The Efficacy of a Genetic Analysis of the BMPR2 Gene in a Patient with Severe Pulmonary Arterial Hypertension and an Atrial Septal Defect Treated with Bilateral Lung Transplantation

Shunsuke Tatebe¹, Koichiro Sugimura¹, Tatsuo Aoki¹, Saori Yamamoto¹, Nobuhiro Yaoita¹, Hideaki Suzuki¹, Haruka Sato¹, Katsuya Kozu¹, Ryo Konno¹, Kimio Satoh¹, Koji Fukuda¹, Osamu Adachi², Ryoko Saito³, Norifumi Nakanishi⁴, Hiroko Morisaki⁵,⁶, Kotaro Oyama⁷, Yoshikatsu Saiki², Yoshinori Okada⁸ and Hiroaki Shimokawa¹

Abstract:
Severe pulmonary arterial hypertension (PAH) rarely develops in children with an atrial septal defect (ASD), even those with a large defect. We herein report the case of a 27-year-old man with a moderate-sized secundum ASD and right ventricular failure due to severe PAH, which developed in his early teens. He was diagnosed as having a genetic mutation of the bone morphogenetic protein receptor-2 (BMPR2) gene and was successfully treated with bilateral lung transplantation with ASD path closure. In patients with congenital heart disease, a genetic analysis may provide information about the lifetime risk of developing PAH.

Key words: BMPR2 mutation, pulmonary arterial hypertension, atrial septal defect, Eisenmenger syndrome, lung transplantation, congenital heart disease

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Introduction
Severe pulmonary arterial hypertension (PAH) rarely occurs in children with atrial septal defect (ASD), even in patients with a large defect (1). This is because, in contrast to ventricular septal defect (VSD)-ASD only imposes a volume overload on the pulmonary circulation. Thus, pulmonary vascular disease (PVD) may not develop until the third or fourth decade of life. This observation raises the possibility that young ASD patients with severe PAH and patients with idiopathic PAH have common risk factors for developing PAH. A mutation in the bone morphogenetic protein receptor-2 (BMPR2) gene has been identified not only familial and idiopathic PAH but also in PAH associated with congenital heart disease (CHD) (2), indicating a common pathogenesis underlying the development of PAH in both groups.

We herein report the case of a 27-year-old man with a moderate-sized secundum ASD and right heart failure (RHF) due to severe PAH, in whom a genetic analysis allowed us to understand the clinical course, including the childhood onset of PAH.

Case Report
A 27-year-old man with severe PAH and ASD was re-
ferred to Tohoku University Hospital to evaluate further treatment options in August 2012. Moderate cyanosis was initially noted at 13 years of age when he caught a cold. Right heart catheterization (RHC) at the referring hospital revealed severe PAH with irreversible PVD (Table) and a moderate-sized secundum ASD of 15 mm in size, with a bidirectional shunt. Because there was no indication for surgical ASD closure, treatment with beraprost sodium was started. However, his shortness of breath on exertion and general fatigue had gradually worsened over the past 5 years, despite the administration of bosentan and sildenafil. Syncope was also noted 2 years prior to his admission to our hospital. There was no family history of PAH or CHD.

On physical examination, his blood pressure was 106/64 mmHg, and his pulse rate 90 bpm. Moderate cyanosis, an euglobulinemia, an anemia, and clubbed fingers were observed. The precordial impulses were visible and palpable. Auscultation revealed an accentuated pulmonic component of S2, a grade 2/6 ejection systolic murmur along the lower left sternal border, and a right-sided S3 in the tricuspid region. Blood examination demonstrated secondary polycythemia with a hemoglobin level of 20.1 g/dL, a hematocrit value of 60%, and elevated brain-type natriuretic peptide (BNP) level of 499 pg/mL. Chest X-ray revealed increased muscularization of the hilar pulmonary arterial shadow and dilatation of the hiliar pulmonary arterial shadow (Fig. 1A). Furthermore, a 12-lead electrocardiogram (ECG) demonstrated a normal sinus rhythm, right axis deviation, first-degree atrioventricular block, and complete right bundle block with a QRS duration of 200 ms (Fig. 1B). Transthoracic echocardiography showed severely impaired systolic function of the right ventricle (RV) with a tricuspid annular plane systolic excursion of 11 mm and an RV fractional area change of 14%. The RV was markedly enlarged (Fig. 1C). Tricuspid regurgitation was moderate with an estimated RV pressure of 75 mmHg. A secundum ASD of 16 mm in diameter with bidirectional flow was also identified (Fig. 1C).

The left ventricular ejection fraction (LVEF), as measured by cardiac magnetic resonance imaging decreased to 35% because of interventricular septal bowing and compression of the left ventricle (LV) (Fig. 1D). Chest CT showed no evidence of thromboembolism, tumors or lung disorders such as chronic obstructive or interstitial lung disease. Spirometry showed a vital capacity of 3.9 L (83% of the predicted) and an forced expiratory volume (FEV)1/forced vital capacity (FVC) ratio of 74%. Although both the lung diffusion capacity of carbon monoxide (DLCO)(89.6% of the predicted value) and the diffusing capacity divided by the alveolar volume (DLCO/VA)(89.6% of the predicted value) were within the normal limits, the patient’s exercise capacity was decreased; the 6-minute-walk distance was 378 m, with a marked drop in SpO2: from 81% to 68% and a peak oxygen consumption of 11.3 mL/kg/min on bicycle ergometer. RHC revealed a pulmonary capillary wedge pressure of 6 mmHg, mean pulmonary arterial pressure of 48 mmHg, mean RA pressure of 7 mmHg (Table). The pulmonary to systemic flow ratio was 0.84. The pulmonary and systemic vascular resistance was 20.4 and 29.7 Wood Units, respectively. No pulmonary vasoreactivity to inhaled nitric oxide (40 ppm for 10 minutes) was noted.

Given the poor prognosis of PAH with medically refractory RHF, the patient was referred for lung transplantation and was registered in the Japan Organ Transplantation Network in December 2012. A genetic analysis revealed a heterozygous missense mutation in exon 12 of the BMPR2 gene (NM_001204.6: c.2474A>G), leading to the substitution of tryptophan to cysteine at amino acid position 825 (p.Tyr825Cys). Parental material was not available.

Three years later, the patient underwent bilateral lung transplantation and concomitant cardiac surgery. A secundum ASD of 16 mm was surgically confirmed and was closed with an autologous pericardial patch. A histological examination revealed increased muscularization of the intra-arterial arterioles, medial hypertrophy and intimal thickening.

### Table. Time-course of Hemodynamic Data.

|                    | 13 years old | 27 years old | 30 years old | (3 months after LTx) | Baseline |
|--------------------|--------------|--------------|--------------|----------------------|---------|
| **PAP, mmHg**      | 102/49 (69)  | 102/43 (66)  | 60/35 (48)   | 62/37 (48)           | 22/8    |
| **mRAP, mmHg**     | 6            | 6            | 7            | 4                    | 0       |
| **mPCWP, mmHg**    | 6            | 7            | 6            | 5                    | 9       |
| **AoP, mmHg**      | 104/78 (87)  | 102/71 (84)  | 95/78 (80)   | 105/73 (80)          | 143/80  |
| **Qp, L/min**      | 3.75         | 3.11         | 2.06         | 2.13                 | 4.64    |
| **Qs, L/min**      | 3.38         | 2.72         | 2.46         | 2.62                 | 4.64    |
| **Qp/Qs**          | 1.05         | 1.15         | 0.84         | 0.81                 | 1.0     |
| **Rp, Wood Units** | 16.8         | 18.6         | 20.4         | 20.2                 | 0.9     |
| **Rs, Wood Units** | 23.9         | 28.7         | 29.7         | 29.0                 | 21.6    |
| **Rp/Rs**          | 0.74         | 0.65         | 0.69         | 0.70                 | 0.04    |

AoP: aortic pressure, LTx: lung transplantation, mPCWP: mean pulmonary capillary wedge pressure, mRAP: mean right atrial pressure, PAP: pulmonary artery pressure, Qp: pulmonary blood flow, Qs: systemic blood flow, Qp/Qs: pulmonary to systemic flow ratio, Rp: pulmonary vascular resistance, Rs: systemic vascular resistance, Rp/Rs: pulmonary to systemic resistance ratio.
of the small pulmonary arteries, and multiple channels at branch points of the large preacinus pulmonary arteries (Fig. 2A-C), the latter of which was thought to have developed as a result of plexiform lesions. The postoperative course was complicated by symptomatic ventricular tachycardia (VT), possibly due to preexisting myocardial damage. The patient’s VT was successfully controlled by the combination of bisoprolol, mexiletine and sotalol. A subcutaneous implantable cardioverter-defibrillator (sICD) was implanted to allow the use of immunosuppressive drugs for preventing allograft rejection. At 6 months after the lung transplantation, the patient was discharged with a marked improvement in his cardiopulmonary symptoms. RHC before discharge confirmed the complete resolution of PAH with a normal cardiac output (Table). However, biventricular dysfunction partially remained with an LVEF of 52% and a right ventricular ejection fraction (RVEF) of 42% on cardiac MRI.

**Discussion**

PVD can occur in various types of CHD, leading to severe PAH and a fatal outcome (3). The causes of PVD associated with CHD are multifactorial and include chronic volume and pressure overload of the pulmonary artery due to left-to-right shunt, hypoxic vasoconstriction, and elevated pulmonary venous pressure (3). Although the time of the onset of advanced PVD varies in each patient according to their anatomy and physiology, an isolated ASD does not usually cause severe PAH in younger patients because the pulmonary vasculature is only exposed to excess pulmonary blood flow (4). In contrast, the vast majority of patients with unrestricted VSD will develop severe PVD if they are not treated within the first years of life because the pulmonary vasculature is exposed to both an increased pulmonary flow and increased systemic arterial pressure (4). The present case was interesting because the patient had severe PAH with irreversible PVD at his initial presentation at 13 years.
Therrien et al. found no without PVD (7.5% vs. 1.2%, p=0.004) (7). In contrast, with PVD was significantly higher in comparison to thoseported that the rate ofpare our patient to these cases. More recently, Liu et al. re-

tricuspid shunt, such as VSD, atrio-ventricular septal defect, or patent ductus arteriosus, and the two of them had addi-
tional genetic syndrome. Thus, it is difficult to simply compare our patient to these cases. More recently, Liu et al. re-
ported that the rate of BMPR2 mutations in CHD patients with PVD was significantly higher in comparison to those without PVD (7.5% vs. 1.2%, p=0.004) (7). In contrast, Therrien et al. found no BMPR2 mutations in Eisenmenger syndrome patients with ASD or ASD controls (1), indicating that a BMPR2 mutation is an important factor for triggering PAH in patients with CHD, whereas hemodynamic stress alone can produce irreversible PVD in patients with a large defect that causes Eisenmenger syndrome.

The BMPR2 gene is located on chromosome 2q33-q34 and has 13 exons (8). We identified a novel heterozygous missense mutation, c.2474A>G, in exon 12 of the BMPR2 gene. Exon 12 encodes an intracellular tail domain with an unknown function (8). To determine whether this mutation had been previously reported, we checked ExAC (http://exac.broadinstitute.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases, and the Human Gene Mutation Da-
tabase (HGMD, http://www.hgmd.org). However, it was not found in these databases and was considered to be novel. An in silico analysis, using SIFT (http://sift.jcvi.org) predicted that the c.2474A>G in BMPR2 was damaging, while an analysis using Mutation Taster (http://www.mutationtaster.org/), predicted that it was disease causing, and an analysis using PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) predicted that it was probably damaging. This suggests that the mutation found in the present case occurred in a highly con-
served site across species and that it may have affected the BMPR2 function. The mode of inheritance was unclear because there were no available material from relatives and because the patient had no family history of PAH or CHD.

The early onset of irreversible PVD in the present case may be explained by the "two-hit" theory. Evans et al. re-
ported that PAH patients with BMPR2 mutations presented at a younger age with more severe disease and had a poor
prognosis in comparison to patients without BMPR2 mutations (9). Interactions between abnormal cell signals [via BMPR2 mutation (5)] and hemodynamic overload (via ASD) can promote the rapid progression of pulmonary vascular lesions, resulting in severe PAH in patients in their teens. Thus, it seems useful for CHD patients with PAH to undergo a genetic analysis for idiopathic or familial PAH to evaluate the pathophysiology of developing PAH and in order to select an appropriate treatment. The determination of BMPR2 mutations appears to help in identifying CHD patients with PAH who have a high-risk of developing severe PVD at a much earlier stage in comparison to patients in whom it is expected based on the hemodynamic overload of the pulmonary circulation and the need for early corrective surgery. It may also be helpful to assess the risk of persistent or recurrent PH after the correction of the defect.

TGF-β/BMP signaling is essential for heart development and the maintenance of the cardiac function; abnormalities in this signaling can cause congenital heart defects, such as atrioventricular defect and truncus arteriosus (10, 11). Talati et al. reported that both impaired fatty acid oxidation and the increased expression of the lipid transporter molecule CD36 are the mechanisms underlying the accumulation of lipids in the BMPR2 mutant RV (12). Van der Bruggen et al. showed that PAH patients with BMPR2 mutations had more severe RV dysfunction in comparison to those without the mutation, despite a similar RV afterload (13). Thus, the BMPR2 mutation in our case may have affected both the progression of PVD and the abnormal atrial septal formation and/or the early development of RHF, which required lung transplantation.

**Conclusion**

In conclusion, we reported the case of a young man with a BMPR2 gene mutation who presented with severe PAH in his early teens. A genetic analysis of CHD patients with PAH may help to evaluate the pathophysiological mechanisms underlying the development of PAH and facilitate the selection of an appropriate treatment.

The authors state that they have no Conflict of Interest (COI).

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