# Individual and joint association of $\alpha_{1A}$-adrenergic receptor Arg347Cys polymorphism and plasma irbesartan concentration with blood pressure therapeutic response in Chinese hypertensive subjects

*Background:* **Individual variability in the therapeutic response to an antihypertensive drug could have a genetic basis. We investigated whether the $\alpha_{1A}$-adrenergic receptor ($\alpha_{1A}$-AR) Arg347Cys polymorphism is associated with the blood pressure (BP) therapeutic response to irbesartan and whether the association could be altered by the plasma irbesartan level.**

*Methods:* **A total of 696 hypertensive subjects were treated with a daily oral dose of 150 mg irbesartan. Baseline BP was measured before the first dose. On the 28th day, after 27 consecutive days of treatment and an overnight fast, BPs and blood samples were obtained before the morning dose (0 hours) and 6 hours after the morning dose was taken. Plasma irbesartan concentrations were measured by use of HPLC-fluorescence.**

*Results:* **BP therapeutic response was defined as baseline BP minus BP on the 28th day of irbesartan treatment. Relative to noncarriers, $\alpha_{1A}$-AR Cys347 allelic carriers had a significantly greater diastolic blood pressure (DBP) response at 0 hours (mean ± SD, 7.5 ± 8.4 mm Hg versus 5.5 ± 8.4 mm Hg; $P = .016$) and at 6 hours (16.2 ± 9.1 mm Hg versus 14.2 ± 8.9 mm Hg, $P = .025$). Although the pattern was similar to the DBP response, $\alpha_{1A}$-AR Cys347 allelic carriers had only a moderately increased systolic blood pressure (SBP) response at the 2 time points. When subjects were stratified into subgroups with high or low plasma irbesartan concentrations (with the median value used as the cutoff point), Cys347 allelic carriers in the high-concentration group, relative to noncarriers, had a more pronounced DBP response at 0 hours (adjusted β [± SE], 3.0 ± 1.0 mm Hg; $P = .004$) and at 6 hours (adjusted β, 3.0 ± 1.2 mm Hg; $P = .014$), and the**

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**same was true for the SBP response at 0 hours (adjusted β, 5.6 ± 2.1 mm Hg; $P = .006$) and at 6 hours (adjusted β, 4.7 ± 2.0 mm Hg; $P = .021$). In contrast, in the low-concentration group, there was no significant association between DBP or SBP responses and Arg347Cys genotypes at 0 hours and 6 hours.** ***Conclusion:*** **Our data suggest that the $\alpha_{1A}$-AR Arg347Cys polymorphism is associated with BP response (particularly DBP) to short-term irbesartan treatment. Our data also showed evidence of an interaction between the $\alpha_{1A}$-AR Arg347Cys polymorphism and the plasma level of irbesartan in relation to BP therapeutic response. The association of the Arg347Cys polymorphism with the BP therapeutic response was more pronounced in those patients with higher plasma concentrations of irbesartan. (Clin Pharmacol Ther 2005; 78:239-48.)**

Irbesartan, a nonpeptide angiotensin II type 1 (AT1) receptor antagonist, acts at the final step of the renin-angiotensin system by selectively blocking the binding of angiotensin II to the AT1 receptor. Several clinical trials have revealed that among mild-to-moderate hypertensive patients who were randomized to oral treatment with 150 mg irbesartan once daily or placebo for 6 to 8 weeks only 56% of irbesartan-treated patients had a favorable response.[1,2] Considerable variations were observed in the distribution of blood pressure (BP) responses to treatment at the usual therapeutic dosages. The reasons for this individual variability are largely unknown. $\alpha_1$-Adrenergic response was associated with BP response to treatment in hypertensive patients.[3] Studies in monozygotic and dizygotic twins[4] and in families[5] suggest that the great variability in $\alpha_1$-adrenergic receptor ($\alpha_1$-AR)–mediated vascular response may be attributable to genetic factors. It is hypothesized that drug pharmacodynamic response may be related to those polymorphic candidate genes coding for the structure, configuration, activity, or quantity of drug-targeted receptors.

In this pharmacogenetic study we focus on gene encoding for $\alpha_1$-ARs, which primarily mediate vasoconstriction by sympathetic increase in peripheral vascular tone[6,7] and regulate BP.[8,9] Studies have shown that angiotensin II pretreatment to vascular smooth muscle cells (VSMCs) increases the rate of $\alpha_1$-AR de novo synthesis, enhances transcription and expression of $\alpha_1$-AR genes,[10] and is associated with an increase in $\alpha_1$-AR–stimulated growth-related c-*fos* expression.[10,11] The predominantly potentiating effect of angiotensin II on $\alpha_1$-AR–mediated periarterial electrical nerve–stimulating double-peaked vasoconstriction can be effectively inhibited by an AT1 receptor antagonist.[12] An AT1 receptor antagonist administered to improve left ventricular hypertrophy also suppresses urinary catecholamine excretion and cardiac $\alpha_1$-AR density.[13] Animal models have demonstrated that endothelium-independent desensitization or vascular deoxyribonucleic acid (DNA) synthesis stimulation induced by norepinephrine or angiotensin II on the smooth muscle contractile response is blocked separately by the AT1 receptor antagonist losartan or the $\alpha_1$-AR antagonist prazosin.[14-16] Among 3 subtypes of adrenergic receptors ($\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$),[17] the $\alpha_{1A}$-adrenergic receptor ($\alpha_{1A}$-AR) may be a major contributor to adrenergic vasoconstriction and systemic arterial pressure in sympathetic regulation of peripheral resistance in animals and humans.[7,8] A common polymorphism of the $\alpha_{1A}$-AR gene, the substitution of a C residue for T at nucleotide 1441, results in the substitution of Cys for Arg at codon 347 (Arg347Cys).[18] The palmitoylation site conferred by the Arg347Cys polymorphism in the carboxy-terminal tail of the $\alpha_{1A}$-AR gene may play a key role in its cellular localization and function.[18] We investigated whether the $\alpha_{1A}$-AR Arg347Cys polymorphism is associated with the BP therapeutic response to irbesartan, an AT1 receptor antagonist. We were also interested in whether there was an interaction between the $\alpha_{1A}$-AR Arg347Cys polymorphism and the plasma irbesartan concentration on the BP therapeutic response to irbesartan.

## METHODS

***Study population.*** Patients with mild to moderate hypertension were enrolled from the Taihu community of the Anhui Province, China, from February 2003 to January 2004. The inclusion criteria were as follows: (1) having systolic blood pressure (SBP) between 140 mm Hg and 200 mm Hg or diastolic blood pressure (DBP) between 90 mm Hg and 120 mm Hg, (2) not currently taking antihypertensive medication (4 weeks before the study), and (3) having no secondary hypertension. To avoid potentially severe adverse effects or possible influences on irbesartan's efficacy, patients who were pregnant or lactating and those who had hyperkalemia, severe arrhythmia, chronic heart failure, severe liver or renal dysfunction, or a history of myocardial infarction or stroke, acute coronary artery syndrome, or acute heart failure in the past 3 months were excluded. The study was approved by the Ethics Committee of Anhui Medical University, Hefei, China. The

purpose and procedures of the study were carefully explained to all participants, and written informed consent was obtained.

***BP measurement, blood sampling, and irbesartan administration.*** Participants were invited to our research center, where they underwent a physical examination. After overnight fasting, at 8 AM on the next day, baseline BP was measured by trained nurses after participants rested in a seated position for 15 minutes. All BPs were measured with a standardized mercury sphygmomanometer with appropriately sized cuffs. SBP was recorded by Korotkoff phase I (appearance of sound), and DBP was recorded by Korotkoff phase V (disappearance of sound). Three consecutive measurements were taken at 30-second intervals between readings. If the difference between the measurements was more than 4 mm Hg, the patient was asked to rest for 5 minutes and the measurements were then retaken. In all of our analyses, the mean of the 3 consecutive BP readings was used.

Afterward, participants were given 150 mg irbesartan orally (Sanofi-Synthelabo Minsheng, Hangzhou, China). This dosage was chosen to treat all participants on the basis of considerations of safety and effectiveness.[1] During the consecutive 27-day treatment period, the participants were required to take irbesartan around 8 AM and to record the time at which they took the drug, as well as any side effects. They were also invited to visit our local study site once a week to report any adverse effects and pick up the drugs for the next week. On the 27th day, the participants returned to our research center and stayed there overnight. On the next morning, at 8 AM, the corresponding 0- and 6-hour BPs were measured according to the same procedures as those performed at baseline. A fasting blood sample (0 hours) was taken, and a second blood sample was taken 6 hours after patients had taken the dose of irbesartan that morning. The plasma was separated and transferred to screw-capped polypropylene tubes and stored at −20°C until analysis.

***Plasma irbesartan concentration measurement.*** Plasma samples that had been stored at −20°C were thawed and pretreated before the concentration of irbesartan was measured by the HPLC-fluorescence method.[19] In brief, plasma samples were centrifuged at 4000*g* for 10 minutes after thawing at room temperature. An aliquot (400 μL) of plasma together with a 40-μL internal standard, flunarizine (Di-Nuo, Hunan, China), and 560 μL acetonitrile was pipetted into 1.5-mL polypropylene tubes. The mixture was blended in a vortex mixer for 30 seconds, incubated for 5 minutes at room temperature, and then centrifuged at 4000*g* for 20 minutes. The supernatant was pipetted and injected into the HPLC system. A fluorescence detector set at an excitation wavelength of 250 nm and an emission wavelength of 375 nm was used to detect the peak value of irbesartan. In analysis, solution and mobile phases were prepared just before use. The mobile phases consisted of acetonitrile/aqueous phosphoric acid–triethylamine solution (39:61 [vol/vol]); the latter was prepared by adding 1 mL triethylamine to 1000 mL double-distilled water and then adjusting the pH value to 4.2 with phosphoric acid. The analytic column was a Diamosil C18 column (5 μm, 150 × 4.6 mm) (Agilent Technologies, Palo Alto, Calif). The flow rate was 1.5 $mL \cdot min^{-1}$, and the column temperature was 30°C. Quantification of the metabolites was performed by comparing HPLC peak areas with those of authentic standards, with reference to an internal standard. To ensure the precision and accuracy of the assay, plasma quality-control samples were also prepared to contain 5 different irbesartan concentrations within the standard curve range and were analyzed with regard to intraday and interday means, SDs, and coefficients of variation.

***Genotyping.*** Venous blood samples were collected from participants, and genomic DNA was then extracted by use of the QIAamp Blood Kit (Qiagen, Valencia, Calif) and stored at −20°C until the genotype analysis was performed. We searched the literature and dbSNP for all potential nonsynonymous single-nucleotide polymorphisms in the $\alpha_{1A}$-AR gene.[20] Only the Arg347Cys locus was polymorphic in the Chinese population, and it was genotyped by use of the TaqMan genotyping assay designed and manufactured by Applied Biosystems (Foster City, Calif). Polymerase chain reaction products were amplified in a 5-μL reaction containing 10 ng genomic DNA, 1× master mix, 900-nmol/L forward and reverse primers, and two 250-nmol/L TaqMan MGB Probes with the use of 384-well plates on a PTC-225 Tetrad Thermal Cycler (MJ Research, Watertown, Mass) under the following conditions: 95°C for 10 minutes, 50 cycles at 92°C for 15 seconds, and 60°C for 1 minute. After polymerase chain reaction amplification, an endpoint plate reading of the fluorescence intensity of each well was performed on an ABI Primer 7900 system (Applied Biosystems). Genotype was scored automatically by use of SDS 2.1 software (Applied Biosystems) and inspected visually on the plot.

***Statistical analysis.*** The SAS 8.0 software package (SAS Institute, Cary, NC) was used to perform all statistical analyses. BP response was defined as BP before the first dose minus BP on the 28th day. Because BP was measured twice on the 28th day (at 0 hours and 6 hours) after the morning dose of irbesartan was taken,

**Table I.** Baseline characteristics of subjects by $\alpha_{1A}$-AR Arg347Cys genotype in Taihu community, Anhui Province, China

| | *$\alpha_{1A}$-AR Arg347Cys* | |
|---|---|---|
| *Characteristics* | *Cys347 noncarriers (n = 580)* | *Cys347 carriers (n = 116)* |
| Age (y) | 54.2 ± 7.0 | 53.4 ± 8.0 |
| Height (cm) | 156.4 ± 7.8 | 156.1 ± 7.4 |
| Weight (kg) | 53.7 ± 8.4 | 54.2 ± 9.3 |
| Body mass index ($kg/m^2$) | 21.9 ± 2.7 | 22.2 ± 2.9 |
| Drug concentration at 0 h (ng/mL) | 78.3 ± 51.9 | 76.9 ± 41.7 |
| Natural logarithm–transformed concentration at 0 h | 4.2 ± 0.7 | 4.2 ± 0.6 |
| Drug concentration at 6 h (ng/mL) | 430.7 ± 254.6 | 433.7 ± 264.2 |
| Natural logarithm–transformed concentration at 6 h | 5.9 ± 0.5 | 5.9 ± 0.5 |
| Baseline SBP (mm Hg) | 166.0 ± 16.8 | 166.8 ± 16.4 |
| Baseline DBP (mm Hg) | 91.7 ± 10.4 | 92.2 ± 10.5 |
| SBP response at 0 h (mm Hg) | 18.2 ± 17.9 | 21.1 ± 18.0 |
| DBP response at 0 h (mm Hg) | 5.5 ± 8.4 | 7.5 ± 8.4* |
| SBP response at 6 h (mm Hg) | 32.2 ± 18.4 | 35.1 ± 17.9 |
| DBP response at 6 h (mm Hg) | 14.2 ± 8.9 | 16.2 ± 9.1* |
| Female | 318 (54.8) | 71 (61.2) |
| Smoking status: Current | 232 (40.0) | 40 (34.5) |
| Alcohol status: Current | 113 (19.5) | 20 (17.2) |
| Occupation: Farmer | 458 (79.0) | 99 (85.3) |
| Education: Middle school or higher | 67 (11.6) | 16 (13.8) |

Data are presented as mean ± SD or number and percent.
$\alpha_{1A}$-AR, $\alpha_{1A}$-Adrenergic receptor; SBP, systolic blood pressure; DBP, diastolic blood pressure.
*Two-sided $P < .05$ by $t$ tests.

2 measures of BP response were generated for each study participant. We tested whether the genotypes of the $\alpha_{1A}$-AR Arg347Cys polymorphism were in Hardy-Weinberg equilibrium by use of a chi-square test. Because only 6 subjects were homozygous (Cys/Cys) for the Arg347Cys polymorphism and had the most pronounced BP response (especially DBP) to irbesartan at 0 hours and 6 hours, we combined Cys/Cys with the Arg/Cys heterozygous genotype in the analyses. Univariate associations between important covariates and Arg347Cys genotypes were investigated by use of chi-square and $t$ tests. Plasma irbesartan concentrations skewed toward the left but showed an approximately normal distribution after natural logarithm transformation (data not shown). The definitions of subgroups with low or high plasma irbesartan concentrations were based on the median natural logarithm–transformed values at 0 hours and 6 hours. We tested the association of Arg347Cys genotypes with BP response (both SBP and DBP) at 0 hours and 6 hours individually and stratified by plasma concentration subgroups by use of generalized linear regression models, with adjustment for baseline BP, age, age squared, gender, body mass index, height, height squared, weight, alcohol consumption, cigarette smoking, education, and occupation. We then repeated this analysis while including both 0-hour and 6-hour BP responses (both SBP and DBP) in the same models using generalized estimation equations[20a] to accommodate correlations in BP responses at the 2 times for the same individual and adding a term for time of BP reading (0 hours or 6 hours).

## RESULTS

In total, 696 subjects were enrolled with complete genotype and phenotype information. The genotype distribution conformed to Hardy-Weinberg equilibrium ($\chi^2 = 0.1$, $P = .757$). The demographic characteristics for the 2 genotype groups ($\alpha_{1A}$-AR Cys347 noncarriers and carriers) are shown in Table I. The means and prevalence of covariates including age, body mass index, height, weight, gender, alcohol consumption, and smoking status showed no significant differences between the 2 groups. The mean baseline SBP and DBP were 166.0 ± 16.8 mm Hg and 91.7 ± 10.4 mm Hg, respectively, in Cys347 noncarriers and 166.8 ± 16.4 mm Hg and 92.2 ± 10.5 mm Hg, respectively, in carriers. These findings suggested that the $\alpha_{1A}$-AR Arg347Cys polymorphism was not associated with baseline SBP or DBP measurements. After plasma irbesartan concentration values underwent natural logarithm transformation to approximate normal distributions, the natural logarithm values did not show marked differences in the 2 genotype groups at 0 hours (mean ± SD, 4.2 ± 0.7 ng/mL for noncarriers and 4.2 ± 0.6 ng/mL for carriers) or at 6 hours (5.9 ± 0.5 ng/mL for noncarriers and 5.9 ± 0.5 ng/mL for carriers). Relative

**Table II.** Relative mean BP responses by $\alpha_{1A}$-AR polymorphisms and plasma irbesartan concentrations (modeled via generalized linear regression)

| | | | *0 h on 28th d* | | | | *6 h on 28th d* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *BP response** | *Irbesartan plasma concentration†* | *Cys347 allele* | *No.* | *Mean ± SD* | β *(±SE)* | *P value‡* | *No.* | *Mean ± SD* | β *(±SE)* | *P value‡* |
| ΔSBP | Overall | Noncarrier | 580 | 18.2 ± 17.9 | 0 | | 580 | 32.2 ± 18.4 | 0 | |
| | | 1 Carrier | 110 | 21.3 ± 18.3 | 2.4 ± 1.7 | .167 | 110 | 35.1 ± 17.9 | 1.9 ± 1.6 | .245 |
| | | 2 Carriers | 6 | 17.5 ± 10.6 | −0.02 ± 6.8 | .998 | 6 | 35.8 ± 20.9 | 4.4 ± 6.3 | .490 |
| | | ≥1 Carrier | 116 | 21.1 ± 18.0 | 2.2 ± 1.6 | .174 | 116 | 35.1 ± 17.9 | 2.0 ± 1.5 | .177 |
| | Low | Noncarrier | 293 | 18.1 ± 19.1 | 0 | | 290 | 32.0 ± 18.8 | 0 | |
| | | ≥1 Carrier | 52 | 17.5 ± 18.8 | −2.1 ± 2.5 | .383 | 58 | 33.2 ± 18.3 | −0.2 ± 2.2 | .921 |
| | High | Noncarrier | 287 | 18.3 ± 16.5 | 0 | | 290 | 32.4 ± 18.0 | 0 | |
| | | ≥1 Carrier | 64 | 24.0 ± 16.9 | 5.6 ± 2.1 | .006 | 58 | 37.0 ± 17.5 | 4.7 ± 2.0 | .021 |
| ΔDBP | Overall | Noncarrier | 580 | 5.5 ± 8.4 | 0 | | 580 | 14.2 ± 8.9 | 0 | |
| | | 1 Carrier | 110 | 7.2 ± 8.0 | 1.6 ± 0.8 | .041 | 110 | 15.8 ± 8.5 | 1.5 ± 0.8 | .075 |
| | | 2 Carriers | 6 | 12.8 ± 13.7 | 4.7 ± 3.1 | .140 | 6 | 23.7 ± 16.0 | 5.7 ± 3.2 | .081 |
| | | ≥1 Carrier | 116 | 7.5 ± 8.4 | 1.8 ± 0.8 | .018 | 116 | 16.2 ± 9.1 | 1.7 ± 0.8 | .040 |
| | Low | Noncarrier | 293 | 5.5 ± 8.9 | 0 | | 290 | 14.8 ± 9.3 | 0 | |
| | | ≥1 Carrier | 52 | 5.7 ± 8.0 | 0.4 ± 1.2 | .750 | 58 | 16.3 ± 8.8 | 0.2 ± 1.1 | .817 |
| | High | Noncarrier | 287 | 5.4 ± 7.9 | 0 | | 290 | 13.6 ± 8.5 | 0 | |
| | | ≥1 Carrier | 64 | 9.0 ± 8.4 | 3.0 ± 1.0 | .004 | 58 | 16.2 ± 9.5 | 3.0 ± 1.2 | .014 |

BP, Blood pressure.
*BP response was defined as BP at baseline minus BP at 0 hours and 6 hours on 28th day.
†Low and high groups were defined by median value of natural logarithm–transformed plasma concentration at 0 hours and 6 hours.
‡Adjusted for baseline DBP and SBP, age, age squared, gender, body mass index, height, height squared, weight, alcohol consumption, cigarette smoking, education, and occupation.

to noncarriers, the DBP response was significantly greater in Cys347 allelic carriers at 0 hours (7.5 ± 8.4 mm Hg versus 5.5 ± 8.4 mm Hg, $P = .016$) and at 6 hours (16.2 ± 9.1 mm Hg versus 14.2 ± 8.9 mm Hg, $P = .025$), but the SBP response was only moderately higher in Cys347 allelic carriers at 0 hours (21.1 ± 18.0 mm Hg versus 18.2 ± 17.9 mm Hg, $P = .112$) and at 6 hours (35.1 ± 17.9 mm Hg versus 32.2 ± 18.4 mm Hg, $P = .117$).

We further used multiple linear regression models to estimate the association of Arg347Cys genotypes with BP responses (both SBP and DBP) at 0 hours and 6 hours in total subjects and in subjects stratified by plasma irbesartan concentration (Table II). Without consideration of plasma concentration, the mean DBP responses for genotype subgroups with no copies, 1 copy, and 2 copies of the Cys347 allele were 5.5 ± 8.4, 7.2 ± 8.0, and 12.8 ± 13.7 mm Hg, respectively, at 0 hours and 14.2 ± 8.9, 15.8 ± 8.5, and 23.7 ± 16.0 mm Hg, respectively, at 6 hours. Only a moderate association was observed for SBP responses in these genotype subgroups, as follows: 18.2 ± 17.9, 21.3 ± 18.3, and 17.5 ± 10.6 mm Hg, respectively, at 0 hours and 32.2 ± 18.4, 35.1 ± 17.9, and 35.8 ± 20.9 mm Hg, respectively, at 6 hours. The group of homozygous 347Cys/Cys carriers, who showed the most pronounced BP response (especially DBP) to irbesartan at 0 hours and 6 hours, comprised only 6 subjects. In the remaining analyses we combined Arg/Cys and Cys/Cys genotype groups because of the sample-size issue. After stratification of plasma concentration by its median values, Cys347 allelic carriers in the high-concentration group, relative to noncarriers, had a more pronounced DBP response at 0 hours (adjusted β [±SE], 3.0 ± 1.0 mm Hg; $P = .004$) and 6 hours (adjusted β, 3.0 ± 1.2 mm Hg; $P = .014$), and the same was true for the SBP response at 0 hours (adjusted β, 5.6 ± 2.1 mm Hg; $P = .006$) and 6 hours (adjusted β, 4.7 ± 2.0 mm Hg; $P = .021$). In contrast, in the low-concentration group, there was no significant association between either DBP or SBP response and Arg347Cys genotypes at 0 hours and 6 hours. These results are shown graphically in Fig 1.

As shown in Table III, we evaluated the potential interaction between Arg347Cys genotypes and plasma irbesartan concentration on SBP and DBP therapeutic responses. We used a generalized estimation equation model to adjust for intraclass correlation within subjects for multiple BP measures. We also adjusted for important covariates including a term for timing of BP measurement (0 hours or 6 hours). The interaction term

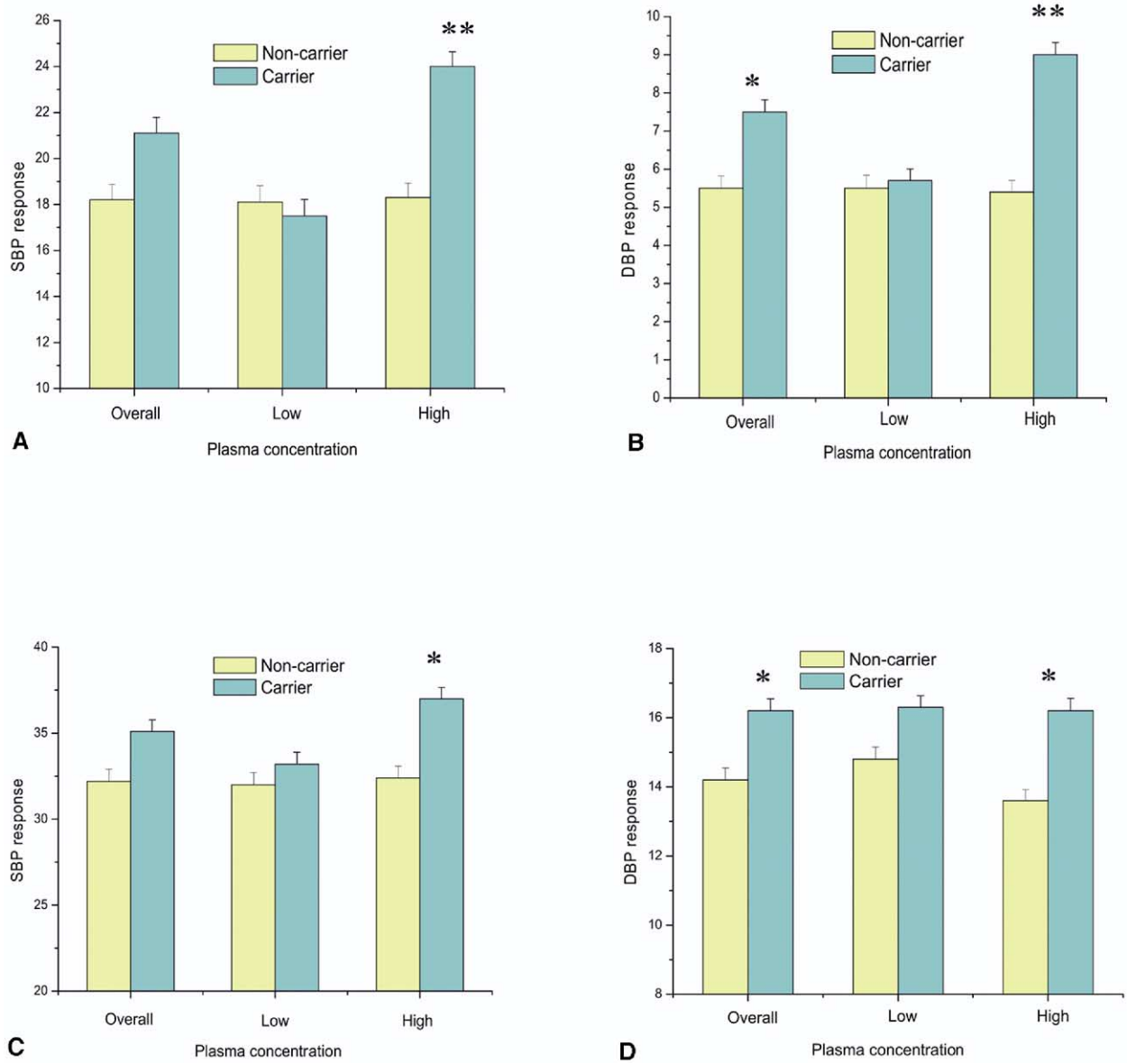


**Fig 1. A**, Systolic blood pressure (SBP) response at 0 hours on 28th day by $\alpha_{1A}$-adrenergic receptor ($\alpha_{1A}$-AR) genotype in overall population and low versus high groups stratified by plasma concentration of irbesartan. Each *column* shows mean values (±SE). Low and high groups were defined by median value of natural logarithm–transformed plasma concentration at 0 hours. *2 Asterisks*, $P < .01$ for Cys347 carriers versus noncarriers in high-concentration group. **B**, Diastolic blood pressure (DBP) response at 0 hours on 28th day by $\alpha_{1A}$-AR genotype in overall population and low versus high groups stratified by plasma concentration of irbesartan. *1 Asterisk*, $P < .05$ for Cys347 carriers versus noncarriers in overall population. *2 Asterisks*, $P < .01$ for Cys347 carriers versus noncarriers in high-concentration group. **C**, SBP response at 6 hours on 28th day by $\alpha_{1A}$-AR genotype in overall population and low versus high groups stratified by plasma concentration of irbesartan. Low and high groups were defined by median value of natural logarithm–transformed plasma concentration at 6 hours. *1 Asterisk*, $P < .05$ for Cys347 carriers versus noncarriers in high-concentration group. **D**, DBP response at 6 hours on 28th day by $\alpha_{1A}$-AR genotype in overall population and low versus high groups stratified by plasma concentration of irbesartan. *1 Asterisk*, $P < .05$ for Cys347 carriers versus noncarriers in overall population and high-concentration group.

**Table III.** Parameter estimates for models of BP response to irbesartan treatment including interaction terms for $\alpha_{1A}$-AR genotype and plasma drug concentration*

| | *Parameter estimates* | | | | | |
|---|---|---|---|---|---|---|
| | *SBP response†* | | | *DBP response†* | | |
| *Variable* | β | *SE* | *P value* | β | *SE* | *P value* |
| Age | 0.9820 | 0.8329 | .2384 | −0.1361 | 0.4831 | .7781 |
| Age$^2$ | −0.0141 | 0.0082 | .0841 | −0.0004 | 0.0046 | .9391 |
| Height | 2.6210 | 1.7783 | .1405 | 0.9341 | 1.0641 | .3800 |
| Height$^2$ | −0.0109 | 0.0060 | .0677 | −0.0031 | 0.0035 | .3757 |
| Weight | 0.7631 | 0.7752 | .3249 | −0.1235 | 0.3890 | .7508 |
| Body mass index | −2.3981 | 1.9830 | .2265 | −0.0177 | 0.9649 | .9854 |
| Gender | | | | | | |
| Female | 0 | | | 0 | | |
| Male | 0.4517 | 1.8545 | .8076 | 0.5775 | 0.8461 | .4949 |
| Smoking status | | | | | | |
| Never | 0 | | | 0 | | |
| Current | −0.0047 | 1.4041 | .9973 | −0.1362 | 0.6932 | .8443 |
| Alcohol status | | | | | | |
| Never | 0 | | | 0 | | |
| Current | 0.6186 | 1.4649 | .6728 | 0.6682 | 0.7611 | .3800 |
| Occupation | | | | | | |
| Other | 0 | | | 0 | | |
| Farmer | −0.3093 | 1.3677 | .8211 | 1.0672 | 0.6180 | .0842 |
| Education | | | | | | |
| Other | 0 | | | 0 | | |
| Middle school or higher | 0.4683 | 1.6563 | .7774 | −0.1301 | 0.8178 | .8736 |
| Baseline SBP | 0.5177 | 0.0366 | < .0001 | — | — | — |
| Baseline DBP | — | — | — | 0.3485 | 0.0288 | < .0001 |
| Time | | | | | | |
| 0 h on 28th d | 0 | | | 0 | | |
| 6 h on 28th d | 14.0452 | 0.5281 | < .0001 | 8.7362 | 0.2638 | < .0001 |
| $\alpha_{1A}$-AR genotype | | | | | | |
| Cys347 noncarrier | 0 | | | 0 | | |
| Cys347 carrier | −0.9547 | 1.9135 | .6178 | 0.3260 | 0.9033 | .7182 |
| Natural logarithm–transformed concentration (drug) | | | | | | |
| Low | 0 | | | 0 | | |
| High | 0.1868 | 1.0499 | .8588 | −0.0040 | 0.4942 | .9935 |
| Interaction: Cys347 carrier × High plasma concentration | 5.9816 | 2.3413 | .0106 | 2.5635 | 1.2112 | .0343 |

*Modeled by use of generalized estimation equations to accommodate correlations within BP responses at the 2 times for the same individual.
†BP response was defined as BP at baseline minus BP on 28th day.

of Plasma irbesartan concentration × Arg347Cys genotypes was significant for SBP response ($P = .0106$) and DBP response ($P = .0343$).

## DISCUSSION

In clinical settings, considerable individual variability in therapeutic responses to antihypertensive drugs has been observed. We hypothesize that such individual variability may be in part a result of genetic variability. This study investigated individual and joint association of the $\alpha_{1A}$-AR Arg347Cys polymorphism and plasma irbesartan concentrations with BP therapeutic responses. The most important findings of our study were that the $\alpha_{1A}$-AR Arg347Cys polymorphism might alter

BP response (particularly DBP) to short-term irbesartan treatment. Our data also provided evidence of an interaction between the $\alpha_{1A}$-AR Arg347Cys polymorphism and plasma irbesartan concentrations in relation to BP therapeutic response. The association of Arg347Cys polymorphism with BP response was more pronounced in those patients with higher plasma concentrations of irbesartan. The antihypertensive steady-state effect of irbesartan was usually seen within 2 weeks of initiation of therapy, with maximum effects occurring between 2 and 6 weeks.[2,21] Thus our major analytic traits, such as BPs and drug concentrations, were precise and reliable because they were measured separately at 0 hours and 6 hours on the 28th day (4 weeks) after the initiation of irbesartan administration.

Although the exact biologic mechanisms remain to be elucidated, our findings appear to be biologically plausible. Studies have demonstrated that there are important interactions between AT1 and $\alpha_1$-ARs in activating a common signal system inducing potential pathophysiologic significance.[10,15,16] Thus functional polymorphic variants in the $\alpha_{1A}$-AR gene might mediate various arterial responses and, to a certain extent, alter the therapeutic effectiveness of the AT1 receptor antagonist irbesartan. The $\alpha_{1A}$-AR is widely distributed and expressed in human vasculature, including resistance arteries and veins.[6] Rat hemodynamic studies have suggested that the vascular $\alpha_{1A}$-AR is the major subtype involved in the sympathetic regulation of peripheral resistance and systemic arterial pressure.[22,23] In in vivo transgenic mice, it was shown that in heterozygous animals the level of $\alpha_{1A}$-AR overexpression is directly related to marked enhancement of cardiac contractility.[24] Most recently, an 8% to 12% reduction in BP dependent on $\alpha_{1A}$-AR gene copy number was shown in $\alpha_{1A}$-AR knockout mice.[8] These findings suggested that the $\alpha_{1A}$-AR is primarily responsible for mediating vasoconstriction to regulate BP by sympathetic vascular tone. A previous study has identified a common polymorphism in the $\alpha_{1A}$-AR gene.[18] The substitution of Cys347 for Arg347 can confer a palmitoylation site and play a key role in its cellular localization and function.

There has been observational evidence that an interaction between angiotensin-converting enzyme (ACE) insertion/deletion polymorphism and ACE inhibitor can result in a difference in AT1 receptor messenger ribonucleic acid expression.[25] The Swedish Irbesartan Left Ventricular Hypertrophy Investigation (SILVHIA) trial evaluated the role of ACE insertion/deletion polymorphism as a potential predictor of BP response to treatment with irbesartan.[26] The result identified that individuals with the insertion/insertion genotype had a significantly greater DBP-lowering effect compared with those with either the deletion/deletion or the insertion/deletion genotype. Further studies are needed to evaluate the role of other potentially important candidate genes, as well as gene-gene interactions, in relation to BP therapeutic response to irbesartan.

Previous studies have shown interactions between AT1 and $\alpha_1$-ARs in VSMCs.[10,15,16] Angiotensin II facilitates neurotransmitter release from the presynaptic nerve terminals, mostly mediated by $\alpha_1$-AR, to cause vasoconstriction and myocardial damage.[27] The $\alpha_1$-adrenergic contractile response to phenylephrine is significantly potentiated by angiotensin II. With the use of phenoxybenzamine to pretreat cells, $\alpha_1$-AR numbers decrease to around 15% of control values and then partially recover after reincubation of the cells in the absence of phenoxybenzamine.[10] However, activation of the angiotensin II receptor also stimulates a marked increase in de novo synthesis of the $\alpha_1$-AR in phenoxybenzamine-pretreated VSMCs, which strongly indicates that a lower number of $\alpha_1$-ARs may in return stimulate the formation of many more activated AT1 receptors that potentiate de novo synthesis of the $\alpha_1$-AR. Therefore we can rationally postulate that Cys347 allelic carriers could harbor lower activities or densities of $\alpha_1$-ARs embedded on the surfaces of VSMCs' membranes, which would cause a decreased pressor response in regulating arterial pressure. In return, a systemically negative feedback would stimulate many more activated AT1 receptors to compensate for the loss of arterial pressure and maintain a normal BP level.

Our study results, similar to those in a previous study,[28] revealed that the $\alpha_{1A}$-AR Arg347Cys polymorphism was not associated with baseline SBP and DBP levels in these hypertensive subjects. In Cys347 allelic carriers, however, higher levels of plasma irbesartan might adequately bind and block those activated AT1 receptors' actions and result in greater SBP and DBP decreases with irbesartan treatment. Several earlier investigations failed to identify a simple association between drug plasma concentration and BP response.[29,30] Our LOWESS smoothing curves of BP therapeutic responses by plasma irbesartan concentration also did not exhibit a linear relationship (data not shown). However, the relationship appeared to differ by $\alpha_{1A}$-AR genotypes. In Cys347 allelic noncarriers, there was little correlation between the steady-state drug level and BP-lowering effect at 0 hours and 6 hours. An explanation may be that 4 weeks of antihypertensive therapy alters the hypertensive disease process and decreases peripheral resistance as a result of a regression of

vascular structural damage. Thus it would be difficult to observe an association between steady-state drug levels and BP-lowering effects. In Cys347 allelic carriers, however, a positive association of drug level with BP response was observed as a result of more stimulations of receptor-mediated vascular sensitivity, which would suggest the physiologic significance of the functional variant.

It appears that pharmacodynamic and pharmacokinetic mechanisms each may play a role in determining interindividual variation in BP responses to the antihypertensive drug. In vitro studies have shown that cytochrome P450 (CYP) 2C9 plays a major part in the metabolism of irbesartan.[31] Therefore genetic variants of CYP2C9 affecting the metabolic enzyme functionality may alter the pharmacokinetic profile of irbesartan, which in turn may influence BP therapeutic responses.

Considerable work remains to be done in this research area. Our findings remain to be replicated in other ethnic populations. The frequency of the Arg347Cys polymorphism is differently distributed among different races,[18,28] with the Cys347 allele being more common in white subjects (53.8%) than in black (29.5%), Japanese (10%), and Chinese subjects (8.8%). To date, there have been no substantial functional studies on the Arg347Cys genetic polymorphism. An in vivo study in small-sized healthy Americans (N = 74) did not show that the Arg347Cys polymorphism in the $\alpha_{1A}$-AR gene alters the agonist-mediated venoconstriction response to phenylephrine.[32] The 2 polymorphic recombinant $\alpha_{1A}$-ARs expressed stably by Chinese hamster ovary cells in an in vitro study also exhibit similar pharmacologic properties in their binding affinities and calcium signal transduction.[18] However, we cannot directly apply this finding to humans because the regulation of the $\alpha_1$-AR messenger ribonucleic acid level and receptor densities by agonists or antagonists is tissue- and cell-specific,[33,34] such as differential sensitivity of venular and arteriolar $\alpha_1$-AR constriction to inhibitors (ie, nifedipine and hypoxia).[35] In vivo functional variant study in human populations is needed. Furthermore, we could not exclude the possibility that Arg347Cys may be in linkage disequilibrium with another causal variant nearby.

In summary, our study suggests that the $\alpha_{1A}$-AR Arg347Cys polymorphism is associated with BP therapeutic responses to short-term irbesartan treatment and such an association is more pronounced at higher levels of plasma irbesartan. Our study represents one of the first steps in our goal to achieve individualized therapy based on an individual's genetic characteristics and plasma drug level.

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