.2%, respectively, and the mean survival time was 49.4±1.6 months. In the 50 carriers, the estimated 1- and 3-year survival rates were 91.7% and 76%, respectively, and the mean survival time was 39.4±2.8 months (Figure 1). Mutation carriers had a younger median age at death (median age at death, 26 years; IQR, 20–30; n=17) than noncarriers (median age at death, 32 years; 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% confidence interval, 1.149–3.506; P=0.014). Age was not associated with the risk of death (HR, 0.991; 95% confidence interval, 0.975–1.008; P=0.305), nor was sex associated with the risk of death (HR, 0.876; 95% confidence interval, 0.499–1.539; P=0.646), which is concordant with 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% confidence interval, 1.121–3.466; P=0.018; Table 2). Thus, BMPR2 mutation carriers were more likely to die than noncarriers. Although sex was not a risk factor of mortality in the univariable Cox proportional regression analysis, a trend 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% confidence interval, 1.416–9.679; P=0.008). In females, the trend did not reach statistical significance (HR, 1.349; 95% confidence interval, 0.625–2.908; P=0.446).

**Survival**

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Noncarriers (n=255)</th>
<th>Carriers (n=50)</th>
<th>P Value</th>
<th>Noncarriers (n=190)</th>
<th>Carriers (n=28)</th>
<th>P Value</th>
<th>Noncarriers (n=65)</th>
<th>Carriers (n=22)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</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>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.3±3.1</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>
</tr>
<tr>
<td>6MWD, m</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>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>Acute vasodilator responsiveness, n</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>
</tr>
</tbody>
</table>

*BMI indicates body mass index; CI, cardiac index; 6MWD, 6-minute walk distance; RAP, right atrial pressure; mPAP, mean pulmonary arterial pressure; and PVR, pulmonary vascular resistance.

*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 comparing mutation carriers and noncarriers, with P<0.05 chosen as the level of significance.

‡Fisher exact test for acute vasodilator responsiveness.

**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, 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.23,24 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 versus 33±12 years, respectively; P=0.02; 1-way ANOVA test, least significant difference). There were no differences among these 3 groups in age at death (24±7, 22±9, and 27±8 years, respectively; P=0.585; 1-way ANOVA test, least significant difference).

**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 guidelines17,19 in Shanghai Pulmonary Hospital. The median age at diagnosis of the patients was similar to that of the NIH registry study,1 but much younger than that of the Registry to Evaluate Early And Long-term pulmonary arterial hypertension disease management.
This difference implies that the disease is phenotypically distinct or that genetic or environmental influences are distinct. Because 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 causes 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 compared with noncarriers further suggests that BMPR2 mutations confer a more severe phenotype. In a study of French patients with PAH, Sztrymf et al reported that BMPR2 mutation carriers had a higher mPAP, lower CI, higher PVR, lower Svo2, 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 \textit{BMPR2} mutation carriers and noncarriers in French patients, whereas 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 \textit{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 attributed to the small numbers in each subgroup. Elliott et al\textsuperscript{15} reported that PAH patients with nonsynonymous \textit{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% confidence interval, 1.416--9.679; $P=0.008$). In females, the risk of mortality was increased by \textit{BMPR2} mutations but this did not reach statistical significance (HR, 1.349; 95% confidence interval, 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 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 PAH. In females, there may be other causes. In this regard, Austin et al noted a decrease in the ratio of the urinary estrogen metabolites 2-OHE (2-hydroxyestradiol) and 16α-OHE, (16α-hydroxyestrone) in females with a BMPR2 mutation compared with nonaffected 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 versus males, n=218 versus 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 pathogenesis 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 causes 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 versus male, n=28 versus 22). The causes may be critical molecules in the BMPR2 signaling pathway, unknown mutations which may predispose patients to PAH. It is also possible that the unknown causes 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.

**Table 2. Hazard Ratios of Mortality Associated With BMPR2 Mutation in Total, Male and Female Patients**

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>BMPR2 Mutation (HR, 95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td></td>
</tr>
<tr>
<td>N 50 255</td>
<td></td>
</tr>
<tr>
<td>Deaths (n, %) 17 (34%) 46 (18%)</td>
<td>Adjusted for age 1.971 (1.121–3.466) 1.0 0.018</td>
</tr>
<tr>
<td>Male patients</td>
<td></td>
</tr>
<tr>
<td>N 22 65</td>
<td></td>
</tr>
<tr>
<td>Deaths (n, %) 9 (40%) 8 (12%)</td>
<td>Adjusted for age 3.702 (1.416–9.679) 1.0 0.008</td>
</tr>
<tr>
<td>Female patients</td>
<td></td>
</tr>
<tr>
<td>N 28 190</td>
<td></td>
</tr>
<tr>
<td>Deaths (n, %) 8 (28%) 38 (20%)</td>
<td>Adjusted for age 1.349 (0.625–2.908) 1.0 0.446</td>
</tr>
</tbody>
</table>

HR indicates hazard ratio.

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

In other studies, Austin and colleagues13 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 al12 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 versus 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. First, because fresh lymphocytes were not available, we did not carry out an experiment to assess whether nonsense or frame-shift mutations predicted to induce premature termination codons and produce unstable mRNA would be degraded by nonsense–mediated decay, a mRNA surveillance mechanism that detects and degrades mRNA transcripts containing premature termination codons leaving only wild-type mRNA detectable.31,32 All truncating mutations should be assessed as nonsense–mediated decay 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 nonsense–mediated decay pathway may only constitute a minority of cases, with 7 of 62 BMPR2 truncating mutation carriers escaping nonsense–mediated decay in the study of Girerd et al12 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 3 mutation groups (calculated using SAS Proc Power with a 1-way ANOVA statement for 1 degree of freedom contrasts and the overall F test in 1-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 causes 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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Disclosures

Dr Jing 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


**CLINICAL PERSPECTIVE**

**BMPR2 mutations** are major predisposing factors for idiopathic and heritable pulmonary arterial hypertension (IPAH and HPAH). The influence of **BMPR2** mutations on clinical outcomes has been reported, but the data are not concordant in different ethnic groups. The current study sought to identify the association of **BMPR2** genotypes on clinical outcomes, and whether any potential association varies by sex in a large group of Chinese IPAH and HPAH patients who underwent genetic counseling in Shanghai Pulmonary Hospital. We compared clinical, functional, and hemodynamic characteristics of **BMPR2** mutation carriers and noncarriers. Our data indicate that **BMPR2** mutation carriers present with a severe phenotype of PAH, with more severe hemodynamic compromise, an earlier age at death, and poor overall survival. **BMPR2** mutations influence outcomes more in male PAH patients, which was reflected by a higher mortality risk of male mutation carriers than that of male noncarriers after adjustment for age at diagnosis (hazard ratio, 3.702; 95% confidence interval, 1.416–9.679; \( P = 0.008 \)). Considering the female predominance in PAH, the pathogenesis of PAH in women is likely more complicated and the influence of **BMPR2** mutations may be modified by other unknown factors, making disparities in the prognosis between female **BMPR2** mutation carriers and noncarriers less evident. The current study underscores the unique characteristics in PAH patients of Chinese origin. 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 with PAH may yield insights into the causes in each of these populations.
BMPR2 Mutations Influence Phenotype More Obviously in Male Patients With Pulmonary Arterial Hypertension
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