and time from PAH diagnosis were 47.6 (25–69) years and 6.21 (0.6–13.9) years, respectively. At baseline, seven patients had IPAH and one had HPAH. The WHO functional class was Class I for one patient, Class II for five patients, and Class III for two patients. Epoprostenol AS was started at the same dose of epoprostenol GM, with a mean (range) dose of 40.13 (17.0–61.0) ng/kg/min. The mean (range) duration of exposure to epoprostenol AS during the 12-week treatment period was 86.9 (78.4–91.6) days. There were no dose adjustments in any patient. All of the patients completed the study schedule during the evaluation period. The continuous treatment period was ongoing as of February 2013.

Safety

Adverse events reported during the 12-week evaluation period are summarized in Table 2 according to system organ class and preferred term using the Medical Dictionary for Regulatory Activities/Japanese version. Seven out of eight patients (87.5%) experienced a total of 18 adverse events. Three patients (37.5%) experienced a total of four adverse events that were considered related to the study drug. Two patients (25.0%) experienced serious adverse events: moderate pneumonia and mild device dislocation in one patient each, both of which were not considered related to the study drug. There were no deaths or adverse events leading to treatment discontinuation during the study. Seven out of eight patients (87.5%) experienced mild adverse events and three (37.5%) experienced moderate adverse events; there were no severe adverse events. The most frequent event was nausea, which was reported by two patients. The other adverse events occurred in one patient only. There were no clinically significant changes from baseline to week 12 in blood pressure, heart rate, body weight, or clinical laboratory tests.

Efficacy

Table 3 shows the hemodynamic factors measured within 60 min before (i.e., baseline) and 60 min after the first dose of epoprostenol, as well as the changes between these two times. As shown in Table 3, there were no marked changes in pulmonary hemodynamic parameters from 60 min before to 60 min after the first dose of epoprostenol AS. Wilcoxon signed rank sum tests revealed no significant differences at the 5% level for the changes from baseline. Table 4 presents the hemodynamic factors measured at baseline and at week 12, together with their changes between these times. As shown in Table 4, there were no remarkable changes in pulmonary hemodynamic factors from baseline to week 12. Additionally, Wilcoxon signed rank sum tests revealed no significant differences at the 5% level for the changes from baseline. The WHO functional class was unchanged from baseline to week 12, as one was categorized as Class I, five as Class II, and two as Class III (Table 5). The mean (range) NT-proBNP concentration was 139 (57–240) pg/mL at baseline and 106 (41–243) pg/mL at week 12. The mean (range) change from baseline to week 12 was −−43.3 (−196 to 43) pg/mL, which was not clinically significant (Wilcoxon signed rank sum test: $P = 0.5781$) (Fig. 1).

Treatment Satisfaction

Table 6 shows the scores for all three domains of the TSQM-9 recorded at baseline and week 12, together with the changes from baseline to week 12. As shown in this table, there were improvements in all three domains during the

Table 2 Summary of treatment-emergent adverse events by intensity and system organ class

	Asymptomatic	Mild	Moderate	Severe	Total ($n = 8$)
All system organ classes					
Total pts with at least one AE	0 (0.0%)	7 (87.5)	3 (37.5%)	0 (0.0%)	7 (87.5%)
Total number of AEs	0	11	7	0	18
Gastrointestinal disorders					
Total pts with at least one AE	0 (0.0%)	3 (37.5%)	3 (37.5%)	0 (0.0%)	5 (62.5%)
Total number of AEs	0	3	3	0	6
Nausea	0 (0.0%)	0 (0.0%)	2 (25.0%)	0 (0.0%)	2 (25.0%)
Diarrhea	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Gastritis	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Vomiting	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Mikulicz's disease	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Infections and infestations					
Total pts with at least one AE	0 (0.0%)	1 (12.5%)	2 (25.0%)	0 (0.0%)	3 (37.5%)
Total number of AEs	0	1	2	0	3
Pharyngitis	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Pneumonia	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Cellulitis	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
General disorders and administrations site conditions					
Total pts with at least one AE	0 (0.0%)	2 (25.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)
Total number of AEs	0	2	0	0	2
Device dislocation	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Injury associated with device	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Skin and subcutaneous tissue disorders					
Total pts with at least one AE	0 (0.0%)	2 (25.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)
Total number of AEs	0	3	0	0	3
Dermatitis allergic	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Drug eruption	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Rash	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Ear and labyrinth disorders					
Total pts with at least one AE	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Total number of AEs	0	1	0	0	1
Vertigo	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)

Table 2 continued

	Asymptomatic	Mild	Moderate	Severe	Total ($n = 8$)
Musculoskeletal and connective tissue disorders					
Total pts with at least one AE	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Total number of AEs	0	1	0	0	1
Arthralgia	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
Nervous system disorders					
Total pts with at least one AE	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Total number of AEs	0	0	1	0	1
Hypoasthesia	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Respiratory, thoracic and mediastinal disorders					
Total pts with at least one AE	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Total number of AEs	0	0	1	0	1
Pleurisy	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)

AE adverse event, *pts* patients

12-week treatment period. The improvement in convenience was statistically significant (Wilcoxon signed rank sum test: $P = 0.0313$).

DISCUSSION

The results of this study demonstrate that patients with PAH can be switched from epoprostenol GM to a new formulation of epoprostenol sodium, epoprostenol AS, with no apparent safety concerns or deteriorations in pulmonary hemodynamic factors. Switching from epoprostenol GM to epoprostenol AS was also associated with an improvement in convenience across three treatment satisfaction domains.

The new formulation of epoprostenol is stable for 24 h at room temperature (1–30 °C) after preparation/dilution in saline, and the prepared solution does not require cooling with a frozen gel pack, which is expected to improve the quality of life of patients, particularly in terms of their daily activities. This expectation is supported by the results of the treatment satisfaction questionnaire, which showed an improvement in convenience. Therefore, patients with PAH treated with conventional formulations of epoprostenol sodium could be switched to epoprostenol AS, a stable formulation at room temperature, and may enhance their quality of life through improvements in treatment convenience.

The outdoor air temperature during the summer in many regions of Japan often exceeds 30 °C, and the temperature around the medication cassette of the diluted solution could be even higher [20]. As part of the risk-management strategy in this study, patients were instructed to use a frozen gel pack during the summer, especially when the environmental temperature exceeded room temperature. In this study, two out of eight patients used a frozen gel pack during the treatment period, but neither of these patients experienced any changes in their overall

Table 3 Changes in hemodynamic parameters from baseline to within 60 min after switching epoprostenol formulations

Hemodynamic parameter	Baseline (n = 8)			After 60 min (n = 8)						P value[a]
	Actual value			Actual value			Changes from baseline			
	Mean ± SD	Median	[Min, Max]	Mean ± SD	Median	[Min, Max]	Mean ± SD	Median	[Min, Max]	
Systolic pulmonary artery pressure, mmHg	50.6 ± 12.4	46.0	[34, 69]	50.5 ± 12.5	48.0	[33, 72]	−0.1 ± 4.2	−0.5	[−7, 6]	0.8438
Diastolic pulmonary artery pressure, mmHg	17.1 ± 2.2	17.0	[14, 22]	16.5 ± 2.2	16.0	[13, 20]	−0.6 ± 1.8	−0.5	[−4, 2]	0.5313
Mean pulmonary artery pressure, mmHg	31.1 ± 5.1	31.5	[22, 40]	30.4 ± 4.6	30.0	[22, 37]	−0.8 ± 2.2	−0.5	[−4, 2]	0.4375
Pulmonary capillary wedge pressure, mmHg	8.4 ± 1.8	8.0	[5, 11]	7.1 ± 1.7	6.5	[5, 10]	−1.3 ± 1.3	−1.5	[−3, 1]	0.0625
Cardiac output, L/min	4.829 ± 1.057	4.440	[2.72, 5.99]	4.834 ± 1.349	4.535	[3.45, 6.76]	0.545 ± 0.782	0.420	[−0.31, 1.84]	0.1094
Mean right arterial pressure, mmHg	4.8 ± 1.8	4.5	[3, 8]	3.6 ± 1.5	3.5	[1, 6]	−1.1 ± 2.0	−1.5	[−3, 3]	0.2031
Mixed venous oxygen saturation, %	73.43 ± 5.50	73.20	[63.4, 83.2]	73.86 ± 5.82	72.15	[66.0, 84.5]	0.44 ± 2.73	0.30	[−3.9, 3.7]	0.8438
Cardiac input, L/min/m^2	2.98 ± 0.86	2.80	[2.0, 4.3]	3.39 ± 1.20	2.95	[2.2, 5.4]	0.41 ± 0.60	0.20	[−0.2, 1.5]	0.1250
Pulmonary vascular resistance, dyn·s/cm^5	448.3 ± 158.1	429.5	[201, 676]	406.0 ± 143.6	380.0	[232, 680]	−42.3 ± 84.1	−14.0	[−166, 41]	0.4609
Pulmonary vascular resistance index, dyn·s/cm^5/m^2	646.5 ± 223.1	598.0	[338, 1,000]	594.8 ± 237.3	534.5	[384, 1,091]	−51.8 ± 124.3	−22.5	[−239, 91]	0.4609

SD standard deviation

[a] P value based upon Wilcoxon signed rank sum test

Table 4 Changes in hemodynamic parameters from baseline to 12 weeks after switching epoprostenol formulations

Hemodynamic parameter	Baseline (n = 8)			After 60 min (n = 8)						P value[a]
	Actual value			Actual value			Changes from baseline			
	Mean ± SD	Median	[Min, Max]	Mean ± SD	Median	[Min, Max]	Mean ± SD	Median	[Min, Max]	
Systolic pulmonary artery pressure, mmHg	50.6 ± 12.4	46.0	[34, 69]	49.8 ± 14.1	44.5	[29, 70]	−0.9 ± 3.5	−1.0	[−5, 4]	0.4375
Diastolic pulmonary artery pressure, mmHg	17.1 ± 2.2	17.0	[14, 22]	18.8 ± 4.6	19.0	[11, 24]	1.6 ± 3.7	2.0	[−3, 7]	0.4844
Mean pulmonary artery pressure, mmHg	31.1 ± 5.1	31.5	[22, 40]	31.4 ± 7.2	32.0	[18, 41]	0.3 ± 2.8	0.5	[−4, 4]	0.8906
Pulmonary capillary wedge pressure, mmHg	8.4 ± 1.8	8.0	[5, 11]	7.3 ± 1.2	7.5	[6, 9]	−1.1 ± 2.3	−2.0	[−4, 3]	0.2344
Cardiac output, L/min	4.829 ± 1.057	4.440	[2.72, 5.99]	4.499 ± 1.005	4.095	[3.25, 5.98]	0.210 ± 0.790	0.310	[−0.91, 1.35]	0.4609
Mean right arterial pressure, mmHg	4.8 ± 1.8	4.5	[3, 8]	4.8 ± 1.8	4.5	[3, 7]	0.0 ± 1.7	0.0	[−3, 3]	1.0000
Mixed venous oxygen saturation, %	73.43 ± 5.50	73.20	[63.4, 83.2]	72.10 ± 3.28	72.75	[67.1, 76.3]	−1.33 ± 4.64	−0.50	[−8.4, 4.7]	0.5469
Cardiac input, L/min/m^2	2.98 ± 0.86	2.80	[2.0, 4.3]	3.11 ± 0.72	3.00	[2.4, 4.3]	0.14 ± 0.52	−0.25	[−0.6, 0.9]	0.6563
Pulmonary vascular resistance, dyn·s/cm^5	448.3 ± 158.1	429.5	[201, 676]	453.6 ± 175.3	424.5	[154, 686]	5.4 ± 78.3	−25.0	[−61, 182]	0.5469
Pulmonary vascular resistance index, dyn·s/cm^5/m^2	646.5 ± 223.1	598.0	[338, 1,000]	648.5 ± 239.6	640.5	[251, 992]	2.4 ± 98.4	−15.5	[−87, 212]	0.7109

SD standard deviation

[a] P value based upon Wilcoxon signed rank sum test

Table 5 Changes in WHO FC from baseline to week 12

	WHO FC					
	Baseline		Week 12			
			I	II	III	IV
N		*N*	[*N*]	[*N*]	[*N*]	[*N*]
8	I	1	1	–	–	–
	II	5	–	5	–	–
	III	2	–	–	2	–
	IV	0	–	–	–	0

FC functional class, *WHO* World Health Organization

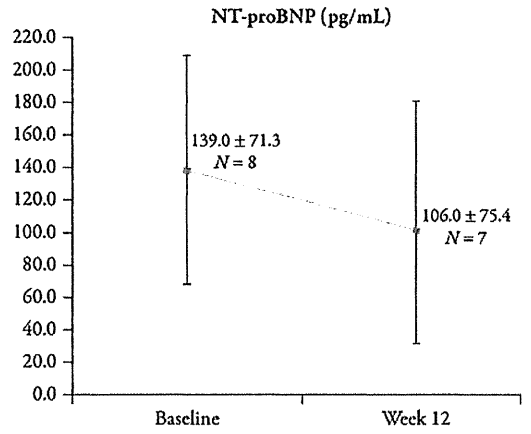

Fig. 1 NT-proBNP concentration measured at baseline and at week 12. $P = 0.5781$ at the 5% level (Wilcoxon signed rank sum test) *NT-proBNP* N-terminal prohormone of brain natriuretic peptide

condition. In addition, the earthquake and tsunami that hit eastern Japan on March 11, 2011, exposed flaws in the systems used to ensure patient safety and maintain a stable supply of medical resources during an emergency [21]. If essential utilities such as electricity and water networks are shut down following a disaster, it is likely that frozen gel packs would not be available, which may be a life-threatening issue for patients with PAH. The authors speculate that epoprostenol AS, which is stable for a longer time at room temperature than the GM formulation, could be valuable for continuing medical treatment during emergencies, and may have a positive impact on the quality of medical care, although additional examination of epoprostenol AS use is required in situations where the temperature exceeds 30 °C. Further studies are needed to evaluate the impact of higher environmental temperatures on the safety/tolerability and efficacy of this formulation, and to accumulate evidence supporting its clinical use.

Some limitations of this study warrant mention. Firstly, the sample size was small, which may prevent detection of small differences in hemodynamic factors or infrequent adverse events. Secondly, as the study was conducted in an open-label manner without a control group (e.g., of patients continuing epoprostenol GM during the 12-week treatment phase), it is possible that a study effect or patient bias contributed to the observed improvements in treatment satisfaction.

CONCLUSION

In conclusion, the present study showed that switching to a new formulation of epoprostenol was associated with an improvement in convenience in relation to treatment satisfaction, without unexpected adverse

Table 6 Changes in treatment satisfaction from baseline to 12 weeks after switching epoprostenol formulations

TSQM-9 domain	Baseline ($n = 8$)			12 weeks of administration ($n = 8$)						
	Actual scale			Actual scale			Changes from baseline			
	Mean ± SD	Median	[Min, Max]	Mean ± SD	Median	[Min, Max]	Mean ± SD	Median	[Min, Max]	*P* value[a]
Effectiveness	56.25 ± 7.55	58.4	[44.4, 66.7]	58.31 ± 14.55	61.1	[33.3, 72.2]	2.06 ± 10.68	0.0	[−11.1, 22.2]	0.9063
Convenience	51.40 ± 10.19	55.6	[33.3, 61.1]	58.33 ± 12.96	61.1	[33.3, 72.2]	6.93 ± 5.73	5.6	[0.0, 16.6]	0.0313
Global satisfaction	54.01 ± 31.30	60.5	[−8.3, 91.7]	54.19 ± 25.94	52.8	[22.2, 93.1]	0.18 ± 23.09	1.4	[−40.2, 30.5]	0.7188

SD standard deviation, *TSQM-9* Treatment Satisfaction Questionnaire for Medication

[a] *P* value based upon Wilcoxon signed rank sum test

events or deteriorations in pulmonary hemodynamic factors. Prospective studies in a larger group of patients are needed to confirm the safety of this formulation in long-term clinical use. Epoprostenol AS was approved in February 2013 in Japan as a generic drug with the same potency and effectiveness as the originally approved drug, epoprostenol GM. As intravenous epoprostenol sodium therapy may result in high medical costs, the introduction of cheaper generic drugs may help to reduce medical expenditure for treating PAH.

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Conflict of interest. Yuichi Tamura declares no conflict of interest. Tomohiko Ono declares no conflict of interest. Keiichi Fukuda declares no conflict of interest. Toru Satoh declares no conflict of interest. Shigetake Sasayama declares no conflict of interest.

Compliance with Ethics Guidelines. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients included in the study.

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Inducible Intrapulmonary Arteriovenous Shunt in a Patient with Beriberi Heart

To the Editor:

Transient intrapulmonary arteriovenous shunt (IPAVS) can be induced by exercise (1–3), a physiological condition producing high cardiac output. However, the involvement of IPAVS in pathological high-output condition, such as beriberi heart, is unclear.

The patient was a 37-year-old male with schizophrenia who was referred with a 2-week history of acute heart failure. His symptoms included dyspnea, hypotension, and prominent edema in the lower extremities. He was mildly hypoxemic with pulse oximeter-oxygen saturation (Sp_{O_2}) of 93% in room air and required subnasal oxygen. His echocardiographic findings were remarkable, showing the exaggerated left ventricular ejection fraction of 84% calculated by the Teichholz method. He received intravenous furosemide and inotropic agents for 2 weeks after the admission, until the diagnosis of beriberi heart was made with a low serum erythrocyte thiamine pyrophosphate level (11.0 ng/ml; normal range, 24–66 ng/ml). After the intravenous thiamine repletion, his hypotension, oligouria, and congestion quickly improved. He was weaned off of the inotropic agents, diuretics, and oxygen over the next few days. The catheter-based hemodynamic data in acute phase revealed excessively high cardiac output (14.0 L/min), decreased systemic vascular resistance (1.9 Wood units), and slightly elevated mean pulmonary artery pressure (26 mm Hg). The cardiac output normalized to 6.6 L/min 5 weeks after thiamine repletion.

The contrast-enhanced echocardiography in the supine position was performed to evaluate IPAVS as a possible contributor to his hypoxemia. In the acute phase, microbubbles opacified the left atrium seven cardiac cycles after the initiation of right-atrial opacification (Figure 1A, *upper panel*; Sp_{O_2} 99% with subnasal oxygen). In contrast, microbubble opacification became negative after the thiamine repletion (Figure 1A, *lower panel*; Sp_{O_2} 99% in room air). The transient, inducible IPAVS was identified in beriberi heart. The patient showed no recurring signs or symptoms of heart failure thereafter without further cardiovascular medications. The chest X-rays demonstrated improvement during this period (Figure 1B).

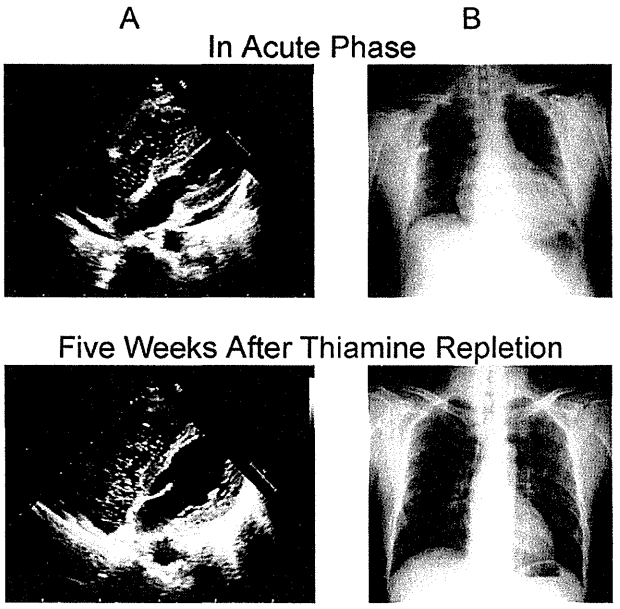

Figure 1. (*A*) Contrast-enhanced echocardiography and (*B*) chest X-rays in the acute phase and 5 weeks after the thiamine repletion. In the acute phase, microbubbles opacified the left atrium seven cardiac cycles after the initiation of right-atrial opacification (*A, upper panel*), whereas 5 weeks after the thiamine repletion, microbubble opacification became negative (*A, lower panel*). (*B*) The chest X-rays display improvement of cardiomegaly and congestion during this period.

The IPAVS not only exists in pathological characteristics such as hepatopulmonary syndrome, but also is induced by the certain physical properties in healthy individuals. The exercise-induced IPAVS using saline contrast microbubbles was demonstrated in subjects breathing room air (2). It is dependent on external environment; breathing hyperoxia prevented the exercise-induced IPAVS, whereas breathing hypoxia and normoxia resulted in a significant exercise-induced IPAVS (4).

The exact role of exercise-induced IPAVS is unclear. It was speculated that shunts might act as "pop-off valves" in response to increases in flow and pulmonary vascular resistance (1, 3) and function to reduce pulmonary vascular resistance and improve right ventricular function during exercise (5). Our patient with beriberi heart revealed high pulmonary flow resulting in slightly elevated pulmonary arterial pressure. This condition resembles exercise, where the inducible IPAVS may emerge in adaptive response to protect the pulmonary vasculature and right ventricle against pressure or volume overloading.

The pathological implications of the exercise-induced IPAVS are directed at two critical conditions: cerebral embolism and hypoxemia. The exercise-induced IPAVS may facilitate a pathway for emboli to circumvent the pulmonary microcirculation (6). It may also contribute to the reduction in pulmonary gas exchange efficiency that occurs during exercise (7). The immobile patients with beriberi heart are presumably more likely to clot than healthy individuals, and thus, predispose themselves to

Author Contributions: S. Nakano dealt with the patients and designed and drafted the manuscript. Y.T. interpreted the data, mostly regarding catheter-based hemodynamics, as he specialized in pulmonary hypertension. He also revised and approved the manuscript, and strongly recommended the clinical importance of intrapulmonary shunt in high-flow pulmonary hypertension. M.A., an echocardiography specialist, performed the contrast-enhanced echocardiography and interpreted the data. She revised and approved the manuscript. Y.S., J.T., T.M., T.S., and S. Nishimura are all members of the cardiology department. They collaborated in designing acquisition, analysis, and interpretation of data over the conference on multiple occasions. They all revised and approved the contents of the manuscript. K.F., who advised the other authors to construct the scientific way of thinking, greatly contributed to the report.

suffer cerebral emboli via inducible IPAVS. The hypoxemia that may develop in some patients with high-output cardiac failure may be a result or, conceivably, an enhancer of inducible IPAVS. Furthermore, although not proven, inducible IPAVS can be theoretically observed in other forms of high-output cardiac conditions in clinical setting, that is, anemia, thyrotoxicosis, sepsis, or administration of high dose of inotropic agents.

In conclusion, our patient with beriberi heart showed transient, inducible IPAVS. The potential impact of inducible IPAVS on unexplainable cerebral embolism or hypoxemia in pathological high-output conditions may become a novel investigational target.

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Erratum: Evolving Epidemiology of Pulmonary Arterial Hypertension

The authors would like to correct an error in the table that appears in their editorial published in the October 15, 2012 issue of the *Journal* (1). The tenth column of the table is labeled "Treatment Status on Enrollment"; in this column, the authors list the medicines that patients with pulmonary arterial hypertension (PAH) were on at the time of enrollment in the various registries. In the entry for the PAH registry in the United Kingdom and Ireland (2), the authors erroneously included the medicines that the patients were prescribed during the time of the registry (thus, the medicines that patients were started on). However, as is correctly mentioned in the text of the editorial, at the time of enrollment these patients were on no medicines. Therefore, that cell in the table should be changed to read "No PAH-specific therapies on enrollment."

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Erratum: Dieulafoy's Disease of the Bronchus

The authors would like to make a correction to their article published in the December 1, 2012 issue of the *Journal* (1). The middle initial was omitted for Dr. Fishman; his name should have appeared as Elliot K. Fishman.

Reference

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ORIGINAL ARTICLE

Bone morphogenetic protein receptor type 2 mutations, clinical phenotypes and outcomes of Japanese patients with sporadic or familial pulmonary hypertension

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ABSTRACT

Background and objective: **Mutation of *bone morphogenetic protein receptor type 2* (*BMPR2*) is a cause of pulmonary arterial hypertension (PAH). We measured the prevalence of this mutation and its impact on the phenotypes and long-term clinical outcomes in Japanese patients.**

Methods: **Between 1999 and 2007, we consecutively enrolled and, until March 2012, followed 49 Japanese patients with PAH, including nine familial cases from seven families. We genotyped *BMPR2*, using direct sequencing and multiplex ligation-dependent probe amplification, to examine (i) the prevalence of *BMPR2* mutations and gene rearrangement, (ii) the relationship between *BMPR2* genotype and clinical phenotypes, and (iii) the long-term clinical outcomes of mutation carriers versus non-carriers under state-of-the-art medical therapy.**

Results: ***BMPR2* mutations were present in four of the seven families (57%) and in 14 of the 40 patients (35%) with sporadic PAH. The mean age at onset of PAH was 37.4 years in *BMPR2* carriers, versus 25.9 years in non-carriers ($P = 0.0025$). The gender distribution and hemodynamic status at time of diagnosis were similar regardless of the mutation status. The 5-year survival rate after diagnosis of PAH was 88.5% in *BMPR2* mutation carriers versus 80.9% in non-carriers (ns).**

Conclusions: **The prevalence of *BMPR2* mutations in Japanese with PAH was similar to that reported in other populations. At onset of PAH, *BMPR2* mutation non-carriers were, on average, younger than carriers, possibly due to the heterogeneity of this subpopulation. With state-of-the-art therapy, the long-term survival of patients with PAH was high, regardless of the mutation status.**

SUMMARY AT A GLANCE

The 57 and 35% prevalence of *BMPR2* mutations measured in Japanese patients with familial and sporadic pulmonary arterial hypertension, was similar to that measured in other populations. In this study of patients on state-of-the art medical therapy, the mean long-term survival of patients with versus without *BMPR2* mutations was similar.

Key words: *bone morphogenetic protein receptor type 2,* Japanese population, mutation, pulmonary arterial hypertension.

Abbreviations: *BMPR2, bone morphogenetic protein receptor type 2;* LTOT, long-term oxygen therapy; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a lethal disease due to abnormal cell proliferation, progressive narrowing and increase in the resistance of the pulmonary arterial vessels and, eventually, right heart failure. The recent development of effective pharmaceuticals, such as prostaglandin (PG) I_2, endothelin receptor antagonists, phosphodiesterase (PDE) 5 inhibitors, and supportive treatment with long-term oxygen therapy (LTOT), have markedly improved the prognosis of patients suffering from PAH. However, a therapy based on treatment of the underlying cause of disease has not been developed, the patients' quality

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of life remains impaired and the life-long treatment has considerable, negative socio-economic consequences.[1] Therefore, besides the search for new treatments, improved management being individualized and based on the expected long-term outcome and predicted response to drug therapy is needed.

In year 2000, *bone morphogenetic protein receptor type 2* (*BMPR2*) was identified as one of the genes responsible for the development of PAH.[2,3] This mutation is mechanistically associated with the abnormal proliferation of pulmonary vascular smooth muscle cells and dysfunction of vascular endothelial cells.[4,5] Missense, nonsense or frame-shift mutations due to the deletion or insertion of 1–4 nucleotides are present in 33–50% of patients suffering from familial PAH and in 12–26% of patients presenting with sporadic PAH.[6–12] In addition, exonal deletion/duplication of *BMPR2* gene is found in substantial frequency, suggesting that direct sequencing alone had underestimated the prevalence of heritable PAH,[13–15] which, in 2008, was classified as an independent disease entity in the Dana Point classification.[16]

Several studies have examined the different ages at time of onset, the disease severity, or both, in patients suffering from PAH with versus without *BMPR2* mutations,[7,17–22] although the clinical genetics of *BMPR2* mutations in Japanese are poorly known. In one study, *BMPR2* mutations were present in four of four (100%) patients presenting with familial PAH, and in 12 of 30 (40%) sporadic cases.[9] No study, however, has determined the frequency of *BMPR2* exonal deletion/duplication or the genotype-phenotype association in Japanese adult patients suffering from PAH.

The aims of this study were to examine (i) the prevalence of *BMPR2* mutations in Japanese patients presenting with PAH, using direct sequencing and multiplex ligation-dependent probe amplification (MLPA) methods, (ii) the relationship between *BMPR2* genotype and clinical phenotypes, (iii) the >5-year clinical outcomes of patients with versus without *BMPR2* mutations under state-of-the-art medical therapy.

METHODS

Patient population

Between October 1999 and March 2007, we enrolled 49 consecutive patients suffering from sporadic or familial PAH diagnosed in the adult or paediatric cardiology divisions of Keio University Hospital or referred from other institutions during the study period. The diagnosis of PAH was based on the measurements of a mean pulmonary artery pressure >25 mm Hg at rest and a pulmonary capillary wedge pressure <15 mm Hg by right heart catheterization.[23] Secondary pulmonary hypertension was excluded by history, blood tests, chest computed tomography scan, nuclear ventilation-perfusion scan and echocardiography. We classified nine patients from seven families as familial cases of PAH. We collected information from the medical records, including age, disease manifestations, New York Heart Association (NYHA) functional class at diagnosis, medications and other treatments and clinical outcomes. The study was reviewed and approved by the Institutional Review Board of Keio University School of Medicine (approval number 13-3-2), and all patients, or their parents if they were < 20 years of age, granted their written consent to participate.

Genotyping of the *BMPR2* gene

Genomic DNA was extracted from peripheral blood, and all exons in the *BMPR2* gene were amplified by polymerase chain reaction (PCR), using the primers described in Supporting Information Table S1. The PCR condition was one cycle at 95° for 9 min, 40 cycles at 95° for 30 s and 60° for 1 min and one cycle at 72° for 5 min. The nucleotide sequences of the amplified fragments were identified by direct sequencing, using an ABI 3700 sequencer (Applied Biosystems Inc, Foster City, CA, USA).

Exonal deletions or duplications were examined by the MLPA method, using the Salsa MLPA kit (MRC-Holland, Amsterdam, the Netherlands) as recommended by the manufacturer.

Statistical analysis

The data are presented as means ± standard deviation (SD) or counts. The phenotypes of patients with versus without *BMPR2* mutations were compared, using Student's *t*- or Fisher's exact tests, as appropriate. Survival and time to onset of LTOT were analysed by the Kaplan–Meier method, and the differences between groups compared by log-rank test. The statistical analyses were performed, using GraphPad Prism, version 4 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Patient characteristics

Important characteristics of the 49 patients, of whom 40 suffered from sporadic and nine from familial PAH from seven families, are shown in Table 1. The male to female ratio was approximately 1:2. The average age at the time of diagnosis was 30.4 years (range 6–59), the most prevalent disease manifestation was dyspnoea on exertion and nearly two-thirds of patients were in NYHA functional class III or IV.

Prevalence and types of *BMPR2* mutations

BMPR2 mutations were identified in 14 of 40 (35%) sporadic cases and in four of seven (57%) familial cases (Table 2). Of these 18 mutations, 10 (56%) were nonsense or frameshift, all resulting in premature stop codons. We identified five missense mutations in the ligand-binding or in the kinase activity domains, in exons 3, 8, and 9. The three nonsense mutations were identical to previously reported mutations,[3,9,21,22] and one (C2617C>T) was found in two unrelated patients. In contrast, all the frameshift or missense mutations identified in this study had not been pre-

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Table 1 Characteristics of patients with familial and sporadic pulmonary arterial hypertension

	Pulmonary arterial hypertension		
	All patients ($n = 49$)	Familial ($n = 9$)	Sporadic ($n = 40$)
Men	17 (34.7)	3 (33.3)	14 (35.0)
Age at time of diagnosis, year	30.4 ± 13.1	36.1 ± 15.5	29.2 ± 12.4
Disease manifestations at time of diagnosis			
Symptoms			
Dyspnoea on exertion alone	25 (51.0)	5 (55.6)	20 (50.0)
Dyspnoea at rest	3 (6.1)	0	3 (7.5)
Syncope	6 (12.2)	0	6 (15.0)
Cough	1 (2.0)	1 (11.1)	0
Haemoptysis	1 (2.0)	0 (0.0)	1 (2.5)
Abnormal ECG at annual health check	13 (26.5)	3 (33.3)	10 (25.0)
NYHA functional class at diagnosis			
I	1 (2.0)	1 (11.1)	0 (0.0)
II	16 (32.7)	2 (22.2)	14 (35.0)
III	28 (57.1)	4 (44.4)	24 (60.0)
IV	1 (2.0)	1 (11.1)	0 (0.0)
Undetermined	4 (8.2)	1 (11.1)	3 (7.5)

Values are means ± standard deviation, or numbers (%) of observations.
ECG, electrocardiogram; NYHA, New York Heart Association.

Table 2 Individual *bone morphogenetic protein receptor type 2* mutations

	Type				
Patient no	Disease	Mutation	Exon	Nucleotide change	References
1	sporadic	Frameshift	3	c.339insA	
2	familial	Missense	3	c.276A>C	
3	sporadic	Nonsense	3	c.274C>T	[26]
4	sporadic	Frameshift	4	c.497delT	
5	sporadic	Missense	8	c.992A>G	
6	sporadic	Missense	8	c.1016T>A	
7	sporadic	Missense	9	c.1151C>T	
8	sporadic	Missense	9	c.1157A>C	
9	familial	Nonsense	9	c.1207C>T	[9]
10	sporadic	Frameshift	12	c.2504insA	
11	sporadic	Nonsense	12	c.2617C>T	[3,21,22,26]
12	sporadic	Nonsense	12	c.2617C>T	[3,21,22,26]
13	sporadic	Frameshift	12	c.2500delCAAA	
14	sporadic	Frameshift	12	c.2128delC	
15	familial	Frameshift	12	c.2009delC	
16	sporadic	exonal deletion	10		
17	familial	exonal deletion	1–3		
18	sporadic	exonal deletion	3		

viously described. Exonal deletions, identified in two sporadic and in one familial cases, represented 17% (3 of 18) of the mutations.

Phenotypes and patients management

Table 3 shows the clinical phenotypes, drug therapy and outcomes of the patients with versus without *BMPR2* mutations. The mean age of the former at the time of diagnosis of PAH was 37.4 years, significantly older ($P = 0.0025$) than the latter (25.9 years; Supporting Information Figure S1). The gender distributions, NYHA functional classes, and hemodynamic status at the time of diagnosis were similar in both groups.

All patients were treated with intravenous PGI_2, endothelin receptor antagonists, PDE5 inhibitors, alone or in combination. In addition, four patients received tyrosine kinase inhibitors in clinical trials. LTOT was introduced during the study period in 57.9% of patients with, and 60.0% of patients without

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Table 3 Characteristics, treatments and outcomes of patients stratified by *bone morphogenetic protein receptor type 2* (*BMPR2*) mutation status

	BMPR2 mutation		
	Present ($n = 19$)†	Absent ($n = 30$)	*P*
Demography			
Men	6 (31.6)	11 (36.7)	ns
Age at time of diagnosis, year	37.4 ± 12.7	25.9 ± 11.3	0.0025
Functional and hemodynamic status			
NYHA functional class ≥2	17 (89.5)	28 (93.3)	ns
Mean pulmonary artery pressure, mm Hg	60.8 ± 15.4	58.8 ± 12.0	ns
Pulmonary vascular resistance, mm Hg/l/min/m^2	21.5 ± 9.4	18.6 ± 8.6	ns
Cardiac output, l/min	3.0 ± 1.4	3.2 ± 0.9	ns
Pharmaceutical treatment			
Intravenous prostaglandin I_2	12 (63.2)	21 (70.0)	ns
Phosphodiesterase 5 inhibitors	13 (68.4)	14 (46.7)	ns
Endothelin receptor antagonists	12 (63.2)	17 (56.7)	ns
Tyrosine kinase inhibitors	2 (10.6)	2 (6.6)	ns
Clinical outcomes			
Long-term oxygen therapy (LTOT)	11 (57.9)	18 (60.0)	ns
Age at initiation of LTOT	44.6 ± 11.1	27.2 ± 12.4	0.0008
5-year survival rate	17 (88.5)	24 (80.9)	ns
Age at time of death	47.5 ± 9.8	27.0 ± 7.2	0.0020
Lung transplantation	0	3 (10.0)	ns
Age at time of death or transplantation	47.5 ± 9.8	23.2 ± 8.9	0.001

Values are means ± standard deviation, or numbers (%) of observations.
†Two members from the same family with mutation 17 were included in the analysis.
NYHA, New York Heart Association.

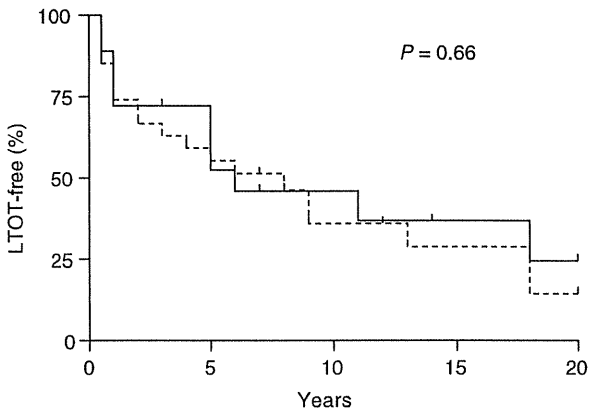

Figure 1 Years between onset of symptoms and introduction of long-term oxygen therapy (LTOT).
Solid lines, *BMPR2* carriers; dotted lines, mutation-free patients.

BMPR2 mutations. When LTOT was initiated, the patients without *BMPR2* mutations were 27.2 ± 12.4 years of age, significantly younger ($P = 0.0008$) than the carriers of mutations (44.6 ± 11.1 years), probably reflecting the age difference at the time of diagnosis. The time between onset of symptoms and introduction of LTOT was similar in both groups (Fig. 1).

Patient survivals

The median and mean ± SD overall follow-up was 79 and 80.3 ± 41.9 months, respectively. The 5-year survival rate after diagnosis of PAH was >80% (Table 3) and the overall patient survival similar (Fig. 2 and Supporting Information Fig. S2) regardless of the presence or absence of *BMPR2* mutations. On average, the patients without *BMPR2* mutations were significantly younger ($P = 0.002$) at the time of death (27.0 ± 7.2 years) than the carriers of mutations (47.5 ± 9.8 years), also reflecting the age difference at the time of diagnosis.

DISCUSSION

Our study showed that the prevalence of *BMPR2* point mutations and exonal deletions in Japanese patients suffering from PAH is similar to those observed in other ethnic populations. Furthermore, during this >5-year prospective observation of patients on optimal medical therapy, the evolution of disease towards lung transplantation or death was not influenced by the presence of *BMPR2* mutations.

Our observations suggest that *BMPR2* mutations are not detected by genotyping with direct sequencing alone in a considerable proportion of patients. In a French PAH registry, 115 *BMPR2* mutations were identified among 382 cases of PAH, of which 20 (17%) were exonal deletions.[20] In a study from Germany,[22] exonal deletions were present in six cases (12%) among 49 mutations,[22] and seven cases were detected among 50 mutations (14%) in 305 Han Chinese patients.[21] The prevalence was similar in our own observations with three cases among 18 mutations

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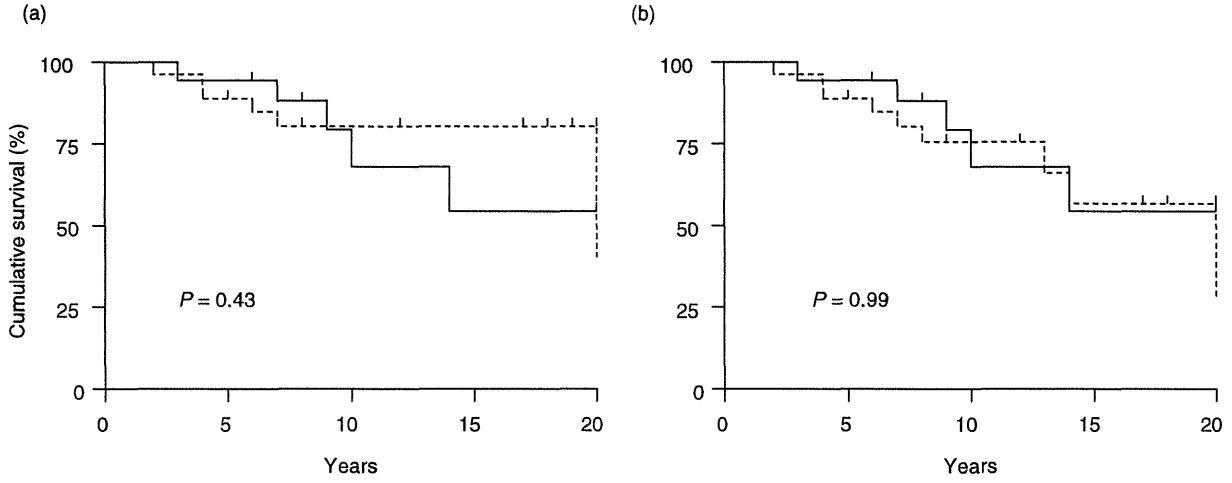

Figure 2 Years between onset of symptoms and death (a) or death/lung transplantation (b). Solid lines, *BMPR2* carriers; dotted lines, mutation-free patients.

(17%) and, combining all results, exonal deletions account for approximately 15% of *BMPR2* mutations among all ethnic groups.

On the other hand, the rates of *BMPR2* mutations have been inconsistent among studies of patients suffering from PAH. In several studies in Caucasians from Europe and the United States, 11–40% of sporadic and 55–70% of familial cases of PAH were *BMPR2* mutation-positive, including point mutations and exonal deletion/duplications.[15,17,18,20,22,24] In contrast, data in Asians are limited. Morisaki *et al.* studied 30 Japanese cases of sporadic PAH by direct sequencing and identified point mutations in 40%,[9] a slightly higher prevalence than the 12 cases among 40 subjects of sporadic PAH in our cohort (30%). In contrast, two studies performed in Han Chinese found a 14–17% prevalence of *BMPR2* mutations in patients presenting with sporadic PAH.[21,25] This discrepancy between Japanese and Han Chinese may be due to genetic backgrounds, different diagnostic means of excluding secondary PAH, or different genotyping techniques.

All missense mutations we identified were new and located in the kinase or ligand-binding domain, as previously reported. The frameshift mutations were also new. On the other hand, the three nonsense mutations (C274T, C1207T, C2617T) and one exonal deletion (exon 10) had been previously described.[3,9,21,22,26]

Except for the significantly older age of *BMPR2* mutation carriers at the time of diagnosis, the demographic and clinical characteristics of the two study groups were similar. In contrast to our observations, some of the previous studies found that patients who carried *BMPR2* mutations developed the disease at a younger age than non-carriers. The mean age of the mutation carriers at the time of diagnosis in our study (37.4 years) was similar with that reported by others (35.8 to 38.5 years).[17,19,20,22,24] However, the average age of our non-mutation carriers at the time of diagnosis was younger compared to other reports, because our *BMPR2* mutation-negative group included six patients who were <15 years old at the time of diagnosis. *BMPR2* mutations are less prevalent in children compared to adults, suggesting that the genetic factors behind the development of PAH are different in children.[27–30] A putative explanation is the high proportion of *ACVRL-1* mutation among children presenting with PAH,[28–30] a genetic defect that affects younger patients and is associated with a worse prognosis than the *BMPR2* mutation.[24] The heterogeneous genetic profile of groups without *BMPR2* mutations might explain the variable age at disease onset between different studies.

The 5-year survival of our patients suffering from PAH was >80%, which was better than the rates previously reported.[31] All study subjects were receiving intravenous PGI2, oral endothelin receptor antagonists, PDE5 inhibitors, either alone or in combination, which probably contributed to improved survival as suggested in meta-analyses and registry data.[32] Recent studies from France,[17] China[33] and Japan[34] also reported 5-year survival rates comparable to our data, although the relatively young age in the subjects in our study might also have affected the outcome. In addition, we were able to demonstrate that the prognosis of carriers and non-carriers of *BMPR2* mutations are similar under standard pharmacotherapy.

Although the prognosis of PAH patients has improved, there is no effective approach to prevent the development of the disease in subjects with *BMPR2* mutation. The transmission rate of *BMPR2* mutations to offspring is 50% with a penetration rate of approximately 20%,[35] suggesting that any offspring from a *BMPR2* mutation-carrier has a 10% risk to develop clinical PAH in the lifetime. Therefore, prenatal or pre-implantation genetic screening for PAH may be considered.[36] Benefits and risks of genetic testing and *in vitro* fertilization have to be thoroughly discussed between physicians and affected families.[37]

One of the limitations of our study is the modest number of patients enrolled at a single-centre. This could have imposed a referral bias, reflected by the

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younger age at the time of diagnosis compared to previous reports, although it is partly due to the inclusion of paediatric patients in our study as discussed above. Another limitation of our study is that our search was limited to *BMPR2* mutations. Although we could not identify *BMPR2* mutation in approximately half of the familial cases, their clinical phenotypes and prognosis were similar to those in sporadic PAH with *BMPR2* mutations, suggesting that '*BMPR2*-negative' familial PAH may be associated with unidentified *BMPR2* mutations in untranslated regions.[38] Future studies should also include an analysis of mutations in other genes, such as *ACVRL-1* and *endoglin*, in hope of developing a personalized management of PAH.

In conclusion, *BMPR2* mutations were present in >1/3 of Japanese patients suffering from PAH. With the currently available treatment options, the long-term clinical outcomes of patients with versus without *BMPR2* mutations were similar.

Acknowledgement

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Age at time of diagnosis of PAH in 19 *BMPR2* mutation carriers versus 30 non-carriers.

Figure S2 Overall survival (a) and survival free from death or lung transplantation (b) after diagnosis of PAH. Solid lines, patients with *BMPR2* mutations; dotted lines, mutation-free patients.

Table S1 Polymerase chain reaction primers.

●シンポジウム：各種の肺高血圧症治療における診断のポイント 5

肺高血圧症の診察所見—特に S3，S4 について—

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はじめに

肺高血圧症の診察所見としては P2 亢進，右心系 S3・S4，三尖弁閉鎖不全，肺動脈弁閉鎖不全，チアノーゼ，浮腫などが挙げられ，各々の出現頻度も原発性肺高血圧症においては，P2 亢進（93%），右心系 S3（23%）・S4（38%），三尖弁閉鎖不全（40%），肺動脈弁閉鎖不全（13%），チアノーゼ（20%），浮腫（32%）と報告されている[1]。本報告では右心カテーテル検査の直前に肺高血圧症患者より記録した心音図を解析して，血行動態との関係を検討したが，S3，S4 の解析を主眼とした。

1　対　　象

2009 年 4 月より 2012 年 3 月の間に右心カテーテル検査（総計 724 回施行）目的で入院し，Danapoint 分類で肺高血圧症の境界領域とされる平均肺動脈圧（mPA）が 20 mmHg 以上の 102 例，138 回の心音図検査を対象とした（最高で 1 例につき 4 回の入院あり）。男女比は 21：81，年齢は 53±15 歳であった。原因疾患は肺動脈性肺高血圧症（PAH）64 例（特発性（IPAH）37 例，膠原病（CTDPH）18 例，先天性心疾患（CHDPH）7 例，門脈性 2 例），慢性肺血栓塞栓症（CTEPH）38 例であった。

2　方　　法

1）心 音 図

右心カテーテル検査の朝，心音聴取をして所見を記載するとともに，心音図計（フクダ電子社製 MES-1000）を使用して心音を電子的に記録し，後日再度聴取して確認した。実際に聴取した心音の所見を優先し，記録した心音図上の所見を補助所見として，両者を総合して心音所見とした。心音の記録部位は左室心尖部（心尖拍動がみえない場合には第 5 肋間，中鎖骨線上と前腋窩線の中間部）と第 3 肋間胸骨左縁とした。

2）心臓カテーテル検査

通常行われている方法で各心内圧（平均右房圧（mRA），右室拡張末期圧（RVEDP），mPA，肺動脈血管抵抗（PVR））を測定後，肺動脈と動脈（左橈骨動脈）より採血を行って酸素飽和度を測定し，Fick 法で心拍出量（CO）を算出した。

3）心音図所見

今回は特に S4 に注目し，カテーテル検査値との関係を対比した。

3　結　　果

1）右心カテーテル検査結果

mRA 6±4 mmHg，PA 72±22/24±10（42±13）mmHg，PVR 9.5±5.4 単位であった。

2）S4 の解析

138 回施行中 2 回は心房細動であったので除外した。残り 136 回（101 例）中，27 回（18%）で S4 を認めた。**図 1** に血行動態との関係を示す。血行動態の四つの指標（mPA，PVR，CO，mRA）は S4 の存否に関して重なりが大きいが，mPA のみは 28 mmHg 以下では S4 は認めてい

Toru Satoh：Physical findings in pulmonary hypertension

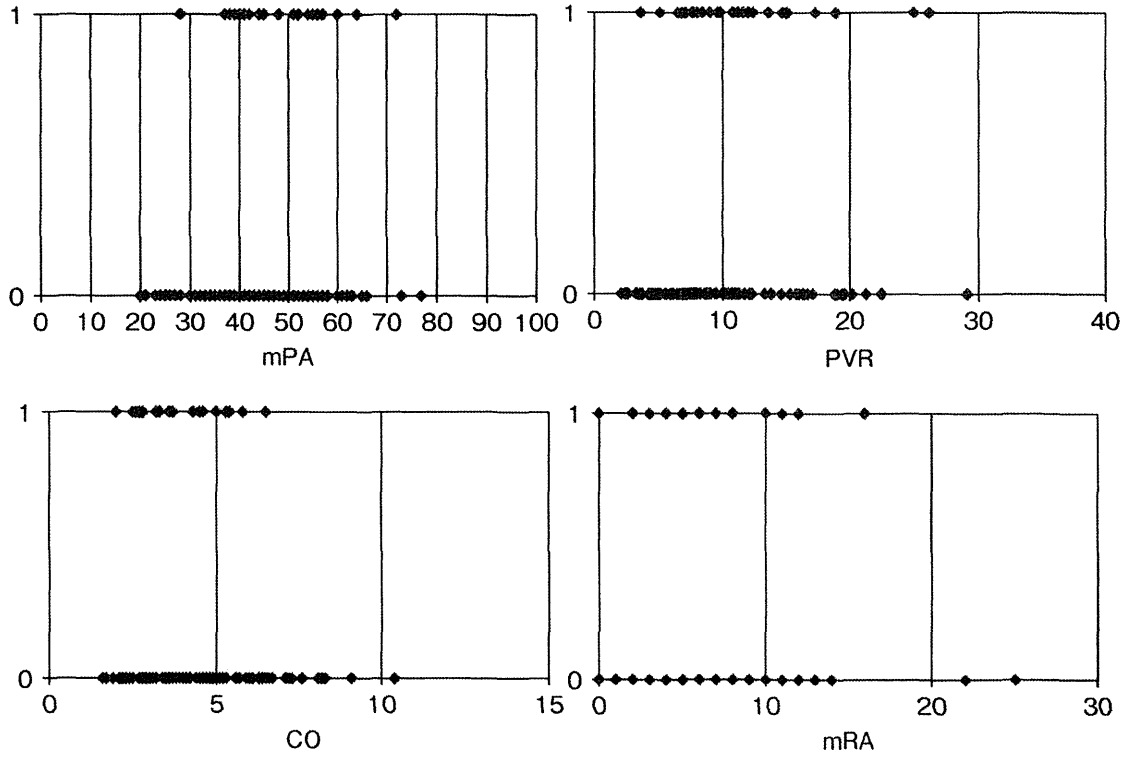

図 1　S4 と血行動態との関係

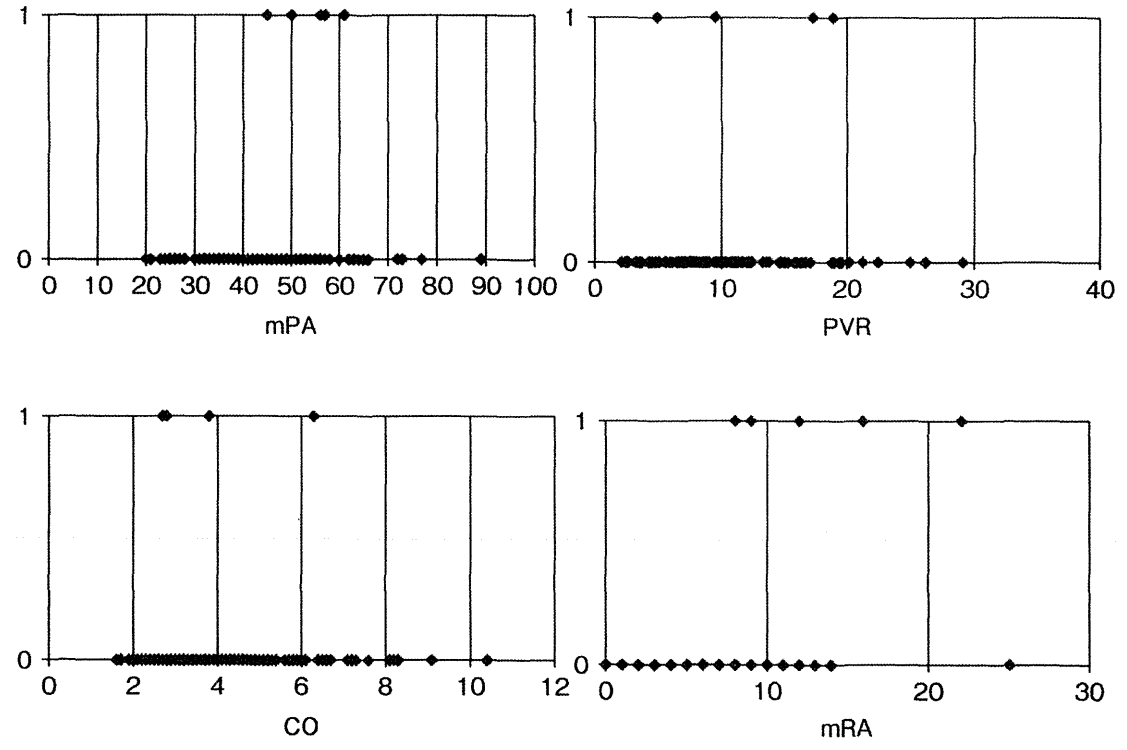

図 2　S3 と血行動態との関係

ない。mPA が 28 mmHg の症例は 62 歳男性で CTEPH が原病であった。高血圧，糖尿病を認め，心音図を見直してみると呼気に S4 は大きくなっており左心性と思われた。他の患者で左心性と思われる S4 を検索したが，認められなかった。したがって S4 を認める患者の mPA の最低値は 37 mmHg であった。mPA により PH を分類すると，25～35 mmHg までを軽症，36～50 mmHg を中等症，51 mmHg 以上を重症とすると，S4 が認められると mPA からみた血行動態は中等症以上といえる。

3）S3 の解析

138 回（102 例）の測定中 5 回（5 例，4%）に S3 を認めた。**図 2** をみると S3 の存否と血行動態指標の間にも重なりが大きかったが，S4 と同様に mPA が最も S3 の存在と関連した。mPA 45mmHg 未満では S3 は認められなかった。すなわち S3 の存在は重症の PH であることを示唆していた。

4　考　　察

日常臨床の経験から，肺高血圧症の診察所見で S3，S4 は重症例に認められるが，実際にどれくらいの血行動態でこれらの過剰心音が聴取されるかを検討した。血行動態指標として mPA がこれらの心音の存否に最も関連が強く，S4 は mPA が中等症以上，S3 はほぼ重症例で聴取された。

1987 年に米国で 200 例近くの原発性肺高血圧症が集められて種々の臨床所見が検討された。診察所見も検討され冒頭に各所見の頻度を示したが，今回の検討では S3，S4 の頻度が少なかった。これには二つの理由が考えられる。第一に，本検討では原疾患が IPAH のみならず CTEPH などの軽症例を含んでおり，S3，S4 の出現頻度が少なかったと思われた。二つ目の理由は，本検討では心音図で両者の区別を行ったが，S3 と S4 の鑑別は聴診だけでは難しい症例が少なからずあり，米国の報告では S4 を S3 と解釈され S3 が過大に評価された可能性があった。

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