Novel Insertional Mutation in the Bone Morphogenetic Protein Receptor Type II Associated With Sporadic Primary Pulmonary Hypertension

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Primary pulmonary hypertension (PPH), which results from occlusion of small pulmonary arteries, is a devastating condition. Mutations of the bone morphogenetic protein receptor type II gene (BMPR2), a component of the transforming growth factor-β (TGF-β) family, which plays a key role in cell growth, have recently been identified as causing familial and sporadic PPH. The first case of BMPR2 mutation found in Japan is reported here in a 19-year-old woman with a clinical diagnosis of PPH and no identifiable family history of pulmonary hypertension. Direct sequencing of the entire coding region and intron/exon boundaries of BMPR2 revealed a frameshift mutation predicted to alter the cell signaling response to specific ligands. A molecular classification of PPH, based upon the presence or absence of BMPR2 mutations, might have important implications for patient management and screening of relatives. (*Circ J 2004; 68: 592–594)

Key Words: Bone morphogenetic protein receptor type II gene; Mutation; Primary pulmonary hypertension

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rimary pulmonary hypertension (PPH) is a progressive disease of unknown etiology that is usually followed by death within 5 years (median, 2.8 years) of diagnosis.1,2 Right ventricle (RV) failure is commonly observed and is a prognostic variable of PPH.3 Although significant advances in the medical treatment of PPH have been achieved in the past decade with continuous intravenous prostacyclin therapy,4 patients who are refractory to vasodilator therapy will ultimately require heart-lung or lung transplantation.5 Recent genetic studies in patients with familial and sporadic PPH have provided new opportunities to address the pathophysiologic mechanisms of this disease.6,7 In the past year, mutations in the bone morphogenetic protein receptor 2 gene (BMPR2), a component of the transforming growth factor-β (TGF-β) family, have been identified in an even higher proportion of cases of familial and sporadic PPH than was first suspected.8 In addition, mutations associated with impaired signaling through other members of the TGF-β receptor family have also been uncovered.9 We report the first case in Japan of PPH associated with a germline insertional mutation of BMPR2.

Case Report

A 19-year-old woman was admitted for evaluation of exertional dyspnea. On admission, her blood pressure was 100/58 mmHg and heart rate was 76 beats/min. The jugular vein was distended and the liver was palpable below the right costal margin. Auscultation revealed no pathological (Received July 18, 2002; revised manuscript received October 9, 2002; accepted October 21, 2002)

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Fig 1. 12-lead ECG on admission.
sounds or arrhythmia. An electrocardiogram demonstrated right atrial (RA) overload and RV hypertrophy (Fig 1), and the chest radiograph revealed mild cardiac enlargement with a cardiothoracic ratio of 0.66 (Fig 2). Echocardiography showed a dilated RA and RV. Right heart catheterization revealed elevated pulmonary arterial pressure (PAP): RA pressure (RAP) 18/16 (15) mmHg; PAP 68/38 (47) mmHg; mean pulmonary wedge pressure (PCWPm) 9 mmHg. The thermodilution cardiac index (CI) was 1.4 L·min−1·m−2 (Table 1). O2 (10 L/min) and prostacyclin (PGI2) (6 ng·kg−1·min−1) were used for acute testing to assess pulmonary vasoreactivity and cardiac performance. When the patient underwent short-term vasodilator testing with PGI2, she responded favorably, with an increase in CI of 47% (Table 1). Based on a diagnosis of PPH, she had started continuous intravenous PGI2 therapy.

The gene encoding BMPR2 contains 13 exons and polymerase chain reaction (PCR) primers have been designed to amplify all 13, as described elsewhere,7 using genomic DNA extracted from whole peripheral blood samples of the patient and a QIAamp Blood Midi kit (QIAGEN Inc, Valencia, CA, USA). The amplified product, purified by a QIAquick PCR purification kit (QIAGEN Inc) is directly sequenced using an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer Biosystems, Foster City, CA, USA). Informed consent was obtained from the patient and her parents. The genetic analysis revealed insertions of the nucleotide residues in the genomic sequence; both an adenine and cytosine were inserted at nucleotide position 1956, a mutation that predicts premature truncation of the BMPR2 protein through shifts of the reading frame (Fig 3). Parental material was not available for analysis. No mutations were detected from normal individuals (n=10) or patients with secondary PPH (n=10).

**Discussion**

We identified novel insertional mutations of BMPR2 in a patient with sporadic PPH and this is the first report of such in Japan. Using direct sequence analysis, 56% of

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**Table 1** Effects of O2 and Intravenous PGI2 on Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Room air O2 PG (–)</th>
<th>O2 10L/min PG (–)</th>
<th>Room air PG (6 ng·kg−1·min−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td>70</td>
<td>61</td>
<td>88</td>
</tr>
<tr>
<td><strong>SAP (mmHg)</strong></td>
<td>96/66 (75)</td>
<td>102/65 (77)</td>
<td>89/56 (66)</td>
</tr>
<tr>
<td><strong>RAP (mmHg)</strong></td>
<td>18/16 (15)</td>
<td>17/16 (15)</td>
<td>20/17 (16)</td>
</tr>
<tr>
<td><strong>PAP (mmHg)</strong></td>
<td>68/38 (47)</td>
<td>67/38 (45)</td>
<td>72/38 (50)</td>
</tr>
<tr>
<td><strong>PCWPm (mmHg)</strong></td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td><strong>CI (L·min−1·m−2)</strong></td>
<td>1.4</td>
<td>1.5</td>
<td>2.2</td>
</tr>
</tbody>
</table>

PG, prostaglandin E; HR, heart rate; SAP, systemic arterial pressure; RAP, right arterial pressure; PAP, pulmonary arterial pressure; PCWPm, mean capillary wedge pressure; CI, cardiac index.

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**Fig 2.** Chest X-ray on admission.

**Fig 3.** Sequence analysis of the genomic DNA of the patient revealed an AC insertion (∗) (1956insAC) at BMPR2 nucleotide, which substitutes a termination codon (tga) for a codon [L654fs (+4 amino acids)] (Lower panel). This mutant (1956insAC) is predicted to produce a truncated protein occurring at the cytoplasmic tail of BMPR2.
families with PPH and approximately 26% of patients with sporadic PPH have been found to have mutations in BMPR2 and a total of 46 unique BMPR2 mutations have been identified in patients with PPH. The mutations are widely dispersed throughout the gene and are found in all exons except 5, 10 and 13. To date, the majority (=50%) of mutations are predicted to lead to a premature termination codon. The mutant (1956insAC) found in the present case is also predicted to produce a truncated protein occurring at the cytoplasmic tail of BMPR2 through shifts of the reading frame (Fig 3). Previous studies have shown that in vitro expression of recombinant BMPR2 containing either the D485G mutation or the I860fs (+10) frameshift mutation, predicted to encode a protein containing 83% of the normal BMPR2, demonstrated complete loss of function. Such alterations may affect either ligand binding, type I receptor binding, or propagation of the signal across the membrane to the kinase domain.

**Conclusion**

We found a frameshift mutation predicted to alter the cell signaling response to specific ligands. A molecular classification of PPH, based upon the presence or absence of BMPR2 mutations, might have important implications for patient management and screening of relatives. However, the detailed molecular mechanisms associated with the pathogenesis of the disorder will require further analysis, including identification of the target genes that have their transcription regulated regulated by BMPR2-mediated cell signaling.

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**References**