Synergistic Effects of *ACE* Insertion/Deletion and *GNB3* C825T Polymorphisms on the Efficacy of PDE-5 Inhibitor in Patients with Pulmonary Hypertension

Rintaro Nishimura^a Nobuhiro Tanabe^{a,b} Ayumi Sekine^a Hajime Kasai^a
Rika Suda^a Fumiaki Kato^a Takayuki Jujo^{a,b} Toshihiko Sugiura^a
Ayako Shigeta^{a,b} Seiichiro Sakao^a Koichiro Tatsumi^a

Departments of ^aRespirology and ^bAdvanced Medicine in Pulmonary Hypertension, Graduate School of Medicine, Chiba University, Chiba, Japan

Key Words

Pulmonary hypertension · Gene polymorphism · *ACE* · *GNB3* · Phosphodiesterase-5 inhibitor

Abstract

Background: The insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme gene (*ACE*) and the C825T polymorphism in the G-protein $\beta 3$ subunit gene (*GNB3*) are associated with the efficacy of phosphodiesterase-5 inhibitor (PDE-5I) in erectile dysfunction. In addition, *GNB3* genotypes could be associated with clinical worsening in pulmonary hypertension (PH) treated with PDE-5I. However, no studies have described the synergistic effects of gene polymorphisms on drug efficacy in patients with PH. **Objectives:** We aimed to examine the effects of combined *ACE*/*GNB3* polymorphisms on the efficacy of PDE-5I in patients with PH. **Methods:** This was a retrospective uncontrolled study. Ninety patients with pulmonary arterial hypertension (PAH) or chronic thromboembolic PH (CTEPH) were treated with PDE-5I. Freedom from clinical worsening and pre- and post-treatment parameters, including the 6-min walk distance (6MWD) and serum brain natriuretic peptide

(BNP) levels, were compared between patients with *ACE*/*GNB3* II/TT and non-II/TT genotypes. **Results:** Time to clinical worsening was significantly longer in patients with the II/TT genotype than in those with the non-II/TT genotype (5-year freedom from clinical worsening: 100 vs. 48.8%, respectively; $p = 0.018$), even in patients with CTEPH alone. Post-treatment 6MWD and BNP levels in patients with the II/TT genotype tended to be better than those in patients with the non-II/TT genotype. The *ACE*/*GNB3* genotype was a significant predictor of clinical worsening, even after adjusting for pulmonary vascular resistance and 6MWD. **Conclusions:** *ACE* and *GNB3* polymorphisms may synergistically influence the efficacy of PDE-5I in patients with PH.

© 2016 S. Karger AG, Basel

Introduction

Pulmonary hypertension (PH) is defined as having a mean pulmonary arterial pressure (Ppa) of ≥ 25 mm Hg at rest as assessed by right heart catheterization [1, 2]. Clinically, PH has been classified into 5 categories [3]. Among them, pulmonary arterial hypertension (PAH) is

characterized by progressive vascular remodeling, which can result in right heart failure and death [2]. Chronic thromboembolic PH (CTEPH) results from pulmonary arterial obstruction by unresolved, organized thrombi. In addition, small vessel diseases similar to PAH also exist with CTEPH [4]. Therefore, medical therapy for PAH has been applied in patients with CTEPH who have inoperable disease or persistent PH after pulmonary endarterectomy [5]. The effects of phosphodiesterase-5 inhibitor (PDE-5I), which has been shown to be efficient in treating PAH, are accomplished by enhancing the downstream response of nitric oxide (NO)-mediated vasodilatation. In addition, sildenafil, a PDE-5I, may have beneficial effects on platelet activation, pulmonary vascular remodeling, and cardiac function [6].

Angiotensin-converting enzyme (ACE) is a key factor in the renin-angiotensin system, which regulates blood flow and plays a fundamental role in the onset and/or progression of cardiovascular diseases [7–9]. The ACE gene contains a polymorphism based on the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 [10]. We previously reported that the ACE D allele may be a factor predicting poor prognosis in medically treated patients with CTEPH [11].

Guanine nucleotide-binding proteins (G proteins) are ubiquitously expressed essential proteins that mediate signal transduction. The G-protein complex consists of three subunits: α , β , and γ . Siffert et al. [12] reported a silent C825T polymorphism in exon 10 of the G-protein $\beta 3$ subunit (*GNB3*) gene encoding the G-protein $\beta 3$ subunit [13].

The drug response to sildenafil in patients with erectile dysfunction differs depending on the ACE I/D and *GNB3* C825T genotypes [14]. In addition, the *GNB3* C825T polymorphism influences the efficacy of sildenafil in the treatment of PH [15]. We thus sought to investigate the association between the combined ACE/*GNB3* polymorphisms and the clinical efficacy of PDE-5I in patients with PH.

Materials and Methods

Study Design and Patients

This study was a retrospective, single-center cohort investigation. Ninety patients with either PAH ($n = 32$) or CTEPH ($n = 58$) were treated with a PDE-5I (sildenafil: $n = 74$; tadalafil: $n = 16$) at Chiba University Hospital between September 2003 and December 2013. PAH or CTEPH was defined as a mean Ppa of ≥ 25 mm Hg with normal wedge pressure on right heart catheterization. In patients with PAH, the inclusion criteria further contained a normal or mottled perfusion pattern with normal ventilation on ven-

tilation-perfusion scans. In patients with CTEPH, the inclusion criteria needed the presence of symptoms such as dyspnea on exertion for more than 6 months, segmental or larger lung perfusion defects concomitant with normal ventilation scans, and chronic thromboembolic findings confirmed on pulmonary angiography.

Determination of the ACE and *GNB3* Genotypes

Genomic DNA was extracted from 200 μ l of peripheral whole blood using a QIAamp DNA Mini Kit (Qiagen, Valencia, Calif., USA), according to the manufacturer's protocols. Genotyping was performed by polymerase chain reaction with an ABI 7300 Prism Sequence Detection System (Applied Biosystems, Carlsbad, Calif., USA). DNA amplification and genotype determination were performed in a single assay using Taqman SNP genotyping assays (Applied Biosystems), with allele-specific fluorogenic probes. Detection of the ACE I/D polymorphism was carried out using primers and methods described by Koch et al. [16]. Genotypes for the C825T polymorphism of the *GNB3* gene were determined as previously described [15]. The patients were blinded to the genotype data for the duration of the trial. The baseline and post-treatment parameters were compared between patients with ACE/*GNB3* II/TT and non-II/TT genotypes.

Study Protocol

Although sildenafil was approved in Japan for the treatment of PAH in 2008, it has been available at our institution since 2003 in a doctor-oriented trial. The disease severity was evaluated prior to administration of the PDE-5I and after treatment within 3 months according to the following parameters: the systolic Ppa estimated on Doppler echocardiography (sPpa), the 6-min walk distance (6MWD) test, serum levels of brain natriuretic peptide (BNP), and World Health Organization (WHO) functional class. Right heart catheterization data obtained within 2 months of initiation of PDE-5I treatment were also evaluated. Starting from the initiation of PDE-5I treatment, the time to clinical worsening defined as death or hospitalization due to right heart failure was compared between genotype groups. Patients who were treated with additional PAH therapies and patients with CTEPH who received surgery or balloon pulmonary angioplasty were censored at that time. The baseline characteristics predicting freedom from clinical worsening were also investigated.

The study protocol was approved by the Research Ethics Committee of Chiba University School of Medicine, and written informed consent was given by each patient.

Statistical Analysis

All data are expressed as the mean $\pm$ standard deviation (SD). Genotype frequencies were compared between patients and controls using the χ^2 test. The Hardy-Weinberg equilibrium for genotype distribution was estimated by the χ^2 test. Comparisons between the two groups were performed by the unpaired t test, Wilcoxon's rank-sum test, or the χ^2 test. Freedom from clinical worsening curves between the two groups were derived from the Kaplan-Meier method and compared by the log-rank test. To determine whether the gene polymorphism was an independent predictor, 6MWD and pulmonary hemodynamic parameters were examined using a multivariate Cox proportional hazard analysis. Differences with p values < 0.05 were considered significant. All statistical analyses were performed using JMP 9.0.0 (SAS Institute Inc., Tokyo, Japan).

Table 1. Characteristics of patients with PAH or CTEPH

<i>ACE/GNB3</i>	All (n = 90)	II/TT (n = 15)	Non-II/TT (n = 75)	p
Age, years	54.2±14.0	48.9±9.32	55.3±14.6	0.03
Female/male	68/22	11/4	57/18	0.83
Disease (PAH/CTEPH)	32/58	3/12	29/46	0.17
Mean Pra, mm Hg	7.24±6.25	5.50±2.81	7.61±6.72	0.33
Mean Ppa, mm Hg	48.0±12.1	46.8±7.46	48.3±12.9	0.59
CI, l/min/m ²	2.54±0.68	2.54±0.45	2.54±0.72	0.76
PVR, Wood units	10.8±5.0	10.4±2.9	10.9±5.4	0.99
PaO ₂ , Torr	61.9±13.4	59.7±10.1	62.4±14.0	0.69
Pretreatment (monotherapy/combination)	53/37	8/7	29/46	0.29
6MWD, m				
Before treatment	358±92.7	375±91.9	355±93.3	0.48
After treatment	380±89.9	404±51.1	375±96.0	0.27
Δ6MWD	24.0±69.3	38.1±53.1	20.8±72.5	0.9
sPpa, mm Hg				
Before treatment	84.7±25.5	82.0±28.9	85.3±25.0	0.67
After treatment	73.6±26.0	67.3±35.6	74.7±24.2	0.39
ΔsPpa	-10.6±18.7	-11.2±24.8	-10.4±17.6	0.72
WHO functional class (I/II/III/IV)				
Before treatment	0/29/46/15	0/5/8/2	0/24/38/13	0.93
After treatment	0/45/42/3	0/10/5/0	0/35/37/3	0.32
BNP, pg/ml				
Before treatment	323±419	244±350	337±430	0.25
After treatment	192±273	105±161	208±288	0.19
BNP <100 pg/ml, %				
Before treatment	34.9	53.9	31.5	0.12
After treatment	59.0	84.6	54.3	0.04

Values are expressed as the mean ± SD or n. Pra = Right atrial pressure; CI = cardiac index, PaO₂ = arterial oxygen tension; Δ = post-treatment data – pretreatment data.

Results

Patient characteristics at baseline are shown in table 1. The mean age was 54.2 ± 14.0 years, and there were more women than men. The mean Ppa and pulmonary vascular resistance (PVR) were 48.0 ± 12.1 mm Hg and 10.8 ± 5.0 Wood units, respectively. Fifty-three patients received PDE-5I as monotherapy, while 37 patients received PDE-5I in addition to other PAH medical therapies including beraprost (n = 13), bosentan (n = 23), and epoprostenol (n = 5).

Table 2 shows the distributions of the *ACE* I/D and *GNB3* C825T genotypes in PH patients and controls, taken from previous reports of Japanese individuals [11, 17]. The observed genotype frequencies were in agreement with the frequencies predicted by the Hardy-Weinberg equilibrium equation. The genotype distribution of *GNB3* polymorphisms in the patient groups was similar to that

in the control group, while that of *ACE* polymorphisms was significantly different between patients with CTEPH and controls (higher frequency of II genotype, p = 0.048).

No significant differences were observed in the baseline characteristics between patients with the II/TT (n = 15) and non-II/TT (n = 75) genotypes, except for higher age in the non-II/TT genotype group (p = 0.03; table 1). The 6MWD, sPpa, BNP, and WHO functional class values improved in both groups after treatment with PDE-5I. There were no significant differences between the two genotype groups in both pre- and post-treatment values, although patients with the II/TT genotype showed a tendency toward longer 6MWD values and lower BNP levels compared to patients with other genotypes after administration of PDE-5I. In addition, the frequency of post-treatment BNP <100 pg/ml was significantly higher in patients with the II/TT genotype compared to those with the non-II/TT genotype (84.6 vs. 54.3%, respectively; p = 0.04).

Fig. 1. Comparison of Kaplan-Meier curves of freedom from clinical worsening between patients with the *ACE*/*GNB3* II/TT genotype and patients with the non-II/TT genotypes in the PDE-5I treatment group. Patients with the II/TT genotype showed significantly longer time to clinical worsening than patients with the non-II/TT genotypes (5-year freedom from clinical worsening: 100 vs. 48.6%, respectively; $p = 0.018$).

The association between the *ACE* I/D polymorphism and freedom from clinical worsening in patients treated with PDE-5I was analyzed. The 5-year freedom from clinical worsening rates in the II and ID/DD groups were 67.0 and 42.9%, respectively ($p = 0.17$). Comparison of the *GNB3* TT genotype with the TC/CC genotype in freedom from clinical worsening showed that the TT group exhibited a significantly longer time to clinical worsening than the TC/CC group (5-year freedom from clinical worsening: 78.7 vs. 48.0%, respectively; $p = 0.04$). The combined effects of *ACE* and *GNB3* polymorphisms showed that no patients with the *ACE*/*GNB3* II/TT genotype ($n = 15$) exhibited clinical worsening. The II/TT group had significantly better clinical courses than the non-II/TT group (5-year freedom from clinical worsening: 100 vs. 48.6%, respectively; $p = 0.018$; fig. 1). In addition, in the monotherapy group, patients with the II/TT genotype also tended to have a longer time to clinical worsening (5-year freedom from clinical worsening: 100 vs. 52.2%, respectively; $p = 0.18$). In contrast, in the combination therapy group, the II/TT genotype was significantly associated with better clinical course (5-year freedom from clinical worsening: 100 vs. 40.8%, respectively; $p = 0.04$).

Table 2. Frequencies of the *ACE* and *GNB3* polymorphisms in patients and controls

<i>ACE</i>	II	ID	DD
Controls (Tanabe, 2006)	40 (41.2)	42 (43.3)	15 (15.5)*
All ($n = 90$)	48 (53.3)	28 (31.1)	14 (15.6)*
CTEPH ($n = 58$)	34 (58.6)	14 (24.1)	10 (17.3)*
PAH ($n = 32$)	14 (43.75)	14 (43.75)	4 (12.5)
<i>GNB3</i>	TT	TC	CC
Controls (Shioji, 2004)	282 (25.5)	536 (48.5)	287 (26)
All ($n = 90$)	24 (26.7)	44 (48.9)	22 (24.4)
CTEPH ($n = 58$)	18 (31)	28 (48.3)	12 (20.7)
PAH ($n = 32$)	6 (18.8)	16 (50)	10 (31.2)

Data are the number of subjects with percentages in parentheses.
* $p < 0.05$ by χ^2 test.

Univariate Cox proportional hazard analysis showed that a higher 6MWD, a lower mean Ppa, a lower PVR, a lower sPpa, and the *ACE*/*GNB3* II/TT genotype were significant predictors of freedom from clinical worsening (table 3). The II/TT genotype was also associated with freedom from clinical worsening when adjusted according to 6MWD and PVR in multivariate analyses. Furthermore, table 4 showed that the *GNB3* genotype (TT vs. TC/CC) alone had a significant effect on clinical worsening in patients with PH treated with a PDE-5I (hazard ratio 0.3, $p = 0.03$), whereas the *ACE* genotype (II vs. ID/DD) alone was not associated (hazard ratio 0.56, $p = 0.17$). However, the combination of two polymorphisms (II/TT vs. non-II/TT) was associated with a substantially improved clinical course (hazard ratio 5.72×10^{-7} , $p = 0.002$), even after adjustment for age (adjusted hazard ratio 6.31×10^{-7} , $p = 0.003$).

Higher age, lower PaO_2 , and higher BNP were observed in patients with CTEPH compared to those with PAH. Table 5 shows baseline characteristics of patients with CTEPH. No significant differences were observed between II/TT and non-II/TT genotypes, except higher age for the non-II/TT genotype (49.5 ± 10.1 and 59.5 ± 12.3 years, respectively; $p = 0.01$). There were no significant differences between the two genotype groups in post-treatment 6MWD, sPpa, and WHO functional class, whereas patients with the II/TT genotype showed lower BNP levels compared with patients having other genotypes after administration of PDE-5I (115 ± 173 and 275 ± 290 pg/ml, respectively; $p = 0.02$).

Table 3. Predictors of freedom from clinical worsening determined by univariate and multivariate Cox proportional hazard analyses

Factor	All patients (n = 90)				CTEPH patients (n = 58)			
	univariate HR (95% CI)	p	multivariate HR (95% CI)	p	univariate HR (95% CI)	p	multivariate HR (95% CI)	p
Age	1.03 (0.996–1.066)	0.08			1.03 (0.983–1.082)	0.22		
Gender (female/male)	1.66	0.33			1.03	0.97		
PAH:CTEPH	1.16	0.73						
<i>ACE</i> / <i>GNB3</i> II/TT (vs. non-II/TT)	5.72×10^{-7}	0.002	3.89×10^{-7}	0.02	4.78×10^{-7}	0.005	3.87×10^{-7}	0.04
Monotherapy/combination	0.6	0.23			0.43	0.13		
sPpa (mm Hg)	1.02 (1.002–1.037)	0.03			1.02 (0.995–1.046)	0.12		
6MWD (m)	0.99 (0.986–0.996)	0.0007	0.99 (0.988–1.001)	0.09	0.99 (0.986–0.999)	0.03	1.00 (0.990–1.007)	0.71
CI (l/min/m ²)	0.47 (0.172–1.134)	0.1			0.51 (0.110–1.981)	0.35		
PaO ₂ (Torr)	0.99 (0.947–1.027)	0.56			0.99 (0.918–1.064)	0.86		
Mean Ppa (mm Hg)	1.07 (1.017–1.115)	0.008			1.03 (0.968–1.102)	0.31		
PVR (Wood unit)	1.16 (1.050–1.268)	0.005	1.12 (1.007–1.245)	0.04	1.15 (0.991–1.321)	0.07	1.16 (0.980–1.391)	0.08

HR = Hazard ratio; 95% CI = 95% confidence interval; CI = cardiac index; PaO₂ = arterial oxygen tension.

Table 4. Genotype interaction between *ACE* and *GNB3* in relation to clinical worsening

	All patients (n = 90)				CTEPH patients (n = 58)			
	HR	p	HR adjusted by age	p	HR	p	HR adjusted by age	p
<i>ACE</i> / <i>GNB3</i> II/TT (vs. non-II/TT)	5.72×10^{-7}	0.002	6.31×10^{-7}	0.003	4.78×10^{-7}	0.005	5.32×10^{-7}	0.009
<i>ACE</i> II (vs. ID or DD)	0.56	0.17	0.57	0.18	0.46	0.16	0.44	0.13
<i>GNB3</i> TT (vs. TC or CC)	0.3	0.03	0.33	0.06	0.46	0.20	0.55	0.37

Patients with the *ACE* II and *GNB3* TT genotypes tended to have better clinical courses than patients with non-II and non-TT genotypes, respectively, but this difference was not significant (5-year freedom from clinical worsening: 73.6 vs. 33.4%, $p = 0.15$; 72.2 vs. 49.6%, $p = 0.22$, respectively). However, patients with CTEPH with the II/TT genotype had significantly longer time to clinical worsening than those with the non-II/TT genotype (5-year freedom from clinical worsening: 100 vs. 45.5%, respectively; $p = 0.03$) as well as in all PH patients (fig. 2). Although there was a similar tendency in patients with PAH, this difference was not statistically significant [5-year freedom from clinical worsening: 100% ($n = 3$) vs. 64.8% ($n = 29$), respectively; $p = 0.31$].

As with all patients, the data from univariate and multivariate Cox proportional hazard regression analyses in patients with CTEPH are shown (table 3). Univariate analysis revealed that the *ACE*/*GNB3* genotype and

6MWD were significant predictors of clinical worsening. In addition, gene polymorphism was an independent factor on multivariate analysis. Evaluation of the synergistic effects of the *ACE*/*GNB3* genotype on clinical worsening was also similar for all patients with PH (table 4).

Discussion

Our results are the first to show that combined *ACE* I/D and *GNB3* C825T polymorphisms were associated with the efficacy of PDE-5I in Japanese patients with PAH or CTEPH. We previously investigated the influence of the *GNB3* C825T polymorphism on the efficacy of sildenafil in patients with PH and found that patients with the TT genotype had a better clinical course than patients with the TC/CC genotype [15]. Although other genetic differences may also contribute to the efficacy of modern

Table 5. Characteristics of patients with CTEPH

<i>ACE/GNB3</i>	All (n = 58)	II/TT (n = 12)	Non-II/TT (n = 46)	p
Age, years	57.4±12.5	49.5±10.1	59.5±12.3	0.01
Female/male	45/13	9/3	36/10	0.81
Mean Pra, mm Hg	6.81±3.97	5.00±2.96	7.29±4.10	0.12
Mean Ppa, mm Hg	47.5±11.5	43.8±5.72	48.5±12.4	0.11
CI, l/min/m ²	2.47±0.55	2.48±0.36	2.46±0.60	0.78
PVR, Wood units	10.9±4.61	9.94±2.49	11.1±5.03	0.33
PaO ₂ , Torr	56.1±9.47	58.5±8.39	55.4±9.75	0.40
Pretreatment (monotherapy/combination)	35/23	7/5	28/18	0.87
6MWD, m				
Before treatment	342±97.0	375±103	333±94.7	0.23
After treatment	356±89.2	404±57.5	343±92.6	0.09
Δ6MWD	25.7±47.6	41.6±59.5	21.0±43.7	0.59
sPpa, mm Hg				
Before treatment	84.3±23.5	79.8±29.2	85.5±22.0	0.48
After treatment	74.9±25.0	66.5±38.2	76.9±21.0	0.45
ΔsPpa	-8.34±15.6	-5.32±19.2	-9.07±14.8	0.52
WHO functional class (I/II/III/IV)				
Before treatment	0/12/33/13	0/3/7/2	0/9/26/11	0.83
After treatment	0/22/35/1	0/7/5/0	0/15/30/1	0.25
BNP, pg/ml				
Before treatment	387±462	256±375	420±479	0.13
After treatment	242±277	115±173	275±290	0.02
BNP <100 pg/ml, %				
Before treatment	32.7	54.5	27.3	0.08
After treatment	43.4	81.8	33.3	0.004

Values are expressed as the mean ± SD or n. CI = Cardiac index; PaO₂ = arterial oxygen tension.

PAH therapies, no further studies have focused on the effects of pharmacogenomic interactions in the treatment of PH. In this study, the *GNB3* C825T polymorphism alone significantly affected the efficacy of PDE-5I in the absence of clinical worsening, as described above, whereas the influence of the *ACE* I/D polymorphism alone on the clinical course was not significant. However, the combined effect of the two gene polymorphisms was substantial and significant in a synergistic manner. Moreover, multivariate analysis showed that the combined gene polymorphism was a significant predictor for a better clinical course after initiation of PDE-5I treatment, independent of 6MWD and PVR.

Our findings are consistent with several previous reports. In patients with erectile dysfunction, patients with the *GNB3* TT genotype or the *ACE* II genotype had a better response to sildenafil [14]. However, the mechanism through which these gene polymorphisms affect the PDE-5I response is not well established.

PDE-5I targets the NO pathway. NO stimulates dilation of vascular smooth muscle through the production of cyclic guanosine monophosphate (cGMP) [6, 18]. Serum and tissue ACE levels in *ACE* DD carriers are higher than those in individuals with the II genotype [19, 20]. Moreover, elevated ACE levels enhance the degradation of bradykinin, which stimulates the release of NO from endothelial cells [21].

The *GNB3* T allele has been shown to enhance vasoconstriction in response to several factors including angiotensin II, and to promote vasodilation of the human hand vein in response to glyceroltrinitrate [22–24]. These data indicate that gene polymorphisms are associated with vasodilation through the NO pathway and interactions between the renin-angiotensin system and G-protein-mediated signaling.

In the current study, we found that patients with the II/TT genotype tended to have a better clinical course than patients with the non-II/TT genotype in patients re-

Fig. 2. Comparison of Kaplan-Meier curves of freedom from clinical worsening between patients with the *ACE/GNB3* II/TT genotype and patients with the non-II/TT genotypes in the CTEPH group. Patients with the II/TT genotype showed significantly longer time to clinical worsening than patients with the non-II/TT genotypes (5-year freedom from clinical worsening: 100 vs. 45.5%, respectively; $p = 0.03$).

ceiving monotherapy. However, in the combination therapy group, the former had a significantly longer time to clinical worsening than the latter. Moreover, we also investigated the association between the gene polymorphisms and the efficacy of bosentan. Clinical worsening occurred in patients with the II/TT genotype and in patients with the non-II/TT genotype ($n = 43$; 3-year absence of clinical worsening: 75.0 vs. 85.5%, $p = 0.25$; data not shown). These results indicated that the *ACE/GNB3* polymorphism may be useful in predicting the efficacy of PDE-5I, regardless of the administration of other vasodilators.

In our study, we could not confirm any significant effects of gene polymorphisms on hemodynamic values, serum BNP levels, WHO functional class, and 6MWD values. However, patients with the II/TT genotype showed a slight tendency to have longer 6MWD values and lower BNP levels than patients with other genotypes after the administration of PDE-5I. In addition, the frequency of BNP <100 pg/ml after PDE-5I treatment was significantly higher in patients with the II/TT genotype than in patients with other genotypes. Moreover, tricuspid annular plane systolic excursion after treatment with PDE-5I was also better in patients with the II/TT genotype (data not

shown). Thus, the observed longer time to clinical worsening in patients with the II/TT genotype may be due to improved right ventricular function. For hemodynamic assessments, we measured only sPpa values after PDE-5I treatment. Therefore, further studies are needed to determine the acute effects of gene polymorphisms on the response to PDE-5I.

Although previously there was no reimbursement for PAH drugs for CTEPH until riociguat was covered by insurance, randomized, placebo-controlled trials with bosentan [25] or sildenafil [26] in patients with CTEPH showed significant improvements in pulmonary hemodynamics using these drugs. Although patients with CTEPH exhibit changes in the pulmonary microvasculature similar to those in patients with PAH, the pathogenesis of CTEPH differs from that of PAH. Considering these two distinct medical conditions, we also evaluated the effects of gene polymorphisms in patients with CTEPH alone. Similar to all PH patients, CTEPH patients with the II/TT genotype had a better clinical course after treatment with PDE-5I compared to that of patients with other genotypes. However, for patients with PAH, this difference did not reach statistical significance, probably due to the small number of patients with the II/TT genotype. In addition, multivariate analysis showed that the *ACE/GNB3* genotype was a significant predictor of clinical worsening, even in patients with CTEPH only.

Although associations between these gene polymorphisms and the risk of developing several diseases, such as atherosclerosis, diabetic nephropathy, and essential hypertension, have been reported [8, 27], no studies have shown associations between these gene polymorphisms and the development of PH. In this study, the two gene polymorphisms were not associated with the risk of PH and PAH. While we observed a higher frequency of the *ACE* II genotype in patients with CTEPH, this difference in our study may have resulted from sample bias due to the small number of patients, because our previous study, which included 95 patients with CTEPH, did not show those differences [11].

In recent years, the long-term effects of certain drugs have become difficult to evaluate due to the popularization of upfront combination therapy in PAH. However, upfront combination therapy is expensive and may not be accepted in every country. Therefore, additional studies are needed to determine the most effective drugs based on the status of gene polymorphisms.

The main limitation of this study was its design as a retrospective observational study performed at a single institution. Therefore, retrieval bias may have been pres-

ent because data were collected from clinical records that had different degrees of completeness. Moreover, there were no untreated control groups for definitely worse prognosis. It is possible that confounding factors could have influenced our results; however, baseline characteristics did not differ significantly between the two genotype groups. Another limitation was that the number of patients was relatively small when patients were divided into groups based on disease category or pretreatment. Additional studies with more patients are needed to confirm our results, particularly in patients with PAH. Despite these limitations, no patients with the *ACE/GNB3* II/TT genotype exhibited clinical worsening after initiation of PDE-5I in our study. Although each polymorphism alone could be an independent predictor of a better clinical course, combination analysis of gene polymorphisms may be more helpful for determining the appropriate treatment regimen in patients with PH, thereby improving efficacy and reducing costs.

In conclusion, the combination of *ACE* and *GNB3* polymorphisms was associated with the efficacy of PDE-5I in the treatment of PH.

Acknowledgments

The present study was supported in part by a grant to the Respiratory Failure Research Group from the Ministry of Health, Labour and Welfare of Japan, and a research grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (25461148).

Financial Disclosure and Conflicts of Interest

N.T. received honoraria for lectures from Actelion and Pfizer. K.T. received honoraria for lectures from Actelion. N.T., R.N., A.S. and T.J. belong to the endowed department sponsored by Actelion.

References

- 1 Fukumoto Y, Shimokawa H: Recent progress in the management of pulmonary hypertension. *Circ J* 2011;75:1801–1810.
- 2 Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G; ESC Committee for Practice Guidelines: Guidelines for the diagnosis and treatment of pulmonary hypertension: the task force for the diagnosis and treatment of pulmonary hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J* 2009;30:2493–2537.
- 3 Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, Gomez Sanchez MA, Krishna Kumar R, Landzberg M, Machado RF, Olschewski H, Robbins IM, Souza R: Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2013;62:D34–D41.
- 4 Hoeper MM, Mayer E, Simonneau G, Rubin LJ: Chronic thromboembolic pulmonary hypertension. *Circulation* 2006;113:2011–2020.
- 5 Pepke-Zaba J, Delcroix M, Lang I, Mayer E, Jansa P, Ambroz D, Treacy C, D'Armini AM, Morsolini M, Snijder R, Bresser P, Torbicki A, Kristensen B, Lewczuk J, Simkova I, Barbera JA, de Perrot M, Hoeper MM, Gaine S, Speich R, Gomez-Sanchez MA, Kovacs G, Hamid AM, Jais X, Simonneau G: Chronic thromboembolic pulmonary hypertension (CTEPH): results from an international prospective registry. *Circulation* 2011;124:1973–1981.
- 6 Archer SL, Michelakis ED: Phosphodiesterase type 5 inhibitors for pulmonary arterial hypertension. *N Engl J Med* 2009;361:1864–1871.
- 7 Lindpaintner K, Pfeiffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH: A prospective evaluation of an angiotensin-converting enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 1995;332:706–711.
- 8 Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witterman JC: Ace polymorphisms. *Circ Res* 2006;98:1123–1133.
- 9 Hamelin BA, Zakrzewski-Jakubiak M, Robitaille NM, Bogaty P, Labbe L, Turgeon J: Increased risk of myocardial infarction associated with angiotensin-converting enzyme gene polymorphism is age dependent. *J Clin Pharmacol* 2011;51:1286–1292.
- 10 Rigat B, Hubert C, Corvol P, Soubrier F: PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). *Nucleic Acids Res* 1992;20:1433.
- 11 Tanabe N, Amano S, Tatsumi K, Kominami S, Igarashi N, Shimura R, Matsubara H, Kasahara Y, Takiguchi Y, Kuriyama T: Angiotensin-converting enzyme gene polymorphisms and prognosis in chronic thromboembolic pulmonary hypertension. *Circ J* 2006;70:1174–1179.
- 12 Siffert W, Roszkopf D, Siffert G, Busch S, Moritz A, Erbel R, Sharma AM, Ritz E, Wichmann HE, Jakobs KH, Horsthemke B: Association of a human G-protein beta3 subunit variant with hypertension. *Nat Genet* 1998;18:45–48.
- 13 Bae Y, Park C, Han J, Hong YJ, Song HH, Shin ES, Lee JE, Han BG, Jang Y, Shin DJ, Yoon SK: Interaction between *GNB3* c825t and *ACE* I/D polymorphisms in essential hypertension in Koreans. *J Hum Hypertens* 2007;21:159–166.
- 14 Eisenhardt A, Siffert W: Genetic risk factors for erectile dysfunction and genetic determinants of drug response – on the way to improve drug safety? *Herz* 2003;28:304–313.
- 15 Sekine A, Tanabe N, Sugiura T, Shigeta A, Jujo T, Nishimura R, Sakao S, Kasahara Y, Tatsumi K: Polymorphism of the G protein beta3 subunit gene influences the efficacy of sildenafil in patients with pulmonary hypertension. *Intern Med* 2014;53:291–297.
- 16 Koch W, Latz W, Eichinger M, Ganser C, Schomig A, Kastrati A: Genotyping of the angiotensin I-converting enzyme gene insertion/deletion polymorphism by the TaqMan method. *Clin Chem* 2005;51:1547–1549.
- 17 Shioji K, Kokubo Y, Mannami T, Inamoto N, Morisaki H, Mino Y, Tagoi N, Yasui N, Iwami N: Association between hypertension and the alpha-adducin, beta1-adrenoreceptor, and G-protein beta3 subunit genes in the Japanese population; the Suita study. *Hypertens Res* 2004;27:31–37.
- 18 Ramani GV, Park MH: Update on the clinical utility of sildenafil in the treatment of pulmonary arterial hypertension. *Drug Des Devel Ther* 2010;4:61–70.

- 19 Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F: An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;86:1343–1346.
- 20 Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, Schunkert H: Angiotensin-converting enzyme in the human heart. Effect of the Deletion/Insertion polymorphism. *Circulation* 1995;92:1387–1388.
- 21 Becker AJ, Uckert S, Stief CG, Truss MC, Machtens S, Scheller F, Knapp WH, Hartmann U, Jonas U: Possible role of bradykinin and angiotensin II in the regulation of penile erection and detumescence. *Urology* 2001;57: 193–198.
- 22 Siffert W: Cardiovascular pharmacogenetics: on the way toward individually tailored drug therapy. *Kidney Int Suppl* 2003;S168–S171.
- 23 Wenzel RR, Siffert W, Bruck H, Philipp T, Schafers RF: Enhanced vasoconstriction to endothelin-1, angiotensin II and noradrenaline in carriers of the GNB3 825T allele in the skin microcirculation. *Pharmacogenetics* 2002;12:489–495.
- 24 Torfgard K, Ahlner J, Axelsson KL: Relaxation of bovine mesenteric arteries by glyceryl trinitrate and other nitro-compounds: evidence for partly different mechanisms of action. *Pharmacol Toxicol* 1990;67:216–221.
- 25 Jais X, D'Armini AM, Jansa P, Torbicki A, Delcroix M, Ghofrani HA, Hoeper MM, Lang IM, Mayer E, Pepke-Zaba J, Perchenet L, Morganti A, Simonneau G, Rubin LJ: Bosentan for treatment of inoperable chronic thromboembolic pulmonary hypertension: benefit (bosentan effects in inoperable forms of chronic thromboembolic pulmonary hypertension), a randomized, placebo-controlled trial. *J Am Coll Cardiol* 2008;52:2127–2134.
- 26 Suntharalingam J, Treacy CM, Doughty NJ, Goldsmith K, Soon E, Toshner MR, Sheares KK, Hughes R, Morrell NW, Pepke-Zaba J: Long-term use of sildenafil in inoperable chronic thromboembolic pulmonary hypertension. *Chest* 2008;134:229–236.
- 27 Zheng H, Xu H, Cui B, Xie N, Wang Z, Luo M: Association between polymorphism of the G-protein beta3 subunit C825T and essential hypertension: an updated meta-analysis involving 36,802 subjects. *Biol Res* 2013;46: 265–273.