Polymorphism of the G Protein β3 Subunit Gene Influences the Efficacy of Sildenafil in Patients with Pulmonary Hypertension

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Abstract

Objective The C825T polymorphism in the G protein β3 subunit gene (GNB3) influences the efficacy of sildenafil in patients with erectile dysfunction. The effects of this polymorphism on the therapeutic response to sildenafil in patients with pulmonary hypertension remains unknown. To investigate whether the GNB3 C825T polymorphism is associated with the clinical efficacy of sildenafil in patients with pulmonary hypertension.

Methods Fifty-nine patients (age: 55.6±13.3 [SD] yrs., mean pulmonary arterial pressure (Ppa): 52±11 mmHg) with pulmonary arterial hypertension or chronic thromboembolic pulmonary hypertension were treated with sildenafil. The pre- and post-treatment parameters, including pulmonary hemodynamics measured using right heart catheterization, the systolic pulmonary arterial pressure estimated on Doppler echocardiography (sPA), the six-minute walk distance (6MWD) and freedom from clinical worsening, were compared between the patients with the TT and CT/CC genotypes.

Results The pretreatment parameters were not significantly different between the two groups, with the exception of a lower mean Ppa in the TT group. The post-treatment World Health Organization (WHO) class was significantly better (p=0.03) and the 6MWD values trended toward improvement in the TT genotype patients compared with that observed in the CC/CT genotype patients (p=0.05). The time to clinical worsening was significantly longer in the TT genotype patients than in the CC/CT genotype patients (3-year freedom from clinical worsening: 83.1% vs. 46.0%, p=0.02), while the TT genotype was found to be a significant predictor of freedom from clinical worsening, even after adjusting for the baseline mean Ppa.

Conclusion The GNB3 C825T polymorphism influences the efficacy of sildenafil in patients with pulmonary hypertension.

Key words: chronic thromboembolic pulmonary hypertension, sildenafil, polymorphism, pharmacogenomics

significant mortality (5-7). Hence, more than one-third of CTEPH patients are also treated medically, approximately half of whom are given oral PAH therapies, such as PDE-5 inhibitors and ERA (8).

In recent randomized-controlled studies, modern PAH therapies have been shown to improve pulmonary hemodynamics, the six-minute walk distance (6MWD), time to clinical worsening, and other parameters, although the response to medical treatment varies among patients. Part of the variability in patient response may be due to underlying genetic differences; however, few studies have investigated the effects of pharmacogenomic variability in the setting of PH (9).

Sildenafil, a selective inhibitor of cyclic guanosine monophosphate-specific PDE-5, has been proven to exhibit efficacy in treating PAH (10). Sildenafil enhances nitric oxide (NO)-mediated vasodilatation and may have additional beneficial effects on platelet activation, pulmonary vascular remodeling and the cardiac function (11). Recently, a small, randomized, double-blind, placebo-controlled pilot study suggested that sildenafil may be a therapeutic option for treating a distal type of CTEPH (12).

The G protein β3 subunit gene (GNB3) may account for the observed variability in the patient response to sildenafil. The C825T polymorphism of GNB3 consists of a C to T base substitution in exon 10, that result in the truncation of 41 amino acids of the protein (13, 14). In vitro studies have shown that this truncated protein is associated with the increased activation of heterometric G proteins. The GNB3 T allele is also associated with significantly altered clinical responses to several cardiovascular drugs. For example, T allele carriers demonstrate coronary vasoconstriction in response to azepexol and decreased blood pressure in response to thiazides (15, 16).

It has previously been reported that the presence of the GNB3 C825T polymorphism influences the efficacy of sildenafil in patients with erectile dysfunction (17). However, to our knowledge, no pharmacogenomic data exist regarding the variable effects of sildenafil or other medical therapies in patients with PH. We thus sought to investigate the association between the GNB3 C825T polymorphism and the clinical efficacy of sildenafil in patients with pulmonary hypertension.

Materials and Methods

Study design

This was a retrospective, single-center, cohort study that enrolled consecutive patients.

Patients

Between March 2003 and October 2011, fifty-nine patients with PAH (n=19) or CTEPH (n=40) were treated with sildenafil at Chiba University Hospital. The patient inclusion criteria included an elevated mean pulmonary arterial pressure (Ppa) of >25 mmHg and a normal wedge pressure as determined using right heart catheterization. In the PAH group, the inclusion criteria further included a normal or mottled perfusion pattern with normal ventilation on ventilation-perfusion scans. Of the nine-teen patients with PAH, ten were classified as having idiopathic or heritable PAH, and nine were classified as having PAH associated with one of the following: connective tissue disease (n=3), HIV (n=1), portal hypertension (n=2), or congenital heart disease (n=3). In the CTEPH group, the inclusion criteria included the presence of dyspnea on exertion for >6 months, segmental or larger perfusion defects with normal ventilation on ventilation-perfusion scans, and chronic thromboembolic findings on pulmonary angiography.

Determination of the GNB3 genotype

Genomic DNA was extracted from 200 μL of peripheral whole blood using the QIAamp® DNA Mini Kit (Qiagen, Valencia, USA). Genotyping was performed with the ABI 7000 Prism Sequence Detection System (Applied Biosystems, Carlsbad, USA). DNA amplification and genotype determination was performed in a single assay using the Taqman® SNP genotyping Assay (Applied Biosystems, Carlsbad), with allele-specific fluorogenic probes. The nucleotide sequences of the primers and probes were as follows: forward primer: 5’-GGC AGA CCA GGA GCT GAT CT-3’; reverse primer: 5’-TCG TCG TAG CCA GCG AAT AGT-3’; T-allele-specific probe : 5’-Fam-ATC ACG TCT GTG GCC-MGB-3’; C-allele-specific probe: 5’-Fam-CAC GTC CGT GCC-MGB-3’. The T- and C- allele-specific probes were labeled with 6-carboxyfluorescein (6FAM) and VIC, respectively, at the 5’ end. Both probes were labeled with the quencher dye 6-carboxytetramethyl-rhodamine (TAMRA) at the 3’ end. The thermo-cycling procedure consisted of 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 62°C for one minute. The patients were blinded to the genotype data for the duration of the trial. The baseline and post-treatment parameters were compared between the CC/CT and TT genotype groups.

Study protocol

Sildenafil was administered at a dose of 30-75 mg/day, with 56 of 59 patients receiving a dose of 60 mg/day. Of note, although sildenafil was approved in Japan for the treatment of PAH in 2008, it has been available at our institution since 2003 due to a doctor oriented clinical trial (approval number 240). The disease severity was evaluated prior to sildenafil administration and after three and six months of treatment (or at the time of clinical worsening if this occurred within three months of treatment) according to the following criteria: World Health Organization (WHO) functional class, the systolic pulmonary arterial pressure estimated on Doppler echocardiography (sPA), the serum level of brain natriuretic peptide (BNP), blood gas analysis parameters (room air), and 6MWD. Right heart catheterization data obtained within one month of sildenafil treatment in-
Statistical analysis

All data are expressed as the mean ± SD. The Hardy-Weinberg equilibrium equation for genotype distribution was estimated using the chi-square test. Comparisons between two groups were made using the unpaired t-test, Wilcoxon-rank-sum test, or chi-square test, as appropriate. Survival and freedom from clinical worsening curves were derived using the Kaplan-Meier method and compared using the log-rank test. Comparisons of the pre- and post-treatment parameters were made using the paired t-test or Wilcoxon-signed-rank test. To determine whether the GNB3 polymorphism was an independent predictor of disease, a multivariate Cox proportional hazards analysis was performed using pulmonary hemodynamic parameters, sPA, category of disease (PAH or CTEPH), and pretreatment (monotherapy versus combination therapy). P-values of less than 0.05 were considered to be significant. All statistical analyses were performed using commercially available software programs for Windows (JMP® 9.0.0, Japanese version, SAS Institute Inc., Tokyo, Japan).

Results

The baseline patient characteristics are shown in Table 1. The mean age was 55.6±13.3 [SD] years, and women outnumbered men (n=46 and 13, respectively). The mean Ppa and pulmonary vascular resistance (PVR) were 51.9±11.3 mmHg and 957±417 dyn.s.cm⁻², respectively. Sixteen patients were homozygous for the CC genotype, 28 were heterozygous for the CT genotype, and 15 were homozygous for the TT genotype. The observed genotype frequencies were in agreement with the frequencies predicted by the Hardy-Weinberg equilibrium equation. The pretreatment medications included beraprost (n=19), bosentan (n=12), and epoprostenol (n=4). Thirty-three patients received sildenafil as monotherapy, while 26 patients received sildenafil in addition to other PAH medical therapies. All patients received warfarin and home oxygen therapy.

No significant differences were identified in the baseline characteristics of the patients with the CC/CT and TT genotypes, with the exception of a higher mean Ppa in the CC/CT genotype groups (p=0.04) (Table 2). The WHO class, BNP levels, and 6MWD values improved in both groups after three to six months of sildenafil treatment. However, the post-treatment WHO class was significantly better in the TT genotype group than in the CC/CT genotype groups (p=0.03), and the change in 6MWD (Δ6MWD) exhibited a better tendency in the TT genotype group compared with that observed in the CC/CT genotype groups (p=0.05) (Table 3).

The association between the GNB3 C825T polymorphism and freedom from clinical worsening in the modern PAH treatment group is shown in Fig. 1. The patients with the TT genotype exhibited a significantly longer time to clinical worsening than those with the CT/CC genotypes (3-year freedom from clinical worsening: 83.1% vs. 46.0% p=0.02). Additionally, in the monotherapy (naïve) group, the TT genotype was associated with a longer time to clinical worsening (p=0.1). No patients with the TT genotype (n=8) demonstrated clinical worsening, while seven of the 26 patients with the CC/CT genotypes exhibited deterioration in their condition or died (p=0.039) (Fig. 2).

The patients with the TT genotype tended to have a longer time to clinical worsening, although this trend did not reach statistical significance when the patients were divided into the PAH and CTEPH groups (p=0.098 and p=0.1).
Table 2. Comparison of Pre- and Post-Treatment Parameters between the CC/CT and TT Genotypes

<table>
<thead>
<tr>
<th></th>
<th>CC/CT genotypes n=44</th>
<th>TT genotype n=15</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>57.4 ± 13.8</td>
<td>50.3 ± 10.5</td>
<td>ns</td>
</tr>
<tr>
<td>Gender (female: male)</td>
<td>37.7</td>
<td>9.6</td>
<td>ns</td>
</tr>
<tr>
<td>PAH/CTEPH</td>
<td>16/28</td>
<td>3/12</td>
<td>ns</td>
</tr>
<tr>
<td>Mean Ppa (mmHg)</td>
<td>53.4 ± 11.6</td>
<td>45.0 ± 7.0</td>
<td>0.04</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>2.57 ± 0.81</td>
<td>2.42 ± 0.83</td>
<td>ns</td>
</tr>
<tr>
<td>PVR (dyn.s.cm⁻⁵)</td>
<td>976 ± 446</td>
<td>870± 258</td>
<td>ns</td>
</tr>
<tr>
<td>PaO₂ (Torr)</td>
<td>57.9 ± 10.9</td>
<td>61.9 ± 16.2</td>
<td>ns</td>
</tr>
</tbody>
</table>

Pre WHO class (1: 2: 3: 4) 0: 6:30:8 0: 5: 5: 5 ns

Pre treatment data-Pre treatment data

Post BNP (pg/mL) 315 ± 357 150 ± 168 ns
Pre BNP (pg/mL) 415 ± 499 412 ± 393 ns
Post 6MWD (m) 354 ± 96 401 ± 63 ns
Pre 6MWD (m) 337 ± 102 360 ± 95 ns
Post sPA (mmHg) 73.0 ± 24.9 76.7 ± 31.9 ns
Pre sPA (mmHg) 84.3 ± 28.1 83.8 ± 26.0 ns

Pre sPA (mmHg) -105 ± 324 -261 ± 321 ns
Pre WHO class (1: 2:3 :4) 13:28:3 :0 0: 9: 6: 0 0.03

PaO₂ (Torr) 57.9 ± 10.9 61.9 ± 16.2 ns

Pre sPA (mmHg) 84.3 ± 28.1 83.8 ± 26.0 ns

Post WHO class (1: 2:3 :4) 13:28:3 :0 0: 9: 6: 0 0.03

Pre sPA (mmHg) 84.3 ± 28.1 83.8 ± 26.0 ns

Post treatment data

Δ: Post-treatment data

Table 3. Predictors of Freedom from Clinical Worsening Determined by Univariate and Multivariate Cox Proportional Hazard Analyses

<table>
<thead>
<tr>
<th>Factor</th>
<th>Univariate HR (95% CI)</th>
<th>Multivariate (invasive) HR (95% CI)</th>
<th>Multivariate (non-invasive) HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02 (0.98-1.06)</td>
<td>1.04 (1.00-1.08)</td>
<td>1.01 (0.98-1.03)</td>
</tr>
<tr>
<td>Gender (female: male)</td>
<td>1.87 (1.62-2.17)</td>
<td>1.02 (0.97-1.07)</td>
<td>1.01 (0.97-1.05)</td>
</tr>
<tr>
<td>Mean Ppa</td>
<td>1.08 (1.03-1.13)</td>
<td>1.07 (1.03-1.12)</td>
<td>1.06 (1.02-1.11)</td>
</tr>
<tr>
<td>CI</td>
<td>0.60 (0.29-1.27)</td>
<td>0.86 (0.52-1.42)</td>
<td>1.01 (0.72-1.41)</td>
</tr>
<tr>
<td>PVR</td>
<td>1.00 (1.00-1.004)</td>
<td>1.00 (1.00-1.004)</td>
<td>1.00 (1.00-1.004)</td>
</tr>
<tr>
<td>PaO₂</td>
<td>0.97 (0.90-1.04)</td>
<td>0.99 (0.93-1.06)</td>
<td>1.00 (0.95-1.05)</td>
</tr>
<tr>
<td>WHO class</td>
<td>1.85 (0.89-3.92)</td>
<td>1.85 (0.89-3.92)</td>
<td>1.85 (0.89-3.92)</td>
</tr>
<tr>
<td>sPA</td>
<td>1.02 (1.00-1.04)</td>
<td>1.02 (1.00-1.04)</td>
<td>1.02 (1.00-1.04)</td>
</tr>
<tr>
<td>6MWD</td>
<td>0.96 (0.990-1.001)</td>
<td>0.96 (0.990-1.001)</td>
<td>0.96 (0.990-1.001)</td>
</tr>
<tr>
<td>BNP</td>
<td>1.0007 (0.999-1.001)</td>
<td>1.0007 (0.999-1.001)</td>
<td>1.0007 (0.999-1.001)</td>
</tr>
<tr>
<td>GNB3</td>
<td>0.2 (0.03-0.71)</td>
<td>0.3 (0.01-0.77)</td>
<td>0.2 (0.01-0.77)</td>
</tr>
<tr>
<td>(TT:CC/CT)</td>
<td>0.03-0.71</td>
<td>0.03-0.74</td>
<td>0.03-0.74</td>
</tr>
<tr>
<td>monotherapy: combination</td>
<td>0.49</td>
<td>0.49</td>
<td>0.49</td>
</tr>
</tbody>
</table>

HR: hazard ratio, CI: confidence of interval

Figure 1. Comparison of Kaplan-Meier curves of freedom from clinical worsening between the patients with the CC/CT and TT genotypes in the modern PAH treatment group. The patients with the TT genotype showed a significantly longer time to clinical worsening than the patients with the CC/CT genotypes. (3-year absence of clinical worsening: 83.1% vs. 46.0%, p=0.02).

Figure 2. Comparison of Kaplan-Meier curves of freedom from clinical worsening between the patients with the CC/CT and TT genotypes in the monotherapy treatment group. No patients with the TT genotype (n=8) exhibited clinical worsening, while seven of the 26 patients with the CC/CT genotypes demonstrated deterioration of their condition or died (p=0.039).
The group receiving sildenafil monotherapy had a significantly better WHO class at baseline \( (p=0.036) \) and demonstrated significantly longer survival than the group receiving combination therapy \( (3\text{-year survival: } 92.8\% \text{ vs. } 67.1\%, \ p=0.038) \). There were no significant differences in survival between the patients with the TT and CC/CT genotypes. A multivariate Cox proportional hazard analysis revealed that a lower mean Ppa was an independent predictor of survival \( \text{(HR 163.1 CI 1.3-4722.1, } \ p=0.04) \); however, neither monotherapy nor the TT genotype were found to be a significant predictor of survival.

**Discussion**

Although modern PAH therapy has been shown to improve pulmonary hemodynamics and survival in patients with PAH and CTEPH, drug responses vary among patients. Genetic differences may contribute to this variability; however, to date, no studies have investigated the effects of pharmacogenomic interactions in the treatment of pulmonary hypertension. This report is the first to show that the \textit{GNB3} C825T polymorphism is associated with freedom from clinical worsening and an improved WHO class following treatment with sildenafil for pulmonary hypertension.

Several issues must be considered when interpreting these results. Our findings are consistent with those of previous reports showing that, among patients with erectile dysfunction, sildenafil treatment is more effective in patients with the TT genotype than in those with the CC/CT genotypes \( (16) \). The mechanism by which the \textit{GNB3} polymorphism affects the sildenafil response remains unknown. Sildenafil is a selective inhibitor of phosphodiesterase-5, the predominant phosphodiesterase expressed by pulmonary artery smooth muscle cells. By preventing cGMP degeneration, sildenafil induces elevated cGMP levels in the smooth muscle, thereby improving vasodilatation and antiproliferative effects \( (11) \). Several reports have documented enhanced G protein-mediated signal transduction in patients carrying the T allele of \textit{GNB3} \( (18) \). Torfgard et al. reported that glycerol trinitrate (GTN) relaxes vascular smooth muscle cells partially via G protein-dependent mechanisms \( (19) \). The nitric oxide (NO)/cyclic GMP pathways are altered in diabetic patients who carry the T allele \( (20) \), and the degree of improved insulin sensitivity and the vasodilatory response to GTN are much larger in T allele carriers \( (14) \). Sildenafil enhances smooth muscle relaxation in the corpora cavernosa, thus resulting in palliation of erectile dysfunction, as well as in the general circulation, as demonstrated by its blood pressure-lowering effects \( (21) \). Therefore, an increase in G protein activation associated with the T allele may enhance the effects of NO on pulmonary smooth muscle cells, leading to improved clinical outcomes in patients with pulmonary hypertension.

We did not observe any significant influence of genotype on the hemodynamic effects of sildenafil; however, the patients with the TT genotype did demonstrate a slightly greater degree of improvement in 6MWD. Sutharalingam et al. reported that, in patients with CTEPH, longer effects (changes in PVR after 12 months) of sildenafil treatment are correlated with the changes in PVR observed during vasoreactivity testing \( (12) \). However, we measured only the sPA values after sildenafil treatment, and further prospective studies of the acute effects of the TT genotype on the response to sildenafil are needed.

Sildenafil is not approved for CTEPH treatment in Japan or any other country. However, we found that sildenafil improved the 6MWD values and BNP levels, even in patients with CTEPH. The patients with the TT genotype had a tendency to exhibit a longer time to clinical worsening, although this trend did not reach statistical significance when the patients were divided into the PAH and CTEPH groups \( (p=0.098 \text{ and } p=0.093, \text{ respectively}) \).

The prognosis can vary in PAH patients, with one study
showing a better prognosis in patients with congenital heart disease than in patients with connective tissue disease or IPAH (22). Even in the IPAH group, the patients with the TT genotype continued to show a tendency towards a longer time to clinical worsening (p=0.098).

The addition of sildenafil to other modern PAH drugs might be less effective than sildenafil therapy alone. We found that the patients receiving sildenafil monotherapy demonstrated a longer survival time compared to those receiving combination therapy. These results may be explained by the more severe WHO classes observed in the patients receiving combination therapy.

On the other hand, pretreatment may enhance the efficacy of sildenafil and result in a longer time to clinical worsening in patients with the TT genotype. However, the monotherapy group showed a slightly longer time to clinical worsening when compared to the combination therapy group. Within both groups, the patients with the TT genotype demonstrated longer times to clinical worsening (3-year freedom from clinical worsening: 100% vs. 68.9%, p=0.1, and 71.4% vs. 35.8%, p=0.09, respectively). Moreover, no patients with the TT genotype (n=8) exhibited clinical worsening, while seven of the 26 patients with the CC/CT genotypes demonstrated deterioration in their condition or died (p=0.039).

We attempted to prevent selection bias by including all patients treated with sildenafil, regardless of the use of other treatment modalities. Several stable patients were treated with other medical PAH therapies, balloon pulmonary angioplasty or surgery to improve their clinical outcomes (23) and were thus removed from the study at that point. Notably, if these patients had been included in the study with the above interventions interpreted as clinical worsening events, the patients with the TT genotype would still have demonstrated significantly longer times to clinical worsening relative to that observed in the patients with the CC/CT genotypes (3-year freedom from clinical worsening: 56.4% vs. 36.7%, p=0.04).

We also investigated the association between the GNB3 polymorphism and the efficacy of bosentan. Following bosentan administration, the 3-year freedom from clinical worsening in the patients with the TT genotype was similar to that observed in the patients with the CC/CT genotypes (TT: 56.1% vs. CC/CT: 64.4%, p=0.60). We found no significant differences between the CC/CT and TT genotype groups in this parameter (data not shown). A longer time to clinical worsening in the patients with the TT genotype was limited to the clinical course after sildenafil therapy. Bosentan or other modern PAH therapies may contribute to improved survival in both patients with the TT and CC/CT genotypes.

Finally, the present study is limited by the fact that it is a retrospective cohort study performed at a single institution and includes a relatively small number of patients. In particular, the association between the GNB3 polymorphism and the efficacy of sildenafil in specific patient populations (based on disease category or treatment with monotherapy versus combination therapy) remains unknown. Despite the several above-described limitations, we believe that this study is unique and that it would be difficult to perform such a study prospectively, as administering upfront combination therapy for PAH (24) and balloon pulmonary angioplasty (BPA) for CTEPH before clinical worsening develops has recently become prevalent, especially in Japan. However, limitations on access to PAH medications in patients with less severe disease, and those receiving combination therapy have previously been reported in the UK and Australia, as a consequence of the cost-effectiveness of these therapies (25). In our study, no patients with the TT genotype exhibited clinical worsening with monotherapy. Therefore, the initial choice of drugs in patients with severe PH can be guided by the patient’s GNB3 genotype, thereby improving efficacy and reducing costs. However, conducting a short-term prospective study on the influence of this polymorphism on the efficacy of sildenafil is warranted to confirm our results.

Conclusion

The GNB3 C825T polymorphism influences the efficacy of sildenafil in the treatment of pulmonary hypertension.

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

Acknowledgement

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