Relationship of BMPR2 Mutations to Vasoreactivity in Pulmonary Arterial Hypertension

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Background—Vasoreactivity tests are fundamental in evaluating pulmonary arterial hypertension (PAH). Mutations of the transforming growth factor-β type II receptor gene, BMPR2, predispose to the development of pulmonary hypertension and may alter the response to vasodilators. Previous investigations have not examined the relationship of BMPR2 mutations to vasoreactivity.

Methods and Results—We identified 133 consecutive unrelated patients with either idiopathic or familial PAH. Sixty-six patients were excluded because we lacked either DNA samples (n=18) or complete data from a vasoreactivity test (n=48). The remaining 67 patients were screened for BMPR2 DNA sequence variations, and specific variations were confirmed by gene sequencing. The vasoreactivity of patients with nonsynonymous BMPR2 variations was compared with that of patients without nonsynonymous BMPR2 variations. We found nonsynonymous BMPR2 variations in 27 of 67 patients with idiopathic (n=16 of 52) or familial (n=11 of 15) PAH. Vasoreactivity was identified in 3.7% of 27 patients with nonsynonymous BMPR2 variations and in 35% of 40 patients without nonsynonymous BMPR2 variations (P=0.003). Five of the 27 nonsynonymous variations occur commonly in healthy individuals. None of the remaining 22 patients with BMPR2 variations demonstrated vasoreactivity, and the analysis remained unchanged when we assumed that nonsynonymous BMPR2 variations were present in all 15 patients with familial PAH.

Conclusions—Patients with familial or idiopathic PAH and nonsynonymous BMPR2 variations are unlikely to demonstrate vasoreactivity. Further trials are required to determine whether long-term therapy can be directed by tests for BMPR2 variations. (Circulation. 2006;113:2509-2515.)

Key Words: genetics ■ molecular biology ■ pulmonary arterial hypertension ■ vasoconstriction ■ vasodilation

Sustained increases of mean pulmonary artery pressure (PAP) without a demonstrable cause define idiopathic pulmonary arterial hypertension (PAH), formerly called primary pulmonary hypertension.1-3 Vasoconstriction, thrombotic obstruction, or dysregulated cellular proliferation that obstructs the vascular lumen may cause increased PAP and increased pulmonary vascular resistance with normal left heart pressures in patients with idiopathic PAH.4 Wood1 first suggested that vasoconstriction was an important pathogenic mechanism for PAH. Subsequent investigators found that patients with marked vasoreactivity were uncommon, but the finding of vasoreactivity identified those patients who had a favorable response to extended treatment with calcium channel blockers.5,6 Recent international consensus has identified vasoreactivity testing as a critical step in the management of patients with idiopathic PAH.5,6 Calcium channel blockers are recommended as initial therapy for patients who respond in the short term to vasodilators.5,6

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Early reports of multiple family members affected by idiopathic PAH suggested an inherited predisposition to this disorder.4,10 In 2000, 2 groups of investigators reported that DNA sequence variations in the gene (BMPR2) that encodes bone morphogenetic protein receptor type II were associated with familial PAH.11,12 BMPR2 variations were also identified in 11% to 40% of patients diagnosed with idiopathic PAH.13-16 These variations may be spontaneous or inherited from a parent who does not express the disease. Bone morphogenetic protein receptor type II, a member of the transforming growth factor (TGF)-β family, regulates cellular proliferation by activating intracellular pathways of Smad (Sma and MAD gene homologues) and LIM (Lin-11, Isl-1, and Mec-3 protein) kinase.17,18 Current evidence suggests that the histopathological and clinical features of familial PAH are...
identical to those of nonfamilial idiopathic PAH, but the relationship of DNA sequence variations found in BMPR2 to acute tests of vasoreactivity remains undefined. We hypothesized that patients with and without nonsynonymous BMPR2 sequence variations do not differ in the likelihood of an acute response to vasodilators. To test this hypothesis, we compared the results of vasoreactivity tests for patients with nonsynonymous BMPR2 variations with vasoreactivity test results for patients without nonsynonymous BMPR2 variations.

Methods
Since 1994, the Utah Pulmonary Hypertension Genetics Project has developed and maintained a patient database to use as a tool to investigate the genetics of pulmonary hypertension. We sought consecutive unrelated patients at the Pulmonary Hypertension Center at LDS Hospital and at biannual meetings of the Pulmonary Hypertension Association. At the time of enrollment, patients provided written, informed consent according to a protocol approved by the institutional review board of the LDS Hospital. Blood samples, detailed family histories, and medical records were obtained. DNA was extracted from lymphocytes with a salting-out protocol (K.W.) (PureGene, Gentra Systems, Minneapolis, Minn). A review of complete medical records confirmed the diagnosis of idiopathic or familial PAH according to consensus standards (C.G.E., M.D.M., S.R.). In brief, the diagnosis of idiopathic PAH required that a consultant with expertise in pulmonary vascular disease confirm the following: (1) mean PAP >25 mm Hg, with a pulmonary capillary wedge pressure ≤15 mm Hg, both measured at rest by right heart catheterization, and (2) the exclusion of other disorders known to cause pulmonary hypertension by clinical evaluation and objective tests, eg, ventilation and perfusion lung scans to exclude pulmonary embolism, contrast echocardiography and/or measurements of oxygen saturation during cardiac catheterization to exclude intracardiac shunting, and echocardiography and cardiac catheterization to exclude left heart disease. The designation of familial PAH required that a consultant with expertise in the differential diagnosis of pulmonary hypertension confirm the diagnosis of PAH and suspect or confirm PAH in 1 or more of the index patient’s blood relatives.

Study Cohort
We (E.G., J.S.) queried the patient database and identified a cohort of 133 consecutive unrelated patients enrolled after June 1994 who had a confirmed diagnosis of idiopathic PAH or familial PAH. Patients were excluded when (1) no DNA was available for analysis (n=18) or (2) complete data from a test of vasoreactivity were not available (n=48). Of 48 patients without complete data, 46 had not had their cardiac output recorded after vasodilator administration, and 2 had received only a single dose of nifedipine. The study cohort (n=67) represented patients who were evaluated at 20 medical centers (Data Supplement, Part A).

Vasoreactivity Testing
Vasoreactivity tests were performed according to each individual hospital’s protocol and choice of vasodilator. Physicians who performed the catheterizations and made the hemodynamic measurements were unaware of the BMPR2 sequence variations. The dose of epoprostenol, adenosine, nifedipine, nitroprusside, prostaglandin E2, or phentolamine was increased until either an intolerable side effect occurred or vasoreactivity was observed. Nitric oxide was inhaled, and the hemodynamic response was measured shortly thereafter according to local institutional protocols. Data as reported in the medical record and stored in the database from the first complete test of vasoreactivity for each patient were analyzed. A priori vasoreactivity was defined by current consensus guidelines as a decrease in mean PAP of at least 10 mm Hg to a level ≤40 mm Hg with either no change or an increase in cardiac output.

Results

Molecular Analysis
We (C.G.E., E.G., J.C., M.K., M.B.S.) defined DNA sequence variations based on current nomenclature. We (M.B.S., M.K., J.C., J.T.M., G.T.H.) identified the wild-type genomic reference sequence from an assembly produced by the Human Genome Project (NCBI contig accession NT-005403). We specifically identified those BMPR2 sequence variations identified in our study sample that have been reported to occur at a frequency of 1% or higher in comparable healthy human populations, but we did not make assumptions about whether or not these sequence variations are associated with PAH.

We (J.T.M., J.C., M.B.S., G.T.H., M.K.) screened genomic DNA for variations in BMPR2 by polymerase chain reaction amplification of exons and analysis of amplicons with use of a Hi-Res melting curve analysis performed on an HR-1 instrument (Idaho Technology, Inc, Salt Lake City, Utah) as described previously. We confirmed specific sequence variations by sequencing the 13 BMPR2 exons with Big Dye Terminator (V3.1) chemistry (Applied Biosystems, Foster City, Calif) and 17 pairs of overlapping primers (M.B.S., J.C., G.T.H., M.K.). The primers and details of these assays are provided (Data Supplement, Part B). Parts of exon 12 were not sequenced because no deviations from wild-type melting profiles were identified by Hi-Res melting curve analysis.

Statistical Analysis
Data were analyzed with Statistica software (StatSoft, Inc, Tulsa, Okla) for means and distribution attributes (R.L.J.). We used the Fisher exact test to compare categorical variables among the cohorts, which were grouped according to either sequence variation status or vasoreactivity status (vasoreactive according to 2004 consensus guidelines or not vasoreactive), and P<0.01 was chosen as the level of significance to account for multiple analyses (R.L.J.). The primary analysis compared vasoreactivity between patients with and without BMPR2 nonsynonymous sequence variations by using Fisher’s exact test, and the test was considered significant when P<0.05. Secondary analyses were performed to assess the possible effect of one common sequence variation (c.2324 G→A) and the possible effect of undetected sequence variations among patients with familial PAH. These secondary analyses also used the Fisher exact test, and the test was considered significant when P<0.05 because these analyses were not independent. All tests were 2 sided.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Clinical Data
The study population included 52 patients with sporadic idiopathic PAH and 15 with familial PAH (15 families; Table 1). Fifty-three of the 67 were women. The mean (±SD) age of the 67 subjects was 38±11 years (range, 14 to 64 years). Sixty-five of 67 patients were in New York Heart Association (NYHA) functional class III at the time of the first vasoreactivity test. Patients with BMPR2 nonsynonymous allelic variants (n=27) did not differ from those without BMPR2 variants (wild type) or those with synonymous BMPR2 variants with respect to age, sex, height, weight, race, baseline hemodynamics, or NYHA functional class at the time of vasoreactivity testing. Patients with nonsynonymous BMPR2 variations were more likely to have familial PAH than were those without nonsynonymous BMPR2 variations.

Molecular Data
We identified sequence variations in 38 of 67 patients: 27 of 52 with idiopathic PAH and 11 of 15 with familial PAH. Twenty-seven of 38 patients had nonsynonymous BMPR2 variations (Table 2). These 27 nonsynonymous variants
Eleven patients, all with idiopathic PAH, had either c. 600 remaining 18 subjects had unique
2.5% in a comparable healthy human population.29 The
2 A( n
7), which are synonymous
2 2.2
2
2 2.3
2
2 0.7
2 0.102
Nonsynonymous
Variations (n=27)
15 (22)
11 (41)
4 (10)†
0.006
Baseline
MPAP, mm Hg
58.4±11.1
60.7±10.5
56.9±11.3
0.163
MRAP, mm Hg
10.1±6.5
10.1±5.6
10.2±7.2
0.978
PAWP, mm Hg
9.7±3.5
8.8±3.3
10.2±3.6
0.115
PVR, Wood units
13.2±5.8
14.6±5.1
12.3±6.1
0.123
CO, L/min
4.1±1.2
3.9±1.3
4.2±1.2
0.377
CI, L·min⁻¹·m⁻²
2.2±0.7
2.1±0.6
2.3±0.7
0.102
NYHA functional class
II, n (%) 1 (1)
0 (0)
1 (2)
0.403
III, n (%) 65 (97)
26 (96)
39 (97)
1.00
IV, n (%) 1 (1)
1 (4)
0 (0)
0.403
†P=0.006 by 2-tailed Fisher exact test for the comparison of family history for patients with BMPR2 nonsynonymous variations and patients without BMPR2 nonsynonymous variations.

Vasoreactivity Data
Epoprostenol, adenosine, nitric oxide, and high doses of nifedipine were used for 94% of the vasoreactivity tests (Table 3). The proportions of patients who were tested with specific vasodilators did not differ significantly for patients with and without BMPR2 sequence variants (Data Supplement, Part C). There were 2 unrelated patients (Nos. 85 and 102 in Table 2) with the same BMPR2 variant who were tested at different pulmonary hypertension centers with different vasodilators with the same result (nonresponders). There was also 1 member of a family and 1 unrelated man (Nos. 137 and 162 in Table 2) with the same BMPR2 nonsynonymous variant who were tested at 2 different pulmonary hypertension centers with 2 different vasodilators, and both were nonresponders. Finally, there were 2 unrelated men (Nos. 114 and 116 in Table 2) with the same BMPR2 variation who were tested at different pulmonary hypertension centers with the same result (nonresponders).

Overall, 15 of 67 patients (22%) were vasoreactive. The baseline cardiac index was higher and right atrial pressure, mean PAP, and pulmonary vascular resistance tended to be lower for these 15 patients (Table 4). Vasoreactivity occurred more commonly among patients without a BMPR2 nonsynonymous variant, and the decrease in mean PAP from baseline to maximal vasodilator effect was greater in the absence of BMPR2 nonsynonymous variations (8.7 versus 3.4 mm Hg, P=0.05). The primary analysis showed that 1 of the 27 patients (3.7%) with a nonsynonymous BMPR2 variation demonstrated vasoreactivity compared with 14 of 40 (35%) without nonsynonymous BMPR2 variants (P=0.003; the Figure). The primary analysis grouped the 5 individuals with the common nonsynonymous c. 2324G→A

TABLE 1. Characteristics of the Study Participants at the Time of Vasoreactivity Testing*

<table>
<thead>
<tr>
<th></th>
<th>All (n=67)</th>
<th>Nonsynonymous BMPR2 Variations (n=27)</th>
<th>No Nonsynonymous BMPR2 Variations (n=40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>37.8±11.3</td>
<td>37.1±12.0</td>
<td>38.2±10.9</td>
<td>0.679</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>14/53</td>
<td>9/18</td>
<td>5/35</td>
<td>0.065</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166.6±9.7</td>
<td>166.9±11.3</td>
<td>166.4±8.6</td>
<td>0.838</td>
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<tr>
<td>Weight, kg</td>
<td>75.9±17.7</td>
<td>80.5±17.5</td>
<td>72.8±17.4</td>
<td>0.083</td>
</tr>
<tr>
<td>Race or ethnic group, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>62 (93)</td>
<td>26 (96)</td>
<td>36 (90)</td>
<td>0.641</td>
</tr>
<tr>
<td>Black</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>0.403</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (5)</td>
<td>1 (4)</td>
<td>2 (5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>15 (22)</td>
<td>11 (41)</td>
<td>4 (10)†</td>
<td>0.006</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPAP, mm Hg</td>
<td>58.4±11.1</td>
<td>60.7±10.5</td>
<td>56.9±11.3</td>
<td>0.163</td>
</tr>
<tr>
<td>MRAP, mm Hg</td>
<td>10.1±6.5</td>
<td>10.1±5.6</td>
<td>10.2±7.2</td>
<td>0.978</td>
</tr>
<tr>
<td>PAWP, mm Hg</td>
<td>9.7±3.5</td>
<td>8.8±3.3</td>
<td>10.2±3.6</td>
<td>0.115</td>
</tr>
<tr>
<td>PVR, Wood units</td>
<td>13.2±5.8</td>
<td>14.6±5.1</td>
<td>12.3±6.1</td>
<td>0.123</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>4.1±1.2</td>
<td>3.9±1.3</td>
<td>4.2±1.2</td>
<td>0.377</td>
</tr>
<tr>
<td>CI, L·min⁻¹·m⁻²</td>
<td>2.2±0.7</td>
<td>2.1±0.6</td>
<td>2.3±0.7</td>
<td>0.102</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II, n (%)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>0.403</td>
</tr>
<tr>
<td>III, n (%)</td>
<td>65 (97)</td>
<td>26 (96)</td>
<td>39 (97)</td>
<td>1.00</td>
</tr>
<tr>
<td>IV, n (%)</td>
<td>1 (1)</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0.403</td>
</tr>
</tbody>
</table>

*Plus-minus values are mean±SD. Percentages may not sum to 100 because of rounding. MPAP indicates mean PAP; MRAP, mean right atrial pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; CO, cardiac output; and CI, cardiac index. P values represent the results of Fisher’s exact test comparing subjects with and without BMPR2 nonsynonymous variations with P<0.01 chosen as the level of significance to account for multiple analyses.

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TABLE 2. Characteristics of 27 Patients With Nonsynonymous BMPR2 Sequence Variations

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex, Age, y</th>
<th>IPAH or FPAH*</th>
<th>Exon</th>
<th>Nucleotide Change†</th>
<th>Amino Acid Predicted Change‡</th>
<th>Type of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>M, 46</td>
<td>FPAH</td>
<td>8</td>
<td>c. 968_G969insT</td>
<td>p. H 324 fs X 3</td>
<td>Frameshift</td>
</tr>
<tr>
<td>5</td>
<td>M, 23</td>
<td>IPAH</td>
<td>9</td>
<td>c. 1248delA</td>
<td>p. F 417 fs X 2</td>
<td>Frameshift</td>
</tr>
<tr>
<td>6</td>
<td>F, 29</td>
<td>IPAH</td>
<td>12</td>
<td>c. 2527delG</td>
<td>p. A 843 fs X 16</td>
<td>Missense</td>
</tr>
<tr>
<td>15§</td>
<td>F, 58</td>
<td>IPAH</td>
<td>12</td>
<td>c. 2324 G&gt;A</td>
<td>p. S 775 N</td>
<td>Missense</td>
</tr>
<tr>
<td>23</td>
<td>F, 33</td>
<td>IPAH</td>
<td>12</td>
<td>c. 2324 G&gt;A</td>
<td>p. S 775 N</td>
<td>Missense</td>
</tr>
<tr>
<td>30</td>
<td>F, 34</td>
<td>IPAH</td>
<td>4</td>
<td>c. 439 C&gt;T</td>
<td>p. R 147 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>35</td>
<td>M, 47</td>
<td>IPAH</td>
<td>12</td>
<td>c. 2324 G&gt;A</td>
<td>p. S 775 N</td>
<td>Missense</td>
</tr>
<tr>
<td>45</td>
<td>F, 42</td>
<td>IPAH</td>
<td>11</td>
<td>c. 1469 C&gt;T</td>
<td>p. A 490 V</td>
<td>Missense</td>
</tr>
<tr>
<td>48</td>
<td>F, 21</td>
<td>IPAH</td>
<td>8</td>
<td>c. 1113_1114insT</td>
<td>p. A 372 fs X 27</td>
<td>Frameshift</td>
</tr>
<tr>
<td>52</td>
<td>F, 43</td>
<td>IPAH</td>
<td>10</td>
<td>c. 1397 G&gt;A</td>
<td>p. W 466 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>60</td>
<td>F, 46</td>
<td>IPAH</td>
<td>2</td>
<td>c. 203 G&gt;A</td>
<td>p. G 68 D</td>
<td>Missense</td>
</tr>
<tr>
<td>85</td>
<td></td>
<td></td>
<td>F, 31</td>
<td>IPAH</td>
<td>12</td>
<td>c. 2617 C&gt;T</td>
</tr>
<tr>
<td>87</td>
<td>M, 43</td>
<td>IPAH</td>
<td>6</td>
<td>c. 631 C&gt;T</td>
<td>p. R 211 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>91</td>
<td>F, 19</td>
<td>IPAH</td>
<td>2</td>
<td>c. 188-208delGTAGCACCTGCTATGGCCTTT</td>
<td>p. S64_W70del</td>
<td>Large deletion</td>
</tr>
<tr>
<td>96 (VR)</td>
<td>F, 26</td>
<td>IPAH</td>
<td>12</td>
<td>c. 2324 G&gt;A</td>
<td>p. S 775 N</td>
<td>Missense</td>
</tr>
<tr>
<td>102</td>
<td>F, 55</td>
<td>IPAH</td>
<td>12</td>
<td>c. 2617 C&gt;T</td>
<td>p. R 873 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>111</td>
<td>M, 47</td>
<td>FPAH</td>
<td>6</td>
<td>c. 727 G&gt;T</td>
<td>p. E 243 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>113</td>
<td>F, 38</td>
<td>FPAH</td>
<td>3</td>
<td>c. 296 T&gt;C</td>
<td>p. C 99 R</td>
<td>Missense</td>
</tr>
<tr>
<td>114#</td>
<td>M, 30</td>
<td>FPAH</td>
<td>8</td>
<td>c. 994 C&gt;T</td>
<td>p. R 332 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>116</td>
<td>M, 54</td>
<td>FPAH</td>
<td>8</td>
<td>c. 994 C&gt;T</td>
<td>p. R 332 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>137</td>
<td>F, 30</td>
<td>FPAH</td>
<td>6</td>
<td>c. 637 C&gt;T</td>
<td>p. R 213 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>146</td>
<td>F, 43</td>
<td>FPAH</td>
<td>Intron 8</td>
<td>c. 1129-3 C&gt;G</td>
<td>Splice site variation</td>
<td></td>
</tr>
<tr>
<td>151</td>
<td>F, 48</td>
<td>IPAH</td>
<td>12</td>
<td>c. 2324 G&gt;A</td>
<td>p. S 775 N</td>
<td>Missense</td>
</tr>
<tr>
<td>159</td>
<td>F, 33</td>
<td>IPAH</td>
<td>2</td>
<td>c. 218 C&gt;G</td>
<td>p. S 73 X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>162</td>
<td></td>
<td>M, 14</td>
<td>IPAH</td>
<td>6</td>
<td>c. 637 C&gt;T</td>
<td>p. R 213 X</td>
</tr>
</tbody>
</table>

*IPAH indicates idiopathic PAH; FPAH, familial PAH; and VR, vasoreactive by current consensus definition.8,9
†Abbreviations are in accord with current nomenclature.25 The letter c. indicates coding DNA, where nucleotide 1 is the A of the ATG translation initiation codon.
‡The letter p. is used to indicate that a change is described at the protein level.
§No known relationship for subjects 15, 23, 35, 96, and 151, all of whom have a nonsynonymous BMPR2 sequence variation reported to occur in 5% of a healthy human population.29
||No known relationship between subjects 85 and 102.
#No known relationship between subjects 114 and 116.
¶No known relationship to subject 137.

variant among the subjects with nonsynonymous BMPR2 variants. However, the finding was not changed when these 5 individuals were combined with those for whom no nonsynonymous variant was identified (0 of 22 versus 15 of 45, \( P=0.001 \)). Thus, the findings remained significant without any assumptions about the functional impact of c. 2324G→A.

BMPR2 variations were not found in 4 of the 15 individuals with familial PAH. If we assume that there were undetected BMPR2 variants in these 4 familial patients, then 2 of 31 individuals with either a BMPR2 nonsynonymous variant (n=27) or familial PAH without a detectable BMPR2 variant (n=4) demonstrated vasoreactivity, whereas 13 of 36 individuals with no BMPR2 variant and without familial PAH (n=25) or synonymous BMPR2 variations (n=11) demonstrated vasoreactivity (\( P=0.008 \)). Four of 38 patients with any sequence variation (synonymous or nonsynonymous) showed vasoreactivity compared with 11 of 29 patients with no sequence variation (\( P=0.016 \)).

### Discussion

The present study is the first to suggest that genotype is an important determinant of the therapeutic response in patients...
with PAH. We found that patients with familial or idiopathic PAH and BMPR2 nonsynonymous sequence variations were unlikely to demonstrate vasoreactivity. This observation suggests that tests for BMPR2 variants identify patients who will not respond to vasodilators in the short term and who are unlikely to benefit from prolonged treatment with calcium channel blockers. Clinical features such as age, sex, and NYHA functional class did not differentiate responders from nonresponders, although responders tended to have lower right atrial pressures and higher cardiac indices, as has been observed previously.6 If our data can be verified prospectively, then identification of a BMPR2 nonsynonymous variation could lead to treatment without the need to perform vasoreactivity tests. Current evidence suggests that at least half of all familial PAH patients31 and 11% to 40% of idiopathic PAH patients32,33 have identifiable BMPR2 variants.

In our study, only 4 of 38 individuals with any variation from the normal DNA sequence demonstrated vasoreactivity, and 3 of these 4 had synonymous BMPR2 variations. Thus, it appears that an acute vasodilator response may occur in patients for whom tests find synonymous DNA variations.34–36 Our findings fit the concept that dysregulated cellular proliferation underlies idiopathic or familial PAH accompanied by BMPR2 mutations.

Our results appear valid and generalizable. The patient population was drawn consecutively from a large number of unrelated adult patients with typical features of idiopathic or familial PAH. Most patients in the present study were middle-aged women with symptoms indicative of NYHA functional class III disease. The patients all had severe PAH at the time of their initial vasoreactivity test. In addition, the majority of patients were identified and studied at major referral centers with experience in the diagnosis and evaluation of PAH. Physicians used drugs commonly accepted for tests of acute vasoreactivity,8 and the protocols for vasoreactivity tests had similar end points. Vasoreactivity tests were performed with vasodilators titrated to doses proven to be effective in patients with PAH with marked vasoreactivity.22–24,32,33 Although multiple drugs were used, there is general consensus that vasoreactivity is independent of the vasodilator.8,32

### TABLE 4. Clinical Characteristics of Patients With Idiopathic or Familial PAH Based on Their Acute Response to a Vasodilator*

<table>
<thead>
<tr>
<th></th>
<th>Vasoreactive (n=15)</th>
<th>Nonreactive (n=52)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35.8±11.2</td>
<td>38.3±11.4</td>
<td>0.46</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>1/14</td>
<td>13/39</td>
<td>0.08</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>1 (7)</td>
<td>14 (37)</td>
<td>0.04</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPAP, mm Hg</td>
<td>52.7±7.7</td>
<td>60.0±11.4</td>
<td>0.02</td>
</tr>
<tr>
<td>MRAp, mm Hg†</td>
<td>6.3±1.1</td>
<td>11.0±6.9</td>
<td>0.02</td>
</tr>
<tr>
<td>PVR, Wood units</td>
<td>10.5±3.7</td>
<td>14.0±6.1</td>
<td>0.04</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>4.6±1.0</td>
<td>4.0±1.3</td>
<td>0.07</td>
</tr>
<tr>
<td>CI, L·min⁻¹·m⁻²</td>
<td>2.7±0.6</td>
<td>2.1±0.6</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II, n (%)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>...</td>
</tr>
<tr>
<td>III, n (%)</td>
<td>15 (100)</td>
<td>50 (96)</td>
<td>...</td>
</tr>
<tr>
<td>IV, n (%)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>...</td>
</tr>
</tbody>
</table>

*Vasoreactivity was defined according to recent international consensus guidelines.8,9
†n=64; right atrial pressure was not recorded for 3 of 67 patients.

BMPR2 nonsynonymous variations were associated with a negative test for vasoreactivity (A). This result was not altered by including the 5 patients with a BMPR2 variation (c. 2324 G→A) found in unrelated northern Europeans with a genotype frequency of 5%29 with the 40 patients who did not have BMPR2 nonsynonymous variations (B), nor was the result altered by assuming that deleterious variants were present in all 15 patients with familial PAH (FPAH) (C). Vasoreactivity was defined by a >10-mm Hg decrease of mean PAP to a level <40-mm Hg. The numbers represent the number of vasoreactive patients (numerator) and the total number of patients in that category (denominator). The probability values represent the results of Fisher exact test.
Furthermore, our observation of 3 groups of individuals with the same BMPR2 nonsynonymous variation who had the same result, although they were tested at different centers with different vasodilators, strengthens the validity and generalizability of our results.

Our study has potential limitations. The study cohort did not include young children. Previous investigation suggests that younger children are more likely to demonstrate vasoreactivity at the time of testing.\(^2,3\) Second, a number of patients in the study population were excluded because DNA or complete data from vasoreactivity tests were unavailable. We obtained DNA samples only from volunteers who provided written, informed consent. It is not clear what if any bias was introduced by excluding patients without DNA samples or complete data from vasoreactivity tests. However, the excluded patients did not differ from the study cohort with respect to age, sex, or hemodynamic variables. A third limitation may be the evaluation of patients with advanced disease. However, it is interesting to note that overall, 15 of 67 (22%) adult patients with idiopathic or familial PAH demonstrated vasoreactivity, even though they presented with severe elevations of mean PAP and pulmonary vascular resistance. This is comparable to the proportion of idiopathic PAH patients reported previously who meet the current consensus definition of vasoreactivity\(^6,22,32\) (Data Supplement, Part D). Nevertheless, it is possible that vasoreactivity is present in individuals with BMPR2 nonsynonymous variants when they are identified earlier in the course of their disease, particularly if BMPR2 nonsynonymous variants induce proliferation of pulmonary vascular smooth muscle cells followed by secondary activation and injury of pulmonary artery endothelial cells that produce obstruction of pulmonary arteries. Future studies may be able to examine this possibility through early identification of PAH in family members with BMPR2 nonsynonymous variants. A fourth limitation may be the definition of vasoreactivity. Other definitions of acute vasoreactivity exist\(^6,22,23,26,28,32,33\). However, our data appear significant, particularly for the patients with nonsynonymous BMPR2 allelic variants. Only 1 of these 27 patients had a mean PAP decrease to <40 mm Hg at the maximally tolerated dose of vasodilator, and none of the 22 patients with uncommon BMPR2 nonsynonymous variants met the current consensus definition of vasoreactivity.\(^8,9\) A final limitation might be that BMPR2 variants went undetected, particularly in 4 patients with familial PAH,\(^31\) but only 1 of these 4 patients displayed vasoreactivity.

In summary, we have shown that patients with familial or idiopathic PAH and BMPR2 nonsynonymous variants are unlikely to display vasoreactivity. This raises the possibility that BMPR2 sequence variation status identifies patients who are unlikely to respond to long-term vasodilator therapy. This hypothesis deserves evaluation in a prospective outcome study.

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Disclosures

Drs Elliott, Carquist, and Jensen are employed by Intermountain Health Care Services. Dr Rich is currently a part-time salaried employee of United Therapeutics. Dr Ward is the director of Tauert Laboratories. Jason McKinney is employed by and holds equity interest in Idaho Technology. The other authors report no conflicts.

References


**CLINICAL PERSPECTIVE**

Pulmonary arteriolar vasoconstriction is a fundamental mechanism that underlies pulmonary hypertension. Research has established a role for acute vasoreactivity tests to identify patients with idiopathic pulmonary arterial hypertension (PAH) who should be treated initially with calcium channel blockers. Patients who respond in the short term to vasodilators often survive for years when treated with high doses of calcium channel blockers. Treatment with other medications is appropriate for patients who do not respond in the short term to vasodilators. Research has established the critical role of mutations in the transforming growth factor (TGF)-β type II receptor gene, BMP2, in the pathogenesis of disease for many patients with idiopathic or familial PAH. Interestingly, loss of TGF-β type II signaling mediated by BMP2 mutations may cause PAH through a mechanism of dysregulated vascular cell proliferation rather than or in addition to vasoconstriction. Our study compared the vasoreactivity of patients with nonsynonymous BMP2 variations with the vasoreactivity of patients without nonsynonymous BMP2 variations. Patients with familial or idiopathic PAH accompanied by nonsynonymous BMP2 variations were unlikely to demonstrate vasoreactivity. The results suggest that dysregulated cellular proliferation is an important cause of idiopathic or familial PAH in patients with BMP2 mutations. Furthermore, these results suggest that genetic tests may be useful to guide the treatment of patients with idiopathic or familial PAH.
Relationship of BMPR2 Mutations to Vasoreactivity in Pulmonary Arterial Hypertension

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