β_1-Adrenergic receptor polymorphisms influence the response to metoprolol monotherapy in patients with essential hypertension

Objectives: The human β_1-adrenergic receptor, an important therapeutic target in cardiovascular diseases, has 2 common functional polymorphisms (Ser49Gly and Gly389Arg). Our study aimed to confirm that β_1-adrenergic receptor polymorphisms affect the blood pressure response to metoprolol monotherapy in the Chinese population with hypertension.

Methods: β_1-Adrenergic receptor genotype was determined by polymerase chain reaction–restriction fragment length polymorphism assay for 223 patients with essential hypertension. Sixty-one patients with certain β_1-adrenergic receptor diplotypes, 18 for 49Ser389Arg/49Ser389Arg, 15 for 49Ser389Arg/49Gly389Arg, 19 for 49Ser389Gly/49Gly389Arg, and 9 for 49Ser389Gly/49Ser389Gly, were selected from those 61 for measurement of the antihypertensive effect of metoprolol. Patients were given 25 mg metoprolol every 12 hours for 4 weeks. Heart rate and blood pressure were measured weekly for the duration of metoprolol therapy.

Results: The descent of systolic blood pressure after metoprolol administration was significantly different among genotype groups (10.4% ± 4.0%, 2.8% ± 4.7%, and 1.1% ± 1.5% for Arg389Arg, Gly389Arg, and Gly389Gly patients, respectively; $P < .001$). We also found a similar difference in changes of diastolic blood pressure (6.1% ± 4.3%, 2.2% ± 4.2%, and 0.9% ± 4.0%, respectively; $P < .001$) and mean arterial pressure (8.1% ± 3.5%, 2.5% ± 3.0%, and 1.0% ± 2.5%, respectively; $P > .001$) for Arg389Arg, Gly389Arg, and Gly389Gly patients. Ser49Gly variance exhibited a smaller contribution to the antihypertensive effect of metoprolol. Systolic blood pressure decreased significantly in Ser49 homozygous patients compared with Ser49Gly patients (8.4% ± 3.2% versus 5.3% ± 5.2%, $P = .047$). There was a highly significant relationship between diplotype and blood pressure during treatment. Systolic blood pressure significantly decreased in 49Ser389Arg/49Ser389Arg (12.0% ± 3.8%, $P < .001$) and 49Ser389Arg/49Gly389Arg (8.4% ± 5.5%, $P < .001$) patients, with the decrease in the former being more pronounced ($P = .023$). We also found a significant decrease in diastolic blood pressure (6.5% ± 4.7% versus 5.7% ± 3.2%, respectively; both $P < .001$) and mean arterial pressure (8.8% ± 3.2% versus 6.9% ± 3.7%, respectively; both $P < .001$) in 49Ser389Arg/49Ser389Arg and 49Ser389Arg/49Gly389Arg patients. However, blood pressure did not change significantly in 49Ser389Gly/49Gly389Arg and 49Ser389Gly/49Ser389Gly patients (all $P > .05$).

Conclusions: β_1-Adrenergic receptor polymorphism was associated with different blood pressure responses to metoprolol therapy in patients with essential hypertension. 49Ser389Arg/49Ser389Arg and 49Ser389Arg/49Gly389Arg patients were good responders to metoprolol therapy; 49Ser389Arg/49Ser389Arg patients had a larger systolic blood pressure reduction than 49Ser389Arg/49Gly389Arg patients did. 49Ser389Gly/49Gly389Arg and 49Ser389Gly/49Ser389Gly patients were nonresponders to metoprolol antihypertensive therapy. (Clin Pharmacol Ther 2006;80:23-32.)

Jie Liu, MD, PhD, Zhao-Qian Liu, MD, PhD, Bang-Ning Yu, MD, PhD, Fang-Hua Xu, MD, Wei Mo, MD, Gan Zhou, MD, Ying-Zi Liu, MD, Qing Li, MD, and Hong-Hao Zhou, MD *Changsha, Hunan, China*

From the Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University.

Supported by National Science Foundation of China grants F30130210 and 30070880 and China Medical Board grant 01-755.

Received for publication Dec 10, 2004; accepted Mar 1, 2006.

Available online June 8, 2006.

Reprint requests: Hong-Hao Zhou, Professor, Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha, Hunan 410078, China.

E-mail: *hhzhou@public.cs.hn.cn*

0009-9236/$32.00

doi:10.1016/j.clpt.2006.03.004

The β_1-adrenergic receptor is an archetypal G-protein–coupled receptor that controls sympathetic responses in the heart, kidney, and adipocytes after activation by endogenous catecholamines.[1] Although this activation leads to beneficial responses, sustained stimulation also has a key role in the development and progression of cardiovascular disease. This realization has led to the widespread use of β_1-adrenergic receptor blocking drugs to treat cardiovascular disease.[2,3] However, there is considerable interindividual and interethnic variability in the response to β_1-adrenergic receptor antagonists.[4-6] Several studies have elucidated that changes in heart rate or blood pressure after administration of β-blockers, even highly β_1-selective ones, vary widely among healthy subjects or subjects with hypertension, with adequate blood pressure control failing to be achieved with β-blocker monotherapy in 30% to 60% of patients.[7,8]

A variety of genetic and nongenetic factors determine β_1-blocker response.[9,10] Besides inherited differences in the metabolism and disposition of drugs, genetic polymorphisms in the targets of drug therapy, for example, receptors, can have a significant influence on the efficacy and toxicity of medications.[11] Gene defects can be associated with a dysfunction in the receptor protein and subsequently with a changed response to drugs.[12]

The β_1-adrenergic receptor is encoded by an intronless gene on chromosome 10q24-26 and has been cloned and sequenced by Frielle et al.[13,14] Recently, several functionally important polymorphisms involving the β_1-adrenergic receptor have been described.[15-17] One of these is located in the amino terminus: An A→G exchange at 145 base pairs (bp) (A145G) results in an amino acid substitution of Ser by Gly at residue 49 (Ser49Gly) with an allele frequency of approximately 14% of individuals in various ethnic groups, including Chinese populations.[18-20] In vitro studies have confirmed that amino acid 49 polymorphism of the β_1-adrenergic receptor gene affects agonist-promoted trafficking, with the Gly49 receptor having enhanced agonist-promoted down-regulation.[16] Another single-nucleotide polymorphism is 1165G→C, causing a nonconservative amino acid substitution at residue 389 from Gly to Arg (Gly389Arg).[17-19,21] The allele frequency of the Gly389 varies between white subjects (about 27%) and black subjects (about 42%).[18,20] An in vitro study using site-directed mutagenesis and recombinant expression in Chinese hamster fibroblasts confirms that the substitution markedly alters the G-protein coupling of the β_1-adrenergic receptor, with the Arg389 receptor form having nearly 2-fold greater basal and 3-fold greater agonist-mediated adenylyl cyclase activities.[17] This dramatic difference suggests that the genetic variation of the β_1-adrenergic receptor gene may be the basis of interindividual differences in the response to therapeutic β-adrenergic receptor agonists and antagonists in cardiovascular and other diseases. A recent study has shown that the codon 49 and 389 polymorphisms are in linkage disequilibrium.[22] In vitro studies have confirmed that there are important functional differences among the common haplotypes in the β_1-adrenergic receptor and that there is a need for consideration of haplotypes in determining the in vivo role of these polymorphisms in this important drug target.[23] More important, Johnson et al[24] studied the impact of the Gly389Arg and Ser49Gly polymorphisms of the β_1-adrenergic receptor on the antihypertensive effect of metoprolol in patients with hypertension. They found that patients homozygous for Arg389 displayed a significantly greater reduction in 24-hour and daytime diastolic blood pressure than did patients with the Gly389 allele. Moreover, they also found that patients with the haplotype 49Ser389Arg/49Ser389Arg showed a significant reduction in diastolic blood pressure, whereas those with the haplotype 49Ser389Gly/49Gly389Arg showed almost no reduction in diastolic blood pressure. A similar tendency was also found for reduction in systolic blood pressure, although this failed to reach statistical significance.[24] The study population included white, African American, and Hispanic men and women with hypertension.[24] There is a considerable interethnic variability in the response to β-adrenergic receptor antagonists.[4,6] Therefore, our study was aimed to test whether the different response to metoprolol according to β_1-adrenergic receptor polymorphisms existed in Chinese subjects with hypertension.

METHODS

Subjects. The study protocol was approved by the Ethics Committee of Xiangya School of Medicine, Central South University. Two hundred twenty-three (male, 127; female, 96) unrelated subjects with primary mild to moderate essential hypertension aged 30 to 65 years were recruited for genetics screening after giving their written informed consent. They were all outpatients at Xiangtan Hospital, affiliated with Nanhua University. Inclusion criteria were an average systolic blood pressure, measured at the 2 visits while the subject was sitting, between 140 and 180 mm Hg or an average diastolic blood pressure of at least 90 mm Hg; secondary hypertension was excluded by physical examination and appropriate laboratory analyses. Patients

with liver or kidney disease or other serious systemic diseases, such as diabetes, hypothyroidism, or systemic lupus erythematosus, were excluded. Patients were also excluded from participation if they had conditions that would limit tolerability to β-blockers.

After genotype detection, 61 untreated patients with certain β_1-adrenergic receptor diplotypes were selected to participate in an open-label, noncontrolled intervention study for measurement of the antihypertensive effect of metoprolol. All of them were of the Chinese Han nationality, and they lived in Xiangtan city, Hunan province, China. All the subjects were nonsmokers and abstained from coffee and alcohol for a week before the study.

Genotyping procedures for β_1-adrenergic receptor. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral lymphocytes with phenol-chloroform followed by ethanol precipitation.[25] Genotyping analysis was conducted by the polymerase chain reaction (PCR)–restriction fragment length polymorphism assay. The PCR of the β_1-adrenergic receptor gene was performed as described previously, with minor modification.[19] For the Ser49Gly locus, we used the primer pair as follows: the sense primer P1 (5′-CCGGGCTTCTGGGGTGTTCC-3′) and the antisense primer P2 (5′-GGCGAGGTGATGGCGAGGTAGC-3′). The final 25-μL PCR mixture contained 5.25 μL PCR grade water, 12.5 μL of 2× PCR buffer (Mg^{2+}), 4 μL deoxyribonucleoside triphosphates (2.5 μmol/L each), 1.5 μL primer (10 μmol/L each), 0.25 μL *Taq* DNA polymerase (5 U/μL; TaKaRa Biotech, Dalian City, China), and 1.5 μL genomic DNA sample. Temperature cycling proceeded as follows: initial denaturation for 1 minute at 94°C followed by 30 cycles of 30 seconds at 94°C, 1 minute at 62°C, 1 minute at 72°C, and a terminal extension for 7 minutes at 72°C. The Gly389Arg polymorphic locus was amplified by the use of the sense primer P3 (5′-CATCATGGGCGTCTTCACGC-3′) and the antisense primer P4 (5′-TGGGCTTCGAGTTCACCTGC-3′). The reaction system and amplification conditions were similar to those of the A145G locus except that the denaturation temperature was 60°C. The amplified DNA fragments including the Ser49Gly or Gly389Arg polymorphic site were separately digested with *EcoO109*I (TaKaRa Biotech) or *Bcg*I (New England Biolabs, Beverly, Mass) at 37°C for 8 hours. The different patterns of the digested fragments were visualized on ethidium bromide–stained 2% agarose gel.

Genotyping procedures for CYP2D6*10. The genotype assay of *CYP2D6*10* was performed as described previously with minor modification.[26] We used the sense primer P1 (5′-CCA TTT GGT AGT GAG GCA GGT AT-3′) and the antisense primer P2 (5′-CAC CAT CCA TGT TTG CTT CTG GT-3′) to amplify DNA fragments including *CYP2D6*10*. The amplified DNA fragments were digested with *Hph* I (TaKaRa Biotech).

Protocol. The volunteers and clinic investigators were blinded to the genotype in the current study. Blood pressure was measured by trained nurses, with an automatic blood pressure monitor (Omron, Tokyo, Japan), which allows the detection of alteration of the heart rate by greater than or equal to 1 beat/min and of the blood pressure by greater than or equal to 1 mm Hg. Monitors were validated against a mercury sphygmomanometer. Baseline heart rate and blood pressure were measured while patients were in a supine position after resting comfortably for at least 30 minutes. Baseline blood pressure was the average of 2 recordings 10 minutes apart. Once baseline studies were completed, drug therapy was initiated. Each patient underwent treatment for 4 weeks with metoprolol (Astra Pharmaceutical Company, Wuxi, China) 25 mg every 12 hours (8 AM and 8 PM every day)[27] and was seen weekly. All clinic visits for blood pressure checks took place in a quiet air-conditioned room with an almost identical time schedule to minimize the impact of diurnal variation on clinic blood pressure.

Haplotype assignment. Because of the strong linkage disequilibrium between variants at 49 and 389 loci, the haplotype Gly49Gly389 seems to be exceedingly rare, so that patients who were double heterozygotes were assigned Ser49Gly389 and Gly49Arg389 as their 2 haplotypes. With the specific genotypic groups chosen in our study, only 3 haplotypes existed in 61 patients.

Statistical analysis. Data analysis was performed by using SPSS (version 10.0 for Windows; SPSS Inc, Chicago, Ill). Hardy-Weinberg equilibrium was tested by the chi-square test. Linkage disequilibrium analysis was performed with the DnaSP version 3.51 software package[28] and the |D′| value was used to evaluate the linkage between Ser49Gly and Gly389Arg polymorphisms. Baseline characteristics among patients with different genotypes or diplotypes were compared by chi-square test or 1-way ANOVA, as appropriate. We used *t* tests to measure the significance of percent changes of heart rate and blood pressure after 4 weeks of metoprolol treatment compared with baseline. The percentage of blood pressure decrease from baseline to the final measurements by 49 and 389 genotype groups was compared by *t* test or 1-way ANOVA. General linear model repeated-measures ANOVA was performed on raw data of heart rate and blood pressure

Table I. Baseline characteristics of patients with hypertension stratified by β_1-adrenergic receptor genotype

Characteristic	*Ser49Ser (n = 27)*	*Ser49Gly (n = 34)*	*Arg389Arg (n = 33)*	*Arg389Gly (n = 19)*	*Gly389Gly (n = 9)*
Age (y)	53 ± 10	56 ± 10	55 ± 10	55 ± 10	52 ± 11
M/F	15/12	19/15	17/16	12/7	5/4
BMI (kg/m^2)	24.9 ± 2.1	23.7 ± 2.2	24.1 ± 2.4	23.6 ± 2.1	25.3 ± 1.8
HR (beats/min)	77 ± 7.0	76 ± 6.0	77 ± 5.9	75 ± 5.8	78 ± 7.1
SBP (mm Hg)	158 ± 7.2	154 ± 7.1	155 ± 8.4	154 ± 7.4	160 ± 7.1
DBP (mm Hg)	96 ± 8.1	95 ± 8.4	94 ± 8.2	96 ± 8.2	99 ± 7.6
MAP (mm Hg)	117 ± 7.3	115 ± 7.2	114 ± 7.3	115 ± 7.4	119 ± 7.3
Disease duration (y)	3.4 ± 2.2	3.8 ± 2.4	3.8 ± 2.4	3.4 ± 2.6	3.4 ± 1.8
*CYP2D6*10* frequency	0.51	0.53	0.52	0.54	0.49

Values are mean ± SD where appropriate. *P* not significant for all characteristics.
BMI, Body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

among diplotype groups, with β_1-adrenergic receptor diplotype as a factor and body mass index as the covariate. When these analyses revealed a significant effect of diplotype, the percentage of blood pressure and heart rate decrease from baseline to the final measurements by diplotype groups was compared by 1-way ANOVA. When ANOVA and repeated-measures ANOVA were used, post-hoc analysis was performed with least significant difference. All values are reported as means ± SD in the figures and text. A 2-tailed value of $P < .05$ was considered statistically significant.

RESULTS

Genotype result. Two hundred twenty-three patients could be genotyped unambiguously for Gly389Arg and Ser49Gly polymorphisms of the β_1-adrenergic receptor. Repeated assays for randomly selected samples reproduced the accuracy of genotyping results in all samples. We observed a 16.1% frequency of the Gly49 allele, and the frequency of the Arg389 allele was 76.1%, consistent with previous reports.[18] At position 49 of the β_1-adrenergic receptor, 68.7% of patients were homozygous for the Ser genotype, 1.0% were homozygous for the Gly genotype, and 30.3% were heterozygous. At position 389, 58.9% were homozygous for the Arg genotype, 6.6% were homozygous for the Gly genotype, and 34.6% were heterozygous. Genotype frequencies for both polymorphisms were in Hardy-Weinberg equilibrium ($P > .05$). As previously reported, we also observed significant linkage disequilibrium between positions 49 and 389 ($|D'| = 0.92$, $P < .001$).

Responses to metoprolol monotherapy by genotype groups. Table I shows the baseline characteristics of the patients stratified by β_1-adrenergic receptor genotypes. There were no differences in blood pressure or other clinical characteristics among groups. The results from the clinical study stratified by the position 49 genotypes are shown in Fig 1. Ser49 homozygous patients had a more significant decrease in systolic blood pressure compared with Ser49Gly patients (8.4% ± 3.2% versus 5.3% ± 5.2%, $P = .047$). Decreases in diastolic blood pressure (4.6% ± 4.1% versus 3.7% ± 4.0%, $P = .447$) and mean arterial pressure (6.3% ± 3.1% versus 4.4% ± 3.9%, $P = .097$) were not different between the 2 cohorts. There was a significant effect of Gly389Arg polymorphism on blood pressure response to metoprolol in patients with hypertension (Fig 2). Systolic blood pressure decrease was less with patients who were homozygous for Gly389 compared with the homozygous Arg389 patients (10.4% ± 4.0% versus 1.1% ± 1.5%, $P < .001$). Heterozygous patients had an intermediate systolic blood pressure decrease of 2.8% ± 4.7%, which was marginally statistically different from the homozygous Arg389 response ($P = .001$). We also found a similar difference in changes of diastolic blood pressure (6.1% ± 4.3%, 2.2% ± 4.2%, and 0.9% ± 4.0% for Arg389Arg, Arg389Gly, and Gly389Arg patients, respectively; $P < .001$) and mean arterial pressure (8.1% ± 3.5%, 2.5% ± 3.0%, and 1% ± 2.5% for Arg389Arg, Arg389Gly, and Gly389Arg patients, respectively; $P < .001$). Our results indicated that neither Ser49Gly nor Gly389Arg genotypes of the β_1-adrenergic receptor had a significant effect on heart rate response to metoprolol (all *P* values >.05; data not shown).

Responses to metoprolol monotherapy by diplotype groups. We next considered whether certain haplotypes of the 2 polymorphisms predict blood pressure decrease. According to the haplotype combinations (diplotype), patients were stratified to 4 groups. The baseline characteristics of the 4 diplotype groups are

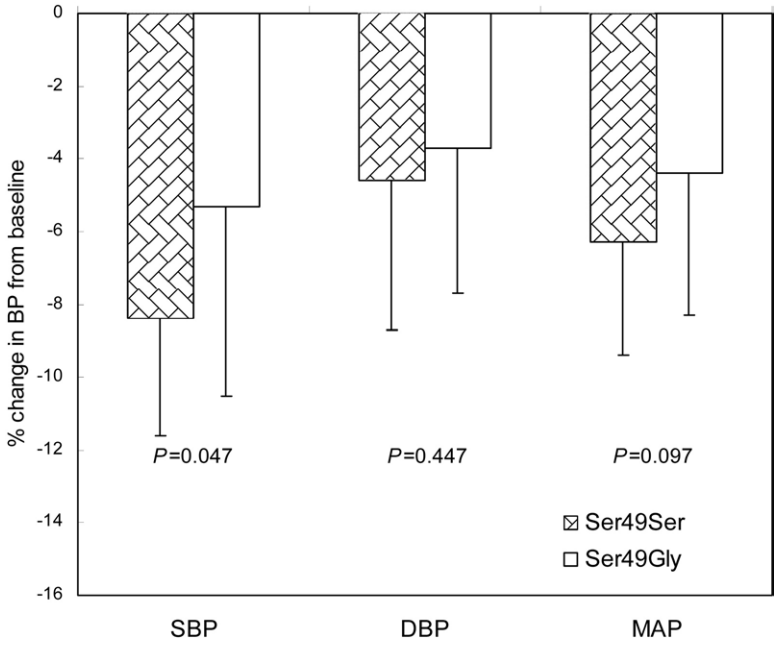

Fig 1. Blood pressure response to metoprolol monotherapy in patients with hypertension stratified according to β_1-adrenergic receptor Ser49Gly genotypes (Ser49Ser, n = 27; Ser49Gly, n = 34). Data are presented as mean percentage decrease with SD. SBP, Systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

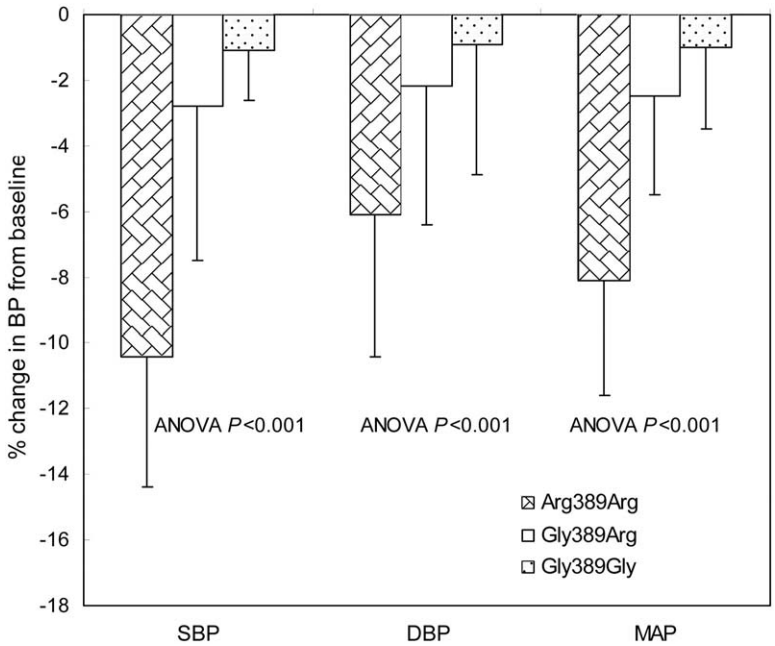

Fig 2. Blood pressure response to metoprolol monotherapy in patients with hypertension stratified according to β_1-adrenergic receptor Gly389Arg genotypes (Arg389Arg, n = 33; Gly389Arg, n = 19; Gly389Gly, n = 9). Data are presented as mean percentage decrease with SD. SBP, Systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Table II. Baseline characteristics of patients with hypertension with different diplotypes of the β_1-adrenergic receptor

	Genotype				
Characteristic	*49S389R/49S389R (n = 18)*	*49S389R/49G389R (n = 15)*	*49S389G/49G389R (n = 19)*	*49S389G/49S389G (n = 9)*	*P value*
Age (y)	53 ± 10	57 ± 10	55 ± 10	52 ± 11	0.622
Sex (M/F)	10/8	7/8	12/7	5/4	0.819
BMI (kg/m^2)	24.4 ± 2.2	23.9 ± 2.3	23.6 ± 2.1	25.3 ± 1.8	0.243
HR(beats/min)	76 ± 6.8	77 ± 4.9	75 ± 5.8	78 ± 7.1	0.603
SBP (mm Hg)	156 ± 6.8	155 ± 7.2	154 ± 7.4	160 ± 7.1	0.194
DBP (mm Hg)	95 ± 8.0	93 ± 8.8	96 ± 8.2	99 ± 7.6	0.415
MAP (mm Hg)	115 ± 7.0	114 ± 7.1	115 ± 7.4	119 ± 7.3	0.327
Disease duration (y)	3.4 ± 2.3	4.2 ± 2.3	3.4 ± 2.6	3.4 ± 1.8	0.698
*CYP2D6*10* frequency	0.52	0.52	0.54	0.49	0.523

Values are mean ± SD where appropriate.

summarized in Table II. There were no differences in blood pressure or other clinical characteristics among groups. Patients in the 4 diplotype groups were well matched for age, sex, and disease duration. The responses of heart rate, systolic blood pressure, diastolic blood pressure, and mean arterial pressure to 4 weeks of metoprolol therapy are summarized in Table III. Repeated-measures data ANOVA suggests that there was a highly significant relationship between diplotype and treatment blood pressure ($P < .001$ for systolic blood pressure and mean arterial pressure; $P = .020$ for diastolic blood pressure), whereas the heart rate response was not significantly different among groups ($P = .508$). Fig 3 shows the different decrease in blood pressure with various diplotypes. There was a significant relationship between diplotype groups and systolic blood pressure, diastolic blood pressure, and mean arterial pressure ($P < .001$ for systolic blood pressure and mean arterial pressure; $P = .001$ for diastolic blood pressure by ANOVA). The percentages of systolic blood pressure decrease for 49Ser389Arg/49Ser389Arg and 49Ser389Arg/49Gly389Arg patients were 12.0% ± 3.8% ($P < .001$) and 8.4% ± 5.5% ($P < .001$), respectively, whereas systolic blood pressure did not change significantly in 49Ser389Gly/49Gly389Arg (2.8% ± 4.7%, $P = .068$) and 49Ser389Gly/49Ser389Gly (1.1% ± 1.5%, $P = .159$) patients. A further comparison between 2 good-response groups indicated that the extent of systolic blood pressure reduction was significantly different between 49Ser389Arg/49Ser389Arg and 49Ser389Arg/49Gly389Arg patients ($P = .023$). A significant decrease during metoprolol therapy for diastolic blood pressure was found in 49Ser389Arg/49Ser389Arg (6.5% ± 4.7%, $P < .001$) and 49Ser389Arg/49Gly389Arg (5.7% ± 3.2%, $P = .001$) patients, whereas diastolic blood pressure did not alter significantly in 49Ser389Gly/49Gly389Arg (2.2% ± 4.2%, $P = .064$) and 49Ser389Gly/49Ser389Gly (0.9% ± 4.0%, $P = .525$) patients. In contrast to a significant difference in systolic blood pressure decrease, the extent of diastolic blood pressure decrease was not significantly different between 49Ser389Arg/49Ser389Arg and 49Ser389Arg/49Gly389Arg groups ($P = .578$). We also found differences among diplotype groups for mean arterial pressure decrease. The percentage of mean arterial pressure decrease was 8.8% ± 3.2% in 49Ser389Arg/49Ser389Arg patients ($P < .001$) versus 6.9% ± 3.7% in 49Ser389Arg/49Gly389Arg patients ($P < .001$), whereas mean arterial pressure did not alter significantly in 49Ser389Gly/49Gly389Arg (2.5% ± 3.0%, $P = .171$) and 49Ser389Gly/49Ser389Gly (1.0% ± 2.5%, $P = .260$) patients.

DISCUSSION

It is well recognized by physicians that some medications exhibit wide interpatient variability in their efficacy and toxicity.[8] There are responders and nonresponders to β-blocker therapy in patients with heart failure and hypertension.[9,10] Factors that contribute to variability in β-blocker therapy have been elucidated, including race,[4,6] renin levels,[29] variability in drug-metabolizing enzymes, and genetic polymorphisms in the β_1-adrenergic receptor.[11]

Besides information from in vitro results, our previous studies and those of others with different designs in both healthy subjects and patients of different ethnicity

Table III. Cardiovascular response to 4 weeks of metoprolol therapy in patients with hypertension stratified by β_1-adrenergic receptor diplotype

	Diplotype				*P value*	
Parameter	*49S389R/49S389R (n = 18)*	*49S389R/49G389R (n = 15)*	*49S389G/49G389R (n = 19)*	*49S389G/49S389G (n = 9)*	*Drug effect*	*Drug × haplotype effect*
HR (beats/min)					$P < .001$	$P = .508$
Baseline	76 ± 6.8	77 ± 4.9	75 ± 5.8	78 ± 7.1		
Week 1	68 ± 6.6	70 ± 6.6	71 ± 4.7	69 ± 5.4		
Week 2	67 ± 6.4	68 ± 5.5	68 ± 5.2	65 ± 7.1		
Week 3	67 ± 6.6	68 ± 5.7	69 ± 5.3	68 ± 5.4		
Week 4	67 ± 6.5	69 ± 5.0	68 ± 4.9	72 ± 6.9		
SBP (mm Hg)					$P < .001$	$P < .001$
Baseline	156 ± 6.8	155 ± 7.2	154 ± 7.4	160 ± 7.1		
Week 1	147 ± 7.3	152 ± 5.5	152 ± 7.8	159 ± 6.2		
Week 2	145 ± 9.0	149 ± 6.6	153 ± 6.7	160 ± 5.8		
Week 3	142 ± 7.1	145 ± 7.1	150 ± 8.5	156 ± 5.7		
Week 4	137 ± 6.9	142 ± 8.7	150 ± 9.2	159 ± 5.8		
DBP (mm Hg)					$P < .001$	$P = .020$
Baseline	95 ± 8.0	93 ± 8.8	96 ± 8.2	99 ± 7.6		
Week 1	93 ± 7.5	90 ± 5.9	95 ± 8.0	100 ± 7.1		
Week 2	91 ± 6.2	88 ± 5.7	94 ± 9.3	99 ± 7.0		
Week 3	90 ± 6.0	88 ± 5.0	93 ± 8.9	98 ± 8.1		
Week 4	89 ± 6.7	87 ± 7.8	93 ± 8.6	98 ± 8.6		
MAP (mm Hg)					$P < .001$	$P < .001$
Baseline	115 ± 7.0	114 ± 7.1	115 ± 7.4	119 ± 7.3		
Week 1	111 ± 6.2	111 ± 4.3	115 ± 7.0	119 ± 6.0		
Week 2	109 ± 5.9	108 ± 3.8	114 ± 7.6	119 ± 6.0		
Week 3	108 ± 5.6	107 ± 3.2	113 ± 7.5	117 ± 7.2		
Week 4	105 ± 5.4	106 ± 5.7	113 ± 7.4	118 ± 7.4		

Values are mean ± SD.

P values were of repeated-measures data ANOVA among diplotype groups. Within-subject variable is drug effect; number of levels is 5 (baseline, week 1, week 2, week 3, and week 4); between-subjects factor is group.

have elucidated the in vivo impact of the β_1-adrenergic receptor Ser49Gly and Gly389Arg variants on cardiovascular responses to β_1-adrenergic receptor selective antagonists.[24,30-32] The published literature has focused on isolated single-nucleotide polymorphisms rather than haplotypes or diplotypes and inconsistencies. For example, in a retrospective study, O'Shaughnessy et al[31] failed to find any significant differences between carriers of the Gly389 and Arg389 alleles in blood pressure and heart rate responses to β-blockers with either 50 mg atenolol or 5 mg bisoprolol in patients with hypertension, whereas Sofowora et al[32] found that atenolol caused a significantly larger decrease in resting systolic and mean arterial blood pressure in volunteers homozygous for Arg389 than it did in volunteers homozygous for Gly389. Recently, Johnson et al[24] found that β_1-adrenergic receptor polymorphisms had a significant effect on the reduction in 24-hour and daytime diastolic blood pressure response to metoprolol in white, black, and Hispanic patients with hypertension. Their data also suggested the importance of diplotypes rather than individual genotypes in determining the in vivo role of these polymorphisms.[24] On the basis of this information, our study was designed to confirm whether the different responses to metoprolol according to β_1-adrenergic receptor genotypes or diplotypes existed in a Chinese population with hypertension.

In this work we found that patients with hypertension who had the homozygous Arg389 β_1-adrenergic receptor polymorphism had a significant decrease in systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure in response to metoprolol compared with those with the Gly389 β_1-adrenergic receptor. This finding is consistent with an in vitro study showing the Arg389 receptor form having nearly twofold greater basal and threefold greater agonist-mediated adenylyl cyclase activities,[17] and with several in vivo studies that demonstrated that the cardiovascular response to β-blockers are enhanced in subjects with homozygous Arg389 genotype.[24,30,32]

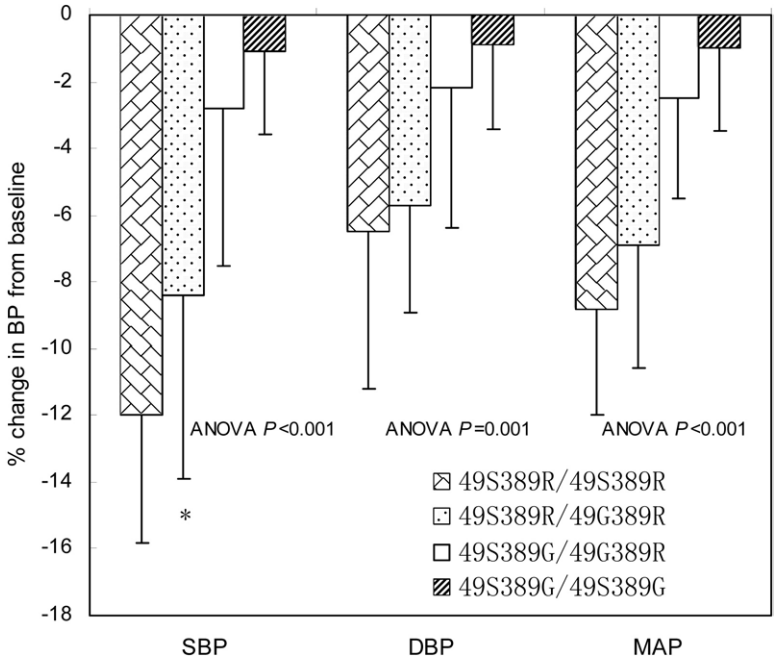

Fig 3. Blood pressure response to metoprolol monotherapy in patients with hypertension stratified according to β_1-adrenergic receptor diplotypes (49S389R/49S389R, n = 18; 49S389R/49G389R, n = 15; 49S389G/49G389R, n = 19; 49S389G/49S389G, n = 9). Data are presented as mean percentage decrease with SD. SBP, Systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. *Asterisk* denotes $P = .023$, compared with 49S389R/49S389R patients.

A smaller contribution to the antihypertensive effect of metoprolol in these patients was the polymorphisms at amino acid position 49. Our result indicated that the Ser49 homozygous patients had more significant decrease in systolic blood pressure compared with Ser49Gly patients, which is consistent with in vitro data that the Gly49 allele undergoes greater agonist-mediated receptor down-regulation.[16] These findings also partly agree with the results from Johnson et al,[24] which found that blood pressure response to metoprolol appears to be a consistently larger magnitude of blood pressure reduction across all ambulatory blood pressure parameters in Ser49 homozygotes compared with Gly49 carriers, with a trend toward significance for 24-hour and daytime diastolic blood pressure.

It has been well documented by Johnson et al[24] that the greatest predictive β_1-adrenergic receptor polymorphisms were haplotypes that included position 49 and 389 polymorphisms. In this study we also found that the β_1-adrenergic receptor diplotype was strongly associated with the reduction in systolic blood pressure, diastolic blood pressure, and mean arterial pressure in response to metoprolol. Carriers of Ser49Arg389/Ser49Arg389 and Ser49Arg389/Gly49Arg389 diplotypes had good response to metoprolol therapy, whereas patients with Ser49Gly389/Gly49Arg389 and Ser49Gly389/Ser49Gly389 were nonresponders. These findings confirmed the rank order of diplotype responsiveness established by Johnson et al[24] in white, black, and Hispanic patients with hypertension and suggested that the genetic associations do not differ by ethnicity.

In contrast to the findings by Johnson et al,[24] which showed a significant reduction only in diastolic blood pressure, our data indicated that both diastolic and systolic blood pressure had a different response to metoprolol according to genotype or diplotype groups. There are several investigative approaches that can be considered to unravel the discrepancy. The larger numbers of subjects used in our study allowed the assessment of significant differences in systolic blood pressure by genotype or diplotype groups. Our study population included 9 Gly389 homozygotes, who had a significantly different response, compared with Arg389 homozygotes, than Gly389Arg heterozygotes did. In addition, untreated systolic blood pressure in our subjects was between 140 mm Hg and 180 mm Hg, whereas the systolic blood pressure above a certain threshold was not required for the study by Johnson et

al,[24] which may lead to a different baseline systolic blood pressure between our subjects and those of Johnson et al. How baseline blood pressure affects the response to metoprolol is still uncertain, but the difference may be a potential contributor to the different findings between our study and that of Johnson et al.[24]

In the current study, metoprolol concentration, which is mainly determined by CYP2D6 activity,[11] was not measured. To exclude the potential effect of different metoprolol concentrations among groups on the blood pressure response to metoprolol, 61 patients were genotyped for *CYP2D6*10* variance, which is the most common genetic polymorphism occurring on *CYP2D6* and has been confirmed to have a good correlation with metoprolol concentration in the Chinese population.[26] Other genetic polymorphisms were extremely rare in the Chinese population and were not found in these 61 patients. In fact, as shown in Tables I and II, *CYP2D6* polymorphisms were equally distributed among the cohorts, which suggested that the different blood pressure response to metoprolol therapy among groups was not confounded by metoprolol plasma levels.

In the Chinese population, the typical initial dosage for metoprolol in antihypertensive therapy is 25 mg every 12 hours with titration every 4 weeks to 50 mg every 12 hours.[27] In contrast to the titration dose of metoprolol used by Johnson et al,[24] the dosage of metoprolol was fixed to 25 mg every 12 hours in our 4-week study. Because data from numerous studies indicate that the observed difference in blood pressure response to metoprolol cannot be addressed through differential doses or plasma concentrations,[24,30,32] we think our findings cannot be ascribed to the effects of dosage regimen.

Because our study was not designed to test the long-term effects of metoprolol, the longer-duration effects of genetic polymorphisms on cardiovascular response to metoprolol need to be further studied. Another flaw in our study was that the effects of genetic polymorphisms on cardiovascular response to metoprolol were not assessed by 24-hour ambulatory blood pressure data, which can provide more accurate and precise information than clinic blood pressure data.

In summary, our study confirms that β_1-adrenergic receptor polymorphism is associated with response to metoprolol therapy in Chinese patients with essential hypertension. Carriers of 49Ser389Arg/49Ser389Arg and 49Ser389Arg/49Gly389Arg were good responders, whereas 49Ser389Gly/49Gly389Arg and 49Ser389Gly/49Ser389Gly patients were nonresponders. These data confirm functional differences among the common β_1-adrenergic receptor diplotypes and the need for consideration of diplotypes in determining antihypertensive therapy.

All authors have no conflict of interest.

References

1. Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. *Annu Rev Pharmacol Toxicol* 2003;43:381-411.
2. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, et al. Decreased catecholamine sensitivity and β_1-adrenergic receptor density in failing human hearts. *N Engl J Med* 1982;307:205-11.
3. Lindholm LH, Carlberg B, Samuelsson O. Should beta blockers remain first choice in the treatment of primary hypertension? A meta-analysis. Lancet 2005; 366:1545-53.
4. Venter CP, Joubert PH. Ethnic differences in response to β_1-adrenoceptor blockade by propranolol. J Cardiovasc Pharmacol 1984;6:361-4.
5. Venter CP, Joubert PH, Strydom WJ. The relevance of ethnic differences in hemodynamic responses to the head-up tilt manoeuvre to clinical pharmacological investigations. J Cardiovasc Pharmacol 1985;7:1009-10.
6. Zhou HH, Koshakji RP, Silberstein DJ, Wilkinson GR, Wood AJJ. Racial differences in drug response: altered sensitivity to and clearance of propranolol in men of Chinese descent as compared with American whites. N Engl J Med 1989;320:565-70.
7. Comparison of propranolol and hydrochlorothiazide for the initial treatment of hypertension. II. Results of long-term therapy. Veterans Administration Cooperative Study Group on Antihypertensive Agents. JAMA 1982; 248:2004-11.
8. Materson BJ, Reda DJ, Cushman WC, Massie BM, Freis ED, Kochar MS, et al. Single-drug therapy for hypertension in men. A comparison of six antihypertensive agents with placebo. The Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. N Engl J Med 1993;328:914-21.
9. Vestal RE, Wood AJ, Shand DG. Reduced β_1-adrenoceptor sensitivity in the elderly. Clin Pharmacol Ther 1979;26:181-6.
10. Feely J, Crooks J, Stevenson IH. The influence of age, smoking and hyperthyroidism on plasma propranolol steady state concentration. Br J Clin Pharmacol 1981;12: 73-8.
11. Lennard MS, Silas JH, Freestone S, Ramsay LE, Tucker GT, Woods HF. Oxidation phenotype: a major determinant of metoprolol metabolism and response. N Engl J Med 1982;307:1158-60.
12. Vesell ES. Advances in pharmacogenetics and pharmacogenomics. J Clin Pharmacol 2000;40:930-8.
13. Frielle T, Collins S, Daniel KW, Caron M, Lefkowitz RJ, Kobilka BK. Cloning of the cDNA for the human β1-adrenergic receptor. Proc Natl Acad Sci U S A 1987; 84:7920-4.

14. Yang-Feng TL, Xue F, Zhong W, Cotecchia S, Frielle T, Francke U. et al. Chromosomal organization of adrenergic receptor genes. Proc Natl Acad Sci U S A 1990;87: 1516-20.
15. Podlowski S, Wenzel K, Lutter HP, Muller J, Bramlage P, Baumann G, et al. β_1-adrenoceptor gene variations: a role in idiopathic dilated cardiomyopathy? J Mol Med 2000;78:87-93.
16. Rathz DA, Brown KM, Kramer LA, Liggett SB. Amino acid 49 polymorphisms of the human β_1-adrenergic receptor affect agonist-promoted trafficking. J Cardiovasc Pharmacol 2002;39:155-60.
17. Mason DA, Moore JD, Green SA, Liggett SB. A gain-of-function polymorphism in a G-protein coupling domain of the human β_1-adrenergic receptor. J Biol Chem 1999;274:12670-4.
18. Moore JD, Mason DA, Green SA, Hsu J, Liggett SB. Racial difference in the frequencies of cardiac β_1-adrenergic receptor polymorphisms: analysis of c145A→G and c1165G→C. Hum Mut 1999;14:271.
19. Maqbool A, Hall AS, Ball SG, Balmforth AJ. Common polymorphisms of β_1-adrenoceptor: identification and rapid screening assay [letter]. Lancet 1999;353:897.
20. Xie HG, Dishy V, Sofowara G, Kim RB, Landau R, Smiley RM, et al. Arg389Gly β_1-adrenoceptor polymorphism varies in frequency among different ethnic groups but does not alter response in vivo. Pharmacogenetics 2001;11:191-7.
21. Tesson F, Charron P, Peuchmaurd M, Nicaud V, Cambien F, Tiret L, et al. Characterization of a unique genetic variant in the β_1-adrenoceptor gene and evaluation of its role in idiopathic dilated cardiomyopathy. CARDIGENE Group. J Mol Cell Cardiol 1999;31:1025-32.
22. Terra SG, McGorray SP, Wallace MR, Picoult-Newberg L, Pepine CJ, Johnson JA. β_1-adrenergic receptor polymorphisms and linkage disequilibrium [abstract]. Clin Pharmacol Ther 2002;71:P70.
23. Sandilands A, Yeo G, Brown MJ, O'Shaughnessy KM. Functional responses of human beta1 adrenoceptors with defined haplotypes for the common 389R>G and 49S>G polymorphisms. Pharmacogenetics 2004;14: 343-9.
24. Johnson JA, Zineh I, Puckett BJ, McGorray SP, Yarandi HN, Pauly DF. β_1-Adrenergic receptor polymorphisms and antihypertensive response to metoprolol. Clin Pharmacol Ther 2003;74:44-52.
25. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1989.
26. Garcia-Barcelo M, Chow LY, Chiu HF, Wing YK, Lee DT, Lam KL, et al. Genetic analysis of the CYP2D6 locus in a Hong Kong Chinese population. Clin Chem 2000;46:18-23.
27. Jiang Z, Ma H, Zhang L. Clinical observation of metoprolol in treatment of essential hypertension. Chin J Hypertension 1994;2:125-6.
28. Rozas J, Rozas R. DnaSP version 3: An integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 1999;15:174-5.
29. Kralberg BE, Tolagen K. Different antihypertensive effect of β-blocking drugs in low and normal-high renin hypertension. Am J Med 1976;60:891-6.
30. Liu J, Liu ZQ, Tan ZR, Chen XP, Wang LS, Zhou G, et al. Gly389Arg polymorphism of beta1-adrenergic receptor is associated with the cardiovascular response to metoprolol. Clin Pharmacol Ther 2003;74:372-9.
31. O'Shaughnessy KM, Fu B, Dickerson C, Thurston D, Brown MJ. The gain-of-function G389R variant of the beta1-adrenoceptor does not influence blood pressure or heart rate response to beta-blockade in hypertensive subjects. Clin Sci 2000;99:233-8.
32. Sofowora G.G, Dishy V, Muszkat M, Xie HG, Kim RB, Stein CM, et al. A common β_1-adrenergic receptor polymorphism (Arg389Gly) affects blood pressure response to β-blockade. Clin Pharmacol Ther 2003;74:366-71.