Introduction

β-Adrenergic receptor (AR) antagonists (β-blockers) are widely used for the treatment of cardiovascular diseases such as hypertension, ischemic heart disease, arrhythmia, and chronic heart failure. The responsiveness to β-blockers does however vary among individuals and may not exceed 60% – 70% in hypertensive patients treated with β-blockers (1). It is generally recognized that the responsiveness differs among races; the Negroid race is less responsive to β-blockers than the Caucasoid and Mongoloid races. Differences in both β-blocker pharmacokinetics and the receptor sensitivity to β-blockers may account for this observed variation.

β1-AR is expressed in multiple organs and tissues including heart, kidney, brain, and pineal gland and is the main target of β-blockers, particularly β1-AR–selective blockers such as metoprolol, atenolol, and bisoprolol. Genetic variants are frequently found in β1-AR at codons 49 (Ser or Gly) and 389 (Arg or Gly), caused by single nucleotide polymorphisms of the β1-AR gene ADRB1 (2, 3). These two genetic polymorphisms have been shown to exhibit linkage disequilibrium (4, 5) and allele frequencies may differ significantly among races (6, 7). The frequencies of Gly49 allele are 1% – 16% in Caucasians, 20% – 21% in Latino-Hispanics, 15% in Asians, and 13% – 28% in African-Americans; and the frequencies of the Gly389 allele of those races are 24% – 34%, 31% – 33%, 20% – 30%, and 39% – 46%, respectively. These genetic polymorphisms have been shown to alter the functions of β1-AR in vitro experiments. Levin et al. (8) reported that cells expressing β1-AR with Gly49 have higher isoproterenol binding affinity, basal and isoproterenol-induced adenylate cyclase activity, and sensitivity to metoprolol and, moreover, more profound down-regulation of isoproterenol-induced receptor than
cells expressing $\beta_1$-AR with Ser49. Rathz et al. (9) also reported that receptor down-regulation was greater in Gly49-$\beta_1$-AR than Ser49-$\beta_1$-AR, although they found no difference in ligand binding affinities or adenylate cyclase activities. For the polymorphism at codon 389, isoproterenol-induced activity of adenylate cyclase was lower in cells expressing Gly389-$\beta_1$-AR than in cells with Arg389-$\beta_1$-AR (10 – 12).

It is therefore possible that these polymorphisms significantly alter the effect of $\beta$-blockers and could, in part, explain the variety in $\beta$-blocker responsiveness. Such associations between these polymorphisms and the effect of $\beta$-blockers have been reported in several human studies (1, 5, 6). Discordance among these results has however failed to establish whether the sensitivity to $\beta$-blockers is significantly altered by such polymorphisms. Therefore, in an effort to determine whether the sensitivity to $\beta_1$-AR–selective blocker is influenced by these polymorphisms, we conducted a comparative study in healthy young Japanese. By selecting only healthy individuals, we excluded the influence of multiple concomitant factors that could not be controlled when studying patients or older individuals.

Materials and Methods

Ethics

This study was conducted in accordance with World Medical Association Declaration of Helsinki (2000) and Ethical Guidelines for Clinical Research (Ministry of Health, Labour, and Welfare, Japan, 2003). The protocol was approved by the Institutional Review Board for Clinical Trials and the Institutional Review Board for Human Genome/Gene Research, Kyushu University in 2004. Written informed consent was obtained from all participants.

Subjects

From 2005 to 2008, we recruited voluntary participants aged 20 – 39 years from third grade clinical pharmacology students (20 years of age or older) of the Faculty of Medicine, Kyushu University. Students who met the following exclusion criteria were excluded from the study: those who 1) had a disease that should be treated; 2) were under medical treatment; 3) had a history of cardiovascular disease; 4) had a history of bronchial asthma; 5) had serious liver damage; 6) had serious renal damage; 7) were pregnant; 8) had a history of adverse reaction to a $\beta$-blocker; 9) had a history of allergic reaction to a drug or a food or who have a history of an allergic disease; 10) had a heart rate (HR) less than 50/min; 11) had a systolic blood pressure (SBP) lower than 90 mmHg; 12) had atrioventricular block (second or third degree), sinoatrial block, or sick sinus syndrome; 13) had a body weight less than 40 kg; or 14) who were judged to be inappropriate for participating in the study by the principal investigator (T.S.).

Sample size calculation

Sofowara et al. (13) reported that Arg389 homozygotes showed larger decreases in resting SBP and mean blood pressure (MBP) in response to atenolol than Gly389 homozygotes (SBP, −8.7 ± 1.3 vs. −0.2 ± 1.7 mmHg; MBP, −7.2 ± 1.0 vs. −2.0 ± 1.7 mmHg). The power calculation using these data indicated that 9 or 16 subjects in each group would be required to detect the differences in SBP or MBP between groups (8.5 or 5.2 mmHg), respectively, with a power of 80% and a $P$-value of 0.05. Liu et al. (14) reported that metoprolol (150 or 225 mg/day) reduced HR more greatly in Arg389 homozygotes than in Gly389 homozygotes (150 mg, −10.1 ± 1.0% vs. −6.2 ± 1.1%; 225 mg, −14.4 ± 1.4% vs. −10.9 ± 1.3%). We calculated that 12 or 13.5 subjects in each group would be required to detect the differences in HR between groups (3.9% or 3.5%, respectively), with a power of 80% and a $P$-value of 0.05. Assuming the allele frequency of Gly389 to be 25%, we expected that the sample size of the pharmacogenomics study should at least be 200.

Study protocols

After fasting for 4 h, subjects who had been at rest without exercise for at least 30 min were randomly allocated 2:1 to the atenolol group, in which subjects took a capsule containing 0.5 g of Atenolol Dry Syrup 10% “EMEC” (Elmed Eisai Co., Ltd., Tokyo) that includes 50 mg of atenolol, or to the placebo group, in which subjects took a capsule containing 0.5 g of potato starch.

After oral administration of the capsules, HR and blood pressure (BP) were measured in sitting position every 30 min until 3 h, with an automatic sphygmomanometer (HEM-770A fuzzy; Omron Corporation, Kyoto). Subjects were at rest for 10 min before each measurement. HR and BP were measured at least twice for each time and mean values recorded. When a difference of more than 10 beats per min (HR) or 10 mmHg (BP) between two values was observed, measurements were repeated and the mean of two values with a minimum difference was recorded. The MBP was calculated from SBP and diastolic BP (DBP) according to the following formula: $MBP = DBP + 1/3(SBP − DBP)$.

Genotyping

Genomic DNA was extracted from peripheral blood using a DNA isolation kit (GenTLE; Takara Bio, Inc., Otsu). $ADRB1$ genotypes at codons 49 and 389 were determined using polymerase chain reaction restriction
fragment length polymorphism (PCR-RFLP). Primer sequences used for PCR are shown in Table 1. PCR products were then digested with EcoO109I (Takara Bio, Inc.) for the codon 49 or BcgI (New England BioLabs, Inc., Ipswich, MA, USA) for the codon 389 at 37°C for 2 h. Digested products were separated on a 2% agarose gel and visualized using ethidium bromide (Fig. 1). For the codon 49 polymorphism, the Gly variant was cut into two fragments (343 and 219 bp) by EcoO109I, while the Ser variant was not. For the codon 389 polymorphism, the Arg variant was cut into two fragments (342 and 154 bp) by BcgI, while the Gly variant was not.

Measurement of plasma atenolol concentration

Venous blood was taken from 41 subjects at 3 h after atenolol administration and the plasma concentration of atenolol was determined by reverse phase high-performance liquid chromatography (HPLC) using an HPLC system (Tosoh Corporation, Tokyo) consisting of a system controller (PX-8010), autosampler (AS-8010), pump (CCPM), column oven (CO-8010), and UV detector (UV-8010).

The plasma sample (500 μL) was shaken at room temperature for 30 min, afterwards mixed with 12.5 μL of 8 N NaOH, 3 mL of dichloromethane, and 25 μL of 20 μg/mL pindolol (Wako Pure Chemical Industries, Ltd., Osaka), an internal standard. After centrifugation at 3,500 rpm for 5 min, the organic phase was transferred into a new tube and evaporated with a nitrogen stream at 40°C. The residue was dissolved in 450 μL of the eluent consisting of 0.1 M NaH2PO4/methanol (70/30, v/v). An aliquot (50 μL) was applied to a column (TSKgel ODS-80Ts, Tosoh Corporation) and eluted at a flow rate of 0.8 mL/min. Atenolol was detected at the wavelength of 227 nm.

Statistical analyses

Hardy-Weinberg equilibrium was assessed by a Pearson’s chi-square test. Comparisons of baseline demographic data and cardiovascular parameters among groups were performed by the chi-square test, Student t-test, and an analysis of variance (ANOVA), as appropriate. The association between polymorphisms and drug effects was evaluated by ANOVA. Statistical analyses were performed using JMP8 (SAS Institute, Cary, NC, USA). P < 0.05 was considered statistically significant. All continuous variable data are shown as the mean ± S.E.M.

Results

A randomized double-blind trial for the effects of atenolol

Three hundred and forty-one students gave informed consent to participate in the study (Fig. 2), of which 307 participated in the study and 34 were excluded by the exclusion criteria. Two hundred and seven students (176 males and 31 females) were randomly allocated to the atenolol group and 100 students (81 males and 19 females) to the placebo group. There were no significant differences in the demographic background between these two groups (Table 2). In the atenolol group, HR, SBP, DBP, and MBP significantly decreased compared with the placebo group from 30 – 60 min after the ad-

<table>
<thead>
<tr>
<th>Table 1. PCR primer sequences</th>
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<tr>
<td>Codon 49</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Codon 389</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Restriction fragment length polymorphism. Amplified DNA fragments were electrophoresed after digestion with EcoO109I or BcgI to determine the genotypes at codon 49 (A) or 389 (B), respectively. M, molecular size marker.
ministration, and the effects continued until 3 h (Fig. 3).

**Genotype analysis**

Genotyping results, along with the physical and habitual characteristics of the subjects allocated to the atenolol group (207 students) are given in Table 3. For the polymorphism at codon 49, the number of Ser/Ser homozygotes, Ser/Gly heterozygotes, and Gly/Gly homozygotes were 159, 46, and 2, respectively. The 2 Gly49 homozygous subjects were both male. For the polymorphism at codon 389, the number of Arg/Arg homozygotes, Arg/Gly heterozygotes, and Gly/Gly homozygotes were 129, 66, and 12, respectively. The allele frequency of Gly49 and Gly389 were 12% and 22%, respectively, and both polymorphisms were in Hardy-Weinberg equilibrium ($P = 0.80$ and 0.66, respectively).

A trend of fewer HR in Gly49 homozygotes and fewer alcohol drinkers in Gly389 homozygotes was observed, although this was not statistically significant.

**Association between ADRB1 polymorphisms and plasma atenolol concentration**

To test whether a significant difference in pharmacokinetics of atenolol among genetic variants of $\beta_1$-AR exists, we measured plasma atenolol concentration in 41 subjects allocated to the atenolol group. As shown in Fig. 4, there were no significant differences in atenolol concentrations among genetic variants for both polymorphisms, although only one Gly49 homozygous subject was identified.

**Association between ADRB1 polymorphisms and the effects of atenolol**

To investigate whether the polymorphisms at codons 49 and 389 were associated with atenolol pharmacological effects, we measured and analyzed HR and BP at 3 h after drug administration. Changes in HR and BP classified by the genotypes of $\beta_1$-AR are shown in Fig. 5. We found no significant differences among the genotypes at codon 49 or 389 (Fig. 5: A and C). Although a trend for larger decreases in SBP, DBP, and MBP was observed in Gly49 homozygotes when compared with Ser49 allele carriers, differences were not statistically significant. HR did not show a larger decrease. Since the number of Gly49 homozygous subjects was small, Ser49 homozygotes were compared with Gly49 allele carriers, with Ser49/Gly49 heterozygotes and Gly49 homozygotes placed together (Fig. 5B). No statistical difference or distinct trend was found between Ser49 homozygotes.

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**Table 2.** Baseline characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Atenolol</th>
<th>Placebo</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>307</td>
<td>207</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Sex (male, %)</strong></td>
<td></td>
<td>83.7</td>
<td>85.0</td>
<td>81.0</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td>21.5 ± 0.1</td>
<td>21.4 ± 0.1</td>
<td>21.7 ± 0.2</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td>21.4 ± 0.1</td>
<td>21.5 ± 0.2</td>
<td>21.0 ± 0.2</td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td></td>
<td>64.5 ± 0.6</td>
<td>64.3 ± 0.8</td>
<td>65.1 ± 1.0</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td></td>
<td>112.7 ± 0.6</td>
<td>113.3 ± 0.8</td>
<td>111.6 ± 1.1</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td></td>
<td>66.7 ± 0.4</td>
<td>67.3 ± 0.5</td>
<td>65.6 ± 0.6</td>
</tr>
<tr>
<td><strong>Smoking (%)</strong></td>
<td></td>
<td>9.8</td>
<td>11.6</td>
<td>6.0</td>
</tr>
<tr>
<td><strong>Drinking (%)</strong></td>
<td></td>
<td>40.1</td>
<td>40.6</td>
<td>39.0</td>
</tr>
</tbody>
</table>

BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Similarly, we could not find any significant differences between Arg389 homozygous subjects and Gly389 allele carriers (Arg389/Gly389 heterozygotes plus Gly389 homozygotes) (Fig. 5D). Finally, we compared HR and BP changes in the haplotype pairs (diplotypes) for the codons 49 and 389 (Fig. 6). Since the Gly49Gly389 combination allele carriers are very rare (15), there seemed to be 6 different diplotypes: Ser49Arg389/Ser49Arg389, Ser49Arg389/Ser49Gly389, Ser49Gly389/Ser49Gly389, Ser49Arg389/Gly49Arg389, Ser49Gly389/Gly49Arg389, and Gly49Arg389/Gly49Arg389. No significant differences were observed among these diplotypes, although only 2 Gly49Arg389/Gly49Arg389 allele carriers were identified.

In addition, no significant differences in HR or BP were found between the polymorphisms at codon 49 or 389, even after the subjects were stratified by their background factors such as sex and smoking habit, or by the degree of changes in BP or HR (not shown).

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Table 3. Baseline characteristics of the subjects in each genotype at codons 49 and 389

<table>
<thead>
<tr>
<th>Codon 49</th>
<th>Codon 389</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser</td>
<td>Ser/Gly</td>
</tr>
<tr>
<td>Number</td>
<td>159</td>
</tr>
<tr>
<td>Sex (male, %)</td>
<td>83.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.4 ± 0.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5 ± 0.2</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>64.4 ± 0.9</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>112.5 ± 0.9</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>67.0 ± 0.6</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>12.6</td>
</tr>
<tr>
<td>Drinking (%)</td>
<td>42.8</td>
</tr>
</tbody>
</table>

| Arg/Arg | Arg/Gly | Gly/Gly |
| Number | 129 | 66 | 12 |
| Sex (male, %) | 84.5 | 84.9 | 91.7 |
| Age (years) | 21.3 ± 0.1 | 21.8 ± 0.3 | 20.9 ± 0.3 |
| BMI (kg/m²) | 21.4 ± 0.2 | 21.7 ± 0.3 | 21.3 ± 0.9 |
| HR (beats/min) | 63.7 ± 0.9 | 65.2 ± 1.4 | 64.9 ± 4.2 |
| SBP (mmHg) | 112.8 ± 1.0 | 115.1 ± 1.3 | 109.0 ± 1.2 |
| DBP (mmHg) | 67.4 ± 0.7 | 67.5 ± 0.9 | 65.2 ± 2.6 |
| Smoking (%) | 13.2 | 10.6 | 0.0 |
| Drinking (%) | 40.3 | 45.5 | 16.7 |

BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Fig. 4. The effect of polymorphisms on plasma atenolol concentrations. Plasma concentrations of atenolol were measured 3 h after administration of the drug. A) polymorphism at codon 49. B) polymorphism at codon 389.

Fig. 5. The effect of β1-AR gene polymorphisms on HR and BP. Percent changes of HR, SBP, DBP, and MBP were compared among polymorphisms at codons 49 and 389. A and B: Codon 49. C and D: Codon 389. A and C: Comparison among three genotypes. B: Comparison between Ser49 homozygotes and Gly49 allele carriers. D: Comparison between Arg389 homozygotes and Gly389 allele carriers. Scales for HR and BP are shown on the left and right sides of the graphs, respectively.
**Discussion**

Although several studies have previously been conducted to assess the association between $\beta_1$-AR genotypes and the effects of $\beta$-blockers (1, 5, 6), discordance among the reported results prevents reliable conclusions to be made on the influence of receptor genotypes on the clinical use of $\beta$-blockers. Several reasons may exist for the discrepancies of the results, including differences in subjects’ characteristics (age, sex, race, ethnicity, diseases, habits, and other medications), study design (sample size, retrospective or prospective, observational or experimental, and open or double-blind), and the class of $\beta$-blocker used in the study ($\beta_1$-selective or non-selective, partial agonist or inverse agonist, and susceptibility to individual pharmacokinetic differences).

To minimize biases caused by the above factors, our study used healthy young Japanese students from the same class in a double-blind experiment under similar environmental conditions. Furthermore, we chose atenolol as the $\beta$-blocker for our study due to the expected smaller interindividual differences in pharmacokinetics and pharmacodynamics of this drug when compared with other $\beta$-blockers. Atenolol is highly $\beta_1$-selective, lacks a partial agonistic activity, is not readily metabolized by drug metabolizing enzymes and has a low plasma protein-binding ratio. Limited amounts are also transferred across the blood-brain barrier and the majority of the absorbed drug is excreted in urine as an unchanged form (16).

Although measurements of subjects were taken under the resting state and not during exercise stress, atenolol clearly lowered HR and BP compared with the placebo. This was most likely due to the sympathetic nervous activity of the subjects being significantly elevated even in the absence of exercise. The study protocol was therefore adequate and valid for analyzing the difference in the response to atenolol among genotypes.

Allele frequencies of Gly49 (12%) and Gly389 (22%) were consistent with those previously reported for Caucasians and Asians (3, 4, 6, 7), and were similar to those recently reported for Japanese subjects (17 – 19). There was no significant difference in baseline HR or BP or plasma atenolol concentrations among the genotypes at codons 49 and 389, although the number of Gly49 homozygotes was too small to compare accurately.

We found no significant differences in hemodynamic responses to atenolol among genotypes of the two polymorphisms of $\beta_1$-AR, a finding that was not consistent with previous studies. These earlier studies were also conducted with healthy volunteers and reported that the codon 389 polymorphism had a marked effect on the response to $\beta_1$-selective blockers (13, 14, 20). Sofowora et al. (13) showed that Arg389-homozygotes exhibited a greater decrease in resting SBP and MBP after atenolol administration (25 mg) in black, white, and Hispanic subjects. Liu et al. (14) also reported a larger decrease in resting and exercise HR and BP after the administration of metoprolol in young male Chinese subjects and Kurnik et al. (20) reported a large reduction in exercise HR in Arg389 allele carriers in black and white subjects treated with atenolol (25 mg). While similarities in basic experimental design exist between these three studies and our own, the conclusions made were quite different. The reason for this discrepancy is unknown; however, ethnic difference, particularly inclusion of Negroid subjects, smaller sample size, loading exercise, use of a different kind of $\beta$-blocker, different dosages, or study designs without the use of placebo may have influenced the results obtained in previous studies.

Several clinical trials have been conducted with hypertensive patients to assess the association between $\beta_1$-AR polymorphisms and responsiveness to $\beta_1$-blockers. O’Shaughnessy et al. (21) reported negative results, that is, the codon 389 polymorphism did not affect pretreatment HR or BP or the hemodynamic changes after chronic $\beta_1$-receptor blockade. Johnson et al. (15) reported that hypertensive patients homozygous for Arg389 showed a nearly 3-fold greater reduction in DBP compared with those with the variant allele, after administration of metoprolol. They also found that the Ser49Arg389/
Ser49Arg389 diplotype showed a larger reduction in DBP compared with other haplotypes. In particular, Gly allele carriers at both codons (Gly49Arg389/ Ser49Gly389) had no response to metoprolol. Karlsson et al. (22) analyzed the association between the response to atenolol and ADRB1 polymorphisms in hypertensive patients with left ventricular hypertrophy who participated in a randomized double-blind study (SILVHIA trial). The effects of irbesartan and atenolol were compared and no significant associations between the changes in HR and BP and either of the two polymorphisms at codons 49 and 389 were observed, although the reduction in HR of the Gly49 allele was greater than in Ser49 homozygous subjects. Recently, Suonsyrjä et al. (23) reported that there were no significant differences in the response to bisoprolol between the polymorphisms at codons 49 or 389. Our results generally support those of O’Shaughnessy et al. (21), Karlsson et al. (22), and Suonsyrjä et al. (23) except for minor inconsistencies. Results reported by Johnson et al. (15), however, appeared inconsistent with ours. Such discrepancies may be explained by differences between healthy subjects and hypertensive patients or between acute and long-term effects of treatments.

Several studies have elucidated the associations between ADRB1 polymorphisms and the outcomes of β-blocker treatments for patients with heart failure (24, 25). Based on in vitro results, Mialet-Perez et al. (26) conducted a clinical trial of carvedilol with 224 heart failure patients genotyped at codon 389 of β1-AR and Liggett et al. (27) genotyped the same codon in heart failure patients from the β-Blocker Evaluation of Survival Trial (BEST), a large-scale randomized placebo-controlled trial of bucindolol. Arg389 homozygous patients treated with carvedilol showed a substantially greater improvement in left ventricular ejection fraction compared with Gly389 homozygous patients (26). Bucindolol also significantly reduced mortality in Arg389 homozygotes but had no clinical response in Gly389 carriers (27). Furthermore, Terra et al. (28) reported that Ser49 homozygotes and Gly389 allele carriers (Arg389/Gly389 heterozygotes and Gly389 homozygotes) treated with metoprolol required increases in other heart failure medications. This suggested a lower efficacy of metoprolol when comparing Gly49 carriers or Arg389 homozygotes, respectively. Conversely, Gly49 carriers and Arg389 homozygotes showed greater improvements in left ventricular remodeling (29). This may also be consistent with the results of a retrospective study in patients with dilated cardiomyopathy, in which Ser49 homozygotes were suggested to respond less beneficially to β-blockers than Gly49 carriers (30). Some studies have however reported negative results (31, 32). In a sub-study of the Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure (MERIT-HF), there was no significant difference in the prognostic benefit obtained by metoprolol between Arg389 homozygotes and Gly389 carriers (31). Two polymorphisms for β1-AR and 3 polymorphisms for β2-AR have also been reported to have no effects on the response to bisoprolol or carvedilol (32). Since cardiovascular functions may vary between healthy young subjects and heart failure patients, a direct comparison cannot be made with our results and previous reports based on heart failure.

Such contradictory results may in part be explained by the nature of the β-blocker action in each study. In addition to differences in selectivity to AR (α1, β1, and β2), some β-blockers may act as neutral antagonists, while others act as partial agonists or inverse agonists for β1-AR. Galandrín and Bouvier (33) reported that labetalol, bucindolol, and carvedilol behaved as partial agonists, whereas propranolol, metoprolol, bisoprolol, and atenolol were inverse agonists for cAMP production through β1-AR. The influence of an ADRB1 polymorphism has also been reported to be different among β-blockers (34). Metoprolol and bisoprolol did not show a significant difference in inverse agonism between recombinant β1-AR with Arg389 and that with Gly389, whereas inverse agonism of carvedilol was markedly enhanced in Arg389 when compared with Gly389. Interpretation of pharmacogenomic data should therefore take into consideration the class of β-blockers used.

Despite careful experimental design and execution of our study, several limitations were still observed. First, the subject sample size was large enough to evaluate the effects of polymorphism at codon 389, but it may have been insufficient to conclude the effects of the Gly49 allele. Sample size calculation for codon 49 was not easy because preceding studies to compare β-blocker efficacy among codon 49 genotypes using healthy volunteers were rare and therefore we could not expect the impact of the Gly49 allele, although the genotype frequencies in the Japanese had been reported (35). The allele frequency of Gly49 was so small that it seemed to be very difficult to find a sufficient number of Gly49 homozygotes within the next 5–6 years of our study without a more efficient method for genotype screening. Second, only the acute effects of atenolol were observed in this study. As suggested from in vitro experiments, long-term administration of β-blockers may have caused down-regulation of β1-AR, which can be modified by genetic variation of the receptor (8, 9). Third, the subjects enrolled in our study were healthy volunteers, not patients with hypertension, ischemic heart disease, heart failure, or arrhythmia. Significantly different results may therefore have been observed if our study had used cardiovascular disease pa-
patients rather than healthy individuals, due to hemodynamic variation between these two groups. Finally, our study did not investigate the effects of other known polymorphisms relevant to adrenergic responses found in α₂c-AR (36 – 38), β₂-AR (39), and G protein–coupled receptor kinase-5 (40).

In conclusion, our results do not support clinical use of genotyping for the common polymorphisms at codons 49 and 389 of ADRB1 to predict responses to β-blockers, although evidence is not definitive for codon 49. To provide further evidence, a comparative study should be conducted with additional Gly49 homozygotes. Reasons for the existence of non-responders to β-blockers may also be elucidated by carefully examining polymorphisms in other components of AR signal transduction.

Acknowledgments

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References

26 Miallet-Perez J, Rathz DA, Petrashevskaya NN, Hahn HS, Wagoner LE, Schwartz A, et al. β₁-adrenergic receptor polymor-


