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β_1-adrenoceptor genetic varians and ethnicity independently affect response to β-blockade

Daniel Kurnik[a], **Chun Li**[b,c], **Gbenga G. Sofowora**[a], **Eitan A. Friedman**[a], **Mordechai Muszkat**[a], **Hong-Guang Xie**[a], **Paul A. Harris**[d], **Scott M. Williams**[b], **Usha B. Nair**[a], **Alastair J.J. Wood**[a], and **C. Michael Stein**[a]

[a]Departments of Medicine and Pharmacology, Division of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee

[b]Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, Tennessee

[c]Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, Tennessee

[d]Department of Biomedical Informatics, Vanderbilt University School of Medicine, Nashville, Tennessee

Abstract

Objectives—Black subjects may be less responsive to β-blockers than whites. Genetic variants in the β_1-adrenergic receptor (β_1-AR) associated with lesser response to β-blockers are more common in blacks than whites. The purpose of this study was to determine whether ethnic differences in response to β-blockade can be explained by differing distributions of functional genetic variants in the β_1-AR.

Methods—We measured sensitivity to β-blockade by the attenuation of exercise-induced tachycardia in 165 subjects (92 whites) who performed a graded bicycle exercise test before and 2.75 hours after oral atenolol (25 mg). We determined heart rate at rest and at 3 exercise levels from continuous ECG recordings and calculated the area-under-the-curve (AUC). We also measured plasma atenolol concentrations and determined genotypes for variants of the β_1-AR (Ser49Gly, Arg389Gly) and α_{2C}-AR (del322–325). The effects of ethnicity, genotype, and other covariates on the heart rate reduction after atenolol were estimated in multiple regression analyses.

Results—Atenolol resulted in a greater reduction in exercise heart rate in whites than blacks (P=0.006). β_1-AR Arg389 (P=0.003), but not the α_{2C}-AR 322–325 insertion allele (P=0.31), was independently associated with a greater reduction in heart rate AUC. Ethnic differences in sensitivity to atenolol remained significant (P=0.006) after adjustment for β_1-AR and α_{2C}-AR genotypes.

Conclusions—Ethnic differences in sensitivity to the β_1-blocker atenolol persist even after accounting for different distributions of functional genetic β_1-AR variants, suggesting that additional, as yet unidentified factors contribute to such ethnic differences.

Keywords

Beta-adrenergic receptor; Genetics; Pharmacology; Populations

Correspondence to: C. Michael Stein, MD, Division of Clinical Pharmacology, 542 RRB, Vanderbilt University School of Medicine, Nashville, TN 37232-6602, Tel: (615) 936-3420, Fax: (615) 936-2746, E-mail: michael.stein@vanderbilt.edu.

Conflicts of interest: None declared

Introduction

β-adrenergic receptor antagonists (β-blockers) are widely used in the treatment of cardiovascular disease [1], but there is considerable interindividual variability in response even after accounting for pharmacokinetic differences [2]. Ethnicity may contribute to this variability in response. In black patients, β-blockers were less effective in the treatment of hypertension [3,4], heart failure [5], and myocardial infarction [6]; it is, however, unclear whether such ethnic differences reflect genetic alterations in sensitivity to β_1-adrenergic receptor blockade or other biologic and non-genetic factors such as diet, baseline blood pressure, body-mass index, and disease severity [7,8].

Attenuation of exercise-induced tachycardia is a robust clinical measure of β-blockade [9] and has been used to study sensitivity to β-blockers in different ethnic groups in healthy subjects, a population in whom confounding factors can be controlled more easily than in patient populations. Some [10–12], but not all studies [13,14] found that whites had a greater reduction in exercise heart rate after β-blockers than blacks, further suggesting that ethnic differences in sensitivity to β-blockers exist.

Studying drug responses in subjects classified according to race or ethnicity is contentious [15], and we and others have suggested that the study of specific genotypes and well-defined phenotypes within and across ethnic groups will be more informative [16]. Two common non-synonymous polymorphisms in the gene coding for the β_1-adrenergic receptor (*ADRB1*), resulting in the Arg389Gly and Ser49Gly substitutions, affect receptor function *in vitro* and response to β-blockers *in vivo* [17–21]. The less responsive Gly389 and Gly49 variants are approximately twice as frequent in black compared to white subjects [17,22–24]. Such differences in the prevalence of less responsive *ADRB1* genotypes could provide a mechanistic explanation for ethnic differences in response to β-blockers [25]; however, whether ethnic differences in the distribution of *ADRB1* genotypes account for ethnic differences in β-blocker responsiveness has not been defined.

Another adrenergic receptor, the α_{2C}-receptor (α_{2C}-AR), is located on cardiac presynaptic sympathetic nerve endings and inhibits norepinephrine release by negative feedback. A deletion variant in its gene (*ADRA2C* del322–325) is 10-fold more common in African-Americans than in whites, and has been associated with a loss of function *in vitro* [26] and raised sympathetic tone *in vivo* [27]. In one study, the deletion variant was associated synergistically with *ADRB1* genotypes with an increased risk of congestive heart failure in African-Americans [28]. Moreover, patients with congestive heart failure who carried both the *ADRB1* Arg389 and the *ADRA2C* deletion allele had a greater improvement in ejection fraction after metoprolol therapy [29].

We therefore determined the differential contribution of ethnicity and *ADRB1* and *ADRA2C* genotypes to variability in response to β-blockade in order to examine the hypothesis that ethnic differences in response to a β-blocker could be explained by *ADRB1* and *ADRA2C* genotypes.

Methods

Subjects

The Institutional Review Board of the Vanderbilt University Medical Center approved the study protocol, and subjects gave written informed consent. We studied 165 subjects, some of whom (n=34) also contributed data to a previous study [18]. Unrelated American white and black subjects were eligible to participate if they were between 18–50 years of age and had no clinically significant abnormality based on medical history, physical examination, electrocardiogram, and routine laboratory testing. Subjects reported their ethnicity and that of

their parents and grandparents using check-boxes to choose among “Caucasian”, “African-American”, “Hispanic”, “Chinese”, “Japanese”, and “other” (the latter to be specified). Multiple choices were permitted. A subject was assigned to an ethnic group when both parents and at least 3 out of 4 grandparents were of the same ethnicity. Body mass index (BMI) was calculated as weight / height^2 [kg /m^2]. Subjects were free of medications for at least 1 week and received a controlled alcohol- and caffeine-free diet (providing 150 mmol of sodium, 70 mmol of potassium, and 600 mmol of calcium daily) for 6 days prior to the study.

Protocol

On the morning (8:00–10:00 am) of study day 7, subjects were admitted to a temperature-controlled room (22–23°C) in the Vanderbilt University Clinical Research Center after an overnight fast. A 20 G intravenous cannula was inserted into an antecubital arm vein for blood sampling. Digitized ECG signals were continuously acquired on a personal computer at 500 MHz using the Windaq software (v. 2.20, Dataq Instruments Inc., Akron, Ohio). After 30 minutes of supine rest, subjects then slid to an adjacent electronically braked supine bicycle ergometer. The exercise protocol consisted of biking at a constant rate (60 revolutions/min) at increasing workloads of 25, 50, and 75 W, for 2 minutes each. After completion of exercise, subjects rested for 10 minutes and then swallowed a 25-mg tablet of atenolol (Mylan Pharmaceuticals Inc., Morgantown, WV). Two hours and 45 minutes after drug ingestion, approximately the time to peak plasma concentrations and maximal effect of atenolol [30], a venous blood sample (10 mL) was drawn for the determination of atenolol plasma concentrations, and the exercise protocol was repeated as described above.

Genotyping and haplotype assignment

Genotyping for the 2 *ADRB1* single nucleotide polymorphisms, rs1801252 and rs1801253 (corresponding to Ser49Gly and Arg389Gly), was performed by allelic discrimination with TaqMan 5’-nuclease assays [31] on an ABI 7900 HT real-time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA) using validated TaqMan probes and a 95% quality value threshold. Genotypes for a subgroup of 34 subjects had been previously determined by detection of restriction fragment length polymorphisms [18], and the TaqMan assay gave concordant results. Genotyping for the *ADRA2C* 322–325 deletion variant was performed by DNA fragment analysis [32] and confirmed by direct sequencing in randomly selected samples (n=13). We used a fluorescently labeled forward primer (5’-6-FAM-AGACGGACGAGAGCAGCGCA-3’) and a reverse primer (5’-AGGCCTCGCGGCAGATGCCGTACA-3’) to amplify DNA fragments by PCR. Amplicons were denatured at 95°C for 5 min, and fragment analysis performed on an ABI 3730 Genetic Analyzer and its Genotyper V.1.0.1 software. The genotyping success rate was 94.5%, 99.4%, and 92.1% for the *ADRB1* Ser49Gly and Arg389Gly and the *ADRA2C* del322–325 variant, respectively. The software Powermarker (http://statgen.ncsu.edu/powermarker/index.html) was used to assign *ADRB1* haplotypes using the expectation-maximization algorithm [33]. Only haplotypes that could be assigned with a probability >90% were used for haplotype analysis.

Plasma atenolol concentrations

Atenolol plasma concentrations were determined by high performance liquid chromatography as previously described [18]. The coefficients of variation of interday precision measurements for 25 ng/mL of the S(−)- and R(+)-enantiomer were 2.3% and 5.7%, respectively. For data analysis, the concentrations of the active S(−)-enantiomer were used.

Data analysis and statistics

Heart rate was determined from the computerized ECG recordings as the average heart rate over a 1-minute period at rest and during the second minute of each exercise step. Decreases in heart rate after atenolol at rest and each exercise level were expressed as absolute change from the heart rates obtained before atenolol at the corresponding time points of the exercise cycle. For each subject, we plotted the decrease in heart rate over time and calculated the area-under-the-curve (AUC) by the trapezoidal method [34]. Data are expressed as mean and 95% confidence interval (CI) or standard deviation (SD), or median and interquartile range (IQR) for non-normally distributed data. Comparisons of demographic data and unadjusted outcomes were performed by chi-square test, Fisher's exact test, and t-test, as appropriate. To examine the effect of ethnicity and genetic variants (*ADRB1* Ser49Gly and Arg389Gly genotypes or haplotypes and *ADRA2C* del322–325 genotype) on atenolol response, multiple linear regression analyses were performed for the following response variables: absolute reduction in heart rate after atenolol at rest and at maximal exercise, and reduction in heart rate AUC. The analysis was adjusted for predetermined variables selected for their potential association with the outcome: The corresponding heart rate (or heart rate AUC) before atenolol and for age, sex, ethnicity, BMI, and S-atenolol plasma concentrations. Since ethnicity and Arg389Gly genotype were significantly associated with the reduction in heart rate AUC, we repeated the analysis after excluding them, respectively, to illustrate their contribution to the model and to the effect sizes for other covariates. *ADRB1* haplotypes were analyzed assuming additive effects, and the F-test (3 degrees of freedom) was used to determine if the haplotype effects as a whole were significant. Based on previous studies that defined the Ser49-Arg389 haplotype as most responsive [19,21], subjects were further classified as carriers or non-carriers of the Ser49-Arg389 haplotype. We tested for Hardy-Weinberg equilibrium using the chi-square test with 1 degree of freedom or by Monte-Carlo permutation test with the genetic software hwsim (http://krunch.med.yale.edu/hwsim/), where appropriate. All tests were two-tailed, and a P-value of <0.05 was considered significant. Analyses were performed with the statistical software R (www.r-project.org).

Sample size calculation

In our preliminary study [18], at the 75 W exercise level atenolol lowered heart rate by 12.4 ±8.6 bpm. A sample size of at least 69 subjects in each ethnic group would provide 90% power at $\alpha=0.05$ to detect a difference of 5 bpm between groups.

Results

Subject characteristics and genotypes

Demographic characteristics and *ADRB1* and *ADRA2C* genotypes for the 165 subjects (92 whites) are shown in Table 1. *ADRB1* Ser49Gly and Arg389Gly and *ADRA2C* del322–325 genotype distributions conformed to Hardy-Weinberg equilibrium within each ethnic group and were significantly different between the two ethnic groups (Table 1). The minor allele frequencies for whites and blacks were 11.0% and 25.7% for *ADRB1* Gly49 (P=0.001), 29.3% and 42.4% for *ADRB1* Gly389 (P=0.014), and 3.6% and 44.9% (P<0.001) for *ADRA2C* del322–325, respectively. *ADRB1* haplotypes could be inferred with > 90% probability in 154 subjects (93.3%). Haplotype frequencies were within the expected range [23,24] and differed significantly between the two ethnic groups (Table 1).

Atenolol concentrations

Plasma concentrations of the active S-enantiomer of atenolol ranged from 15.7 – 175.8 ng/mL (median 62.2 ng/mL; interquartile range [44.8, 82.4]) and were not different between blacks (64.2 ± 27.6) and whites (67.5 ± 30.1; P=0.46).

Baseline cardiovascular measures

In the unadjusted analysis, there were no ethnic differences in baseline heart rate or blood pressure (Table 1). Female sex (P<0.001) and higher BMI (P=0.042) were associated with higher heart rate after adjustment for ethnicity and age. *ADRB1* and *ADRA2C* genotypes had no effect on any of the baseline measures (P-values >0.33).

Responses to atenolol: Effects of ethnicity

At rest, atenolol reduced heart rate by a mean of 7.3 bpm (95% CI, 6.2 to 8.3; P<0.001). White subjects had a 1.75-fold greater decrease in resting heart rate than blacks (mean difference between ethnic groups, 3.8 bpm; 95% CI, 1.8 to 5.8; P<0.001; Figure 1a). The ethnic difference was weakened after adjustment for multiple covariates (baseline resting heart rate, sex, BMI, age, plasma S-atenolol concentrations, and *ADRB1* and *ADRA2C* genotypes; Table 2).

As the level of exercise increased, atenolol resulted in greater reductions in exercise-induced tachycardia in both ethnicities, but heart rate reduction was consistently greater in whites than blacks (Figure 1a). Accordingly, the unadjusted mean reduction in heart rate AUC was greater in whites (mean ethnic difference, 18.3 bpm*min; 95% CI, 5.6 to 31.1; P=0.006; Figure 1a). This ethnic difference was essentially unaffected after adjustment for all non-genetic covariates and *ADRB1* and *ADRA2C* genotypes (P=0.006; Table 2 and Figure 1b). Excluding the *ADRB1* Arg389Gly genotype as a covariate increased the estimated ethnic effect slightly (mean ethnic difference, 25.1 bpm*min; 95% CI, 11.1 to 39.1; P<0.001; Figure 1b) and reduced the goodness of model fit (adjusted R^2 with (0.49) and without (0.47) *ADRB1* Arg389Gly genotype; P-value for difference, 0.003). In contrast, excluding *ADRA2C* genotype increased the estimated ethnic difference (24.5 bpm*min; 95% CI, 13.5 to 35.4; P<0.001; Figure 1b), but did not change the goodness of model fit (adjusted R^2 with and without *ADRA2C* genotype, 0.49; P=0.31).

Responses to atenolol: Effects of *ADRB1* genotypes

Figure 2a shows the unadjusted decrease in heart rate after atenolol at rest and at exercise stratified by Arg389Gly genotype. Heart rate reduction was greatest in the Arg/Arg group, intermediate in the heterozygotes, and smallest in the Gly/Gly group; this effect was seen in both ethnic groups. The unadjusted reduction in heart rate AUC was significantly different among the three genotype groups (P=0.014). After adjustment for all non-genetic and genetic covariates, the Arg389 allele was associated with the reduction in heart rate AUC (P=0.003, Table 2). Excluding ethnicity from this analysis affected the genotype effect only slightly (Figure 2b), and a test for interaction between ethnicity and Arg389Gly was not significant (P=0.62). These results suggest that the gene effect was similar in both ethnic groups and that the effects of ethnicity and Arg389Gly genotype were largely independent. The Ser49 allele had a less marked effect on heart rate reduction at maximal exercise (P=0.039), but no significant effect on the reduction in heart rate AUC (P=0.45; Table 2). When subjects were grouped into carriers and non-carriers of the responsive Ser49-Arg389 haplotype, carriers had a 27% greater adjusted reduction in heart rate at maximal exercise (mean difference, 3.7 bpm; 95% CI, 1.2 to 6.2; P=0.004) and a 25% greater reduction of heart rate AUC (mean difference, 15.4 bpm*min; 95% CI, 3.7 to 27.1; P=0.011) than non-carriers, suggesting that *ADRB1* haplotypes determined approximately a quarter of atenolol's total negative chronotropic effect.

Responses to atenolol: Effects of *ADRA2C* genotype

In the unadjusted analysis, there was a trend toward an association of the *ADRA2C* insertion allele with greater reduction of heart rate AUC by atenolol (P=0.062). After adjusting for all covariates except ethnicity, this association was stronger (P<0.001; Figure 2b); however, after additional adjustment for ethnicity, it was greatly attenuated and no longer statistically

significant (P=0.30; Table 2; Figure 2b), suggesting that the effect of the *ADRA2C* deletion variant on heart rate response to atenolol was not independent, but largely explained by its association with ethnicity.

Responses to atenolol: Other determinants

Only the pre-atenolol heart rate was associated with the reduction in resting heart rate after atenolol. In addition to pre-atenolol heart rate or heart rate AUC, white ethnicity, *ADRB1* genotypes, male sex, and higher plasma S-atenolol concentrations were associated with a greater reduction in heart rate at maximal exercise and in heart rate AUC (Table 2).

Discussion

This is the largest study to examine the effect of ethnicity on sensitivity to a β-blocker in a controlled setting, and the first to examine the respective contributions of ethnicity and β_1-adrenoceptor genetic variants. Our main findings are that β_1-adrenoceptor blockade results in a greater reduction in exercise heart rate in whites compared to blacks, and that the higher frequency of responsive *ADRB1* genotypes in whites contributes only little to this difference, suggesting that additional mechanisms for ethnic differences exist.

In hypertensive patients, monotherpay with a β-blocker lowered blood pressure less in blacks than whites [3,35–38]. Such findings led to several small, well-controlled studies in healthy subjects to determine whether the ethnic differences observed in hypertensive patients reflected pharmacodynamic differences in β_1-adrenergic sensitivity [10–14]. As in the present study, those studies assessed the reduction in exercise tachycardia after a β-blocker, since this phenotype reflects most specifically sensitivity to β_1-adrenergic blockade [9]. However, the results of those studies were variable, likely because of the small sample sizes ($n \leq 32$), and in hindsight, the lack of control for *ADRB1* variants.

We set out to compare sensitivity to atenolol in a large number of black and white subjects under controlled conditions. White subjects had a significantly greater reduction in heart rate after atenolol than blacks, a difference that persisted after adjustment for non-genetic potential confounding covariates such as gender and body-mass index. Dietary confounders were minimized by providing a standardized diet before the study. Ethnic differences in response to β-blockade were of borderline statistical significance at rest, when heart rate is under both vagal and β-adrenergic control, but were enhanced during exercise, where β-adrenergic control predominates [9]. Concordant with the greater contribution of the β-adrenergic system to heart rate during exercise, covariates directly linked to the β_1-adrenergic pathway, such as plasma atenolol concentrations and *ADRB1* variants, were strongly associated with the decrease in heart rate during exercise, but not at rest.

The discovery of functional variants in the β_1-adrenergic receptor (β_1-AR) has renewed interest in understanding ethnic differences in responses to β-blockers. The two common genetic variants in the β_1-AR, Ser49Gly and Arg389Gly, confer a reduction in β_1-AR mediated signaling *in vitro* [39,40]. Additionally, in healthy subjects and hypertensive patients the Gly389 allele and, to a lesser extent, the Gly49 allele were associated with decreased blood pressure [18–21] and heart rate [20] responses to β-blockers. In more heterogeneous populations, such as patients with congestive heart failure, some [41–43], but not all studies [44,45] have shown such an association.

In the present carefully controlled study, *ADRB1* Arg389 and Ser49 variants did indeed affect the attenuation of exercise tachycardia by atenolol at maximal exercise, accounting for approximately 25% of the total negative chronotropic effect, and the Arg389Gly genotype additionally affected the summary response measure, reduction in heart rate AUC. However,

the effects of ethnicity and Arg389Gly genotype on heart rate reduction were largely independent of each other, illustrating that ethnic differences in the distribution of *ADRB1* genotypes do not explain ethnic differences in heart rate response. In contrast, the association between the *ADRA2C* 322–325 deletion variant and reduced heart rate response to atenolol was not independent, but largely explained by its association with ethnicity. These examples illustrate the importance of statistical adjustment for ethnicity even when the genotypes of interest have been determined.

The discovery of functional genetic variants that affect the response to a drug is of particular interest when there are ethnic differences in both drug response and genotype distribution. In this setting, known genetic variants may provide a mechanistic explanation for ethnic differences, suggesting that specific genotypes would allow better prediction of drug response than would inexact and heterogeneous characteristics such as race or ethnicity. Our study with a β-blocker illustrates the complexity underlying this notion. Thus, despite careful control of environmental factors and adjustment for potential confounding variables, ethnic differences in sensitivity to atenolol persisted even after adjusting for common *ADRB1* and *ADRA2C* variants. It is therefore likely that other, as yet undefined environmental or genetic factors contributed to these ethnic differences.

The study design had many strengths. We used a selective β_1-blocker in order to specifically examine the β_1-adrenergic signaling pathway. Moreover, atenolol is not metabolized to a significant extent [46], which reduces confounding introduced by variability in drug metabolism. Nevertheless, we measured plasma atenolol concentrations to take account of interindividual pharmacokinetic variability. Our findings may apply to other β-blockers with similar characteristics; however, we cannot extrapolate our results to β-blockers that differ substantially from atenolol in their receptor-subtype specificity, agonist/antagonist potency, pharmacokinetics, and other characteristics. We studied young, healthy subjects and not patients with cardiovascular disease. This strategy maximizes the ability to detect ethnic and genotypic pharmacological differences in the β_1-adrenergic signaling pathway, since response is not modified by disease or regulatory changes secondary to disease. Therefore, our findings cannot necessarily be extrapolated to patients with cardiovascular diseases or to other outcomes such as reduction in blood pressure, but provide a firm scientific base for future clinical studies. Interestingly, the β-blocker Evaluation of Survival Trial (BEST) recently showed that patients with congestive heart failure had clinical benefits from the β-blocker bucindolol only if they were homozygous for the Arg389 allele [41]. Furthermore, although no formal statistical analysis was performed, the authors argued that this difference was unlikely to be explained by the racial difference in Arg389Gly genotype distribution between blacks and whites [41]. Our study provides the experimental underpinning for this observation, showing for the first time and under highly controlled conditions that, indeed, ethnicity and Arg389Gly genotype independently affect response to a β-blocker.

In conclusion, our results suggest that ethnic differences in heart rate response to the β_1-adrenergic blocker atenolol are not accounted for by differing ethnic distributions of common genetic variants in the β_1- and α_{2C}-adrenergic receptor and that other, currently unidentified factors explain these differences. This study illustrates the utility of investigating the biologic mechanisms of ethnic differences by accounting for known genetic variability to identify differences that remain unexplained. Further studies to identify other genetic or environmental factors that alter sensitivity to β-blockers will be of great interest.

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References

1. Lopez-Sendon J, Swedberg K, McMurray J, Tamargo J, Maggioni AP, Dargie H, et al. Expert consensus document on beta-adrenergic receptor blockers. Eur Heart J 2004;25:1341–1362. [PubMed: 15288162]
2. Zineh I, Beitelshees AL, Gaedigk A, Walker JR, Pauly DF, Eberst K, et al. Pharmacokinetics and CYP2D6 genotypes do not predict metoprolol adverse events or efficacy in hypertension. Clin Pharmacol Ther 2004;76:536–544. [PubMed: 15592325]
3. Materson BJ, Reda DJ, Cushman WC, Massie BM, Freis ED, Kochar MS, et al. The Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. Single-drug therapy for hypertension in men. A comparison of six antihypertensive agents with placebo. N Engl J Med 1993;328:914–921. [PubMed: 8446138]
4. Cushman WC, Reda DJ, Perry HM, Williams D, Abdellatif M, Materson BJ. Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. Regional and racial differences in response to antihypertensive medication use in a randomized controlled trial of men with hypertension in the United States. Arch Intern Med 2000;160:825–831. [PubMed: 10737282]
5. Beta-Blocker Evaluation of Survival Trial Investigators. A trial of the beta-blocker bucindolol in patients with advanced chronic heart failure. N Engl J Med 2001;344:1659–1667. [PubMed: 11386264]
6. Gottlieb SS, McCarter RJ, Vogel RA. Effect of beta-blockade on mortality among high-risk and low-risk patients after myocardial infarction. N Engl J Med 1998;20(339):489–497. [PubMed: 9709041]
7. Materson BJ. Variability in response to antihypertensive drug treatment. Hypertension 2004;43:1166–1167. [PubMed: 15117911]
8. Douglas JG, Bakris GL, Epstein M, Ferdinand KC, Ferrario C, Flack JM, et al. Management of high blood pressure in African Americans: consensus statement of the Hypertension in African Americans Working Group of the International Society on Hypertension in Blacks. Arch Intern Med 2003;163:525–541. [PubMed: 12622600]
9. McDevitt DG. In vivo studies on the function of cardiac beta-adrenoceptors in man. Eur Heart J 1989;10:22–28. [PubMed: 2553409]
10. Venter CP, Joubert PH. Ethnic differences in beta-1-adrenoceptor sensitivity. S Afr Med J 1982;62:849–850. [PubMed: 6755764]
11. Venter CP, Joubert PH. Ethnic differences in response to beta 1-adrenoceptor blockade by propranolol. J Cardiovasc Pharmacol 1984;6:361–364. [PubMed: 6200729]
12. Joubert PH, Venter CP, Wellstein A. Ethnic differences in response to beta-blockade: fact or artefact? A study with bisoprolol and propranolol. Eur J Clin Pharmacol 1988;34:363–368. [PubMed: 2900144]
13. Sowinski KM, Burlew BS, Johnson JA. Racial differences in sensitivity to the negative chronotropic effects of propranolol in healthy men. Clin Pharmacol Ther 1995;57:678–683. [PubMed: 7781268]
14. Johnson JA, Burlew BS, Stiles RN. Racial differences in beta-adrenoceptor-mediated responsiveness. J Cardiovasc Pharmacol 1995;25:90–96. [PubMed: 7723360]
15. Schwartz RS. Racial profiling in medical research. N Engl J Med 2001;344:1392–1393. [PubMed: 11333999]
16. Stein CM, Lang CC, Xie HG, Wood AJ. Hypertension in black people: study of specific genotypes and phenotypes will provide a greater understanding of interindividual and interethnic variability in blood pressure regulation than studies based on race. Pharmacogenetics 2001;11:95–110. [PubMed: 11266083]
17. Kirstein SL, Insel PA. Autonomic nervous system pharmacogenomics: a progress report. Pharmacol Rev 2004;56:31–52. [PubMed: 15001662]
18. Sofowora GG, Dishy V, Muszkat M, Xie HG, Kim RB, Harris PA, et al. A common beta1-adrenergic receptor polymorphism (Arg389Gly) affects blood pressure response to beta-blockade. Clin Pharmacol Ther 2003;73:366–371. [PubMed: 12709726]

19. Johnson JA, Zineh I, Puckett BJ, McGorray SP, Yarandi HN, Pauly DF. DF Beta 1-adrenergic receptor polymorphisms and antihypertensive response to metoprolol. Clin Pharmacol Ther 2003;74:44–52. [PubMed: 12844134]
20. 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;74:372–379. [PubMed: 14534524]
21. Liu J, Liu ZQ, Yu BN, Xu FH, Mo W, Zhou G, et al. beta(1)-Adrenergic receptor polymorphisms influence the response to metoprolol monotherapy in patients with essential hypertension. Clin Pharmacol Ther 2006;80:23–32. [PubMed: 16815314]
22. Moore JD, Mason DA, Green SA, Hsu J, Liggett SB. Racial differences in the frequencies of cardiac beta(1)-adrenergic receptor polymorphisms: analysis of c145A>G and c1165G>C. Hum Mutat 1999;14:271. [PubMed: 10477438]
23. Wilk JB, Myers RH, Pankow JS, Hunt SC, Leppert MF, Freedman BI, et al. Adrenergic receptor polymorphisms associated with resting heart rate: the HyperGEN Study. Ann Hum Genet 2006;70:566–573. [PubMed: 16907703]
24. Belfer I, Buzas B, Evans C, Hipp H, Phillips G, Taubman J, et al. Haplotype structure of the beta adrenergic receptor genes in US Caucasians and African Americans. Eur J Hum Genet 2005;13:341–351. [PubMed: 15523499]
25. Brodde OE, Stein CM. The Gly389Arg beta1-adrenergic receptor polymorphism: a predictor of response to beta-blocker treatment? Clin Pharmacol Ther 2003;74:299–302. [PubMed: 14534516]
26. Small KM, Forbes SL, Rahman FF, Bridges KM, Liggett SB. A four amino acid deletion polymorphism in the third intracellular loop of the human alpha 2C-adrenergic receptor confers impaired coupling to multiple effectors. J Biol Chem 2000;275:23059–23064. [PubMed: 10801795]
27. Neumeister A, Charney DS, Belfer I, Geraci M, Holmes C, Sharabi Y, et al. Sympathoneural and adrenomedullary functional effects of alpha2C-adrenoreceptor gene polymorphism in healthy humans. Pharmacogenet Genomics 2005;15:143–149. [PubMed: 15861038]
28. Small KM, Wagoner LE, Levin AM, Kardia SL, Liggett SB. Synergistic polymorphisms of beta1- and alpha2C-adrenergic receptors and the risk of congestive heart failure. N Engl J Med 2002;347:1135–1142. [PubMed: 12374873]
29. Lobmeyer MT, Gong Y, Terra SG, Beitelshees AL, Langaee TY, Pauly DF, et al. Synergistic polymorphisms of beta1 and alpha2C-adrenergic receptors and the influence on left ventricular ejection fraction response to beta-blocker therapy in heart failure. Pharmacogenet Genomics 2007;17:277–282. [PubMed: 17496726]
30. Brown HC, Carruthers SG, Johnston GD, Kelly JG, McAinsh J, McDevitt DG, et al. Clinical pharmacologic observations on atenolol, a beta-adrenoceptor blocker. Clin Pharmacol Ther 1976;20:524–534. [PubMed: 10125]
31. Livak KJ. SNP genotyping by the 5'-nuclease reaction. Methods Mol Biol 2003;212:129–147. [PubMed: 12491907]
32. Kurnik D, Muszkat M, Friedman EA, Sofowora GG, Diedrich A, Xie HG, et al. Effect of the alpha2C-adrenoreceptor deletion322–325 variant on sympathetic activity and cardiovascular measures in healthy subjects. J Hypertens 2007;25:763–771. [PubMed: 17351367]
33. Liu K, Muse SV. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 2005;21:2128–2129. [PubMed: 15705655]
34. Rowland, M.; Tozer, TN. Clinical Pharmacokinetics: Concepts and Applications. Vol. 3 edition. Lippincott Williams & Wilkins; 1995.
35. Wassertheil-Smoller S, Oberman A, Blaufox MD, Davis B, Langford H. The Trial of Antihypertensive Interventions and Management (TAIM) Study. Final results with regard to blood pressure, cardiovascular risk, and quality of life. Am J Hypertens 1992;5:37–44. [PubMed: 1736933]
36. Wright JT Jr, DiPette DJ, Goodman RP, Townsend R, McKenney JM. Renin profile, race, and antihypertensive efficacy with atenolol and labetalol. J Hum Hypertens 1991;5:193–198. [PubMed: 1920342]
37. Cubeddu LX, Aranda J, Singh B, Klein M, Brachfeld J, Freis E, et al. A comparison of verapamil and propranolol for the initial treatment of hypertension. Racial differences in response. JAMA 1986;256:2214–2221. [PubMed: 3531560]

38. Veterans Administration Cooperative Study Group on Antihypertensive Agents. Comparison of propranolol and hydrochlorothiazide for the initial treatment of hypertension. I. Results of short-term titration with emphasis on racial differences in response. JAMA 1982;248:1996–2003. [PubMed: 6750166]

39. Levin MC, Marullo S, Muntaner O, Andersson B, Magnussonq Y. The myocardium-protective Gly-49 variant of the beta 1-adrenergic receptor exhibits constitutive activity and increased desensitization and down-regulation. J Biol Chem 2002;277:30429–30435. [PubMed: 12034720]

40. Mason DA, Moore JD, Green SA, Liggett SB. A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. J Bio Chem 1999;274:12670–12674. [PubMed: 10212248]

41. Liggett SB, Mialet-Perez J, Thaneemit-Chen S, Weber SA, Greene SM, Hodne D, et al. A polymorphism within a conserved beta(1)-adrenergic receptor motif alters cardiac function and beta-blocker response in human heart failure. Proc Natl Acad Sci U S A 2006;103:11288–11293. [PubMed: 16844790]

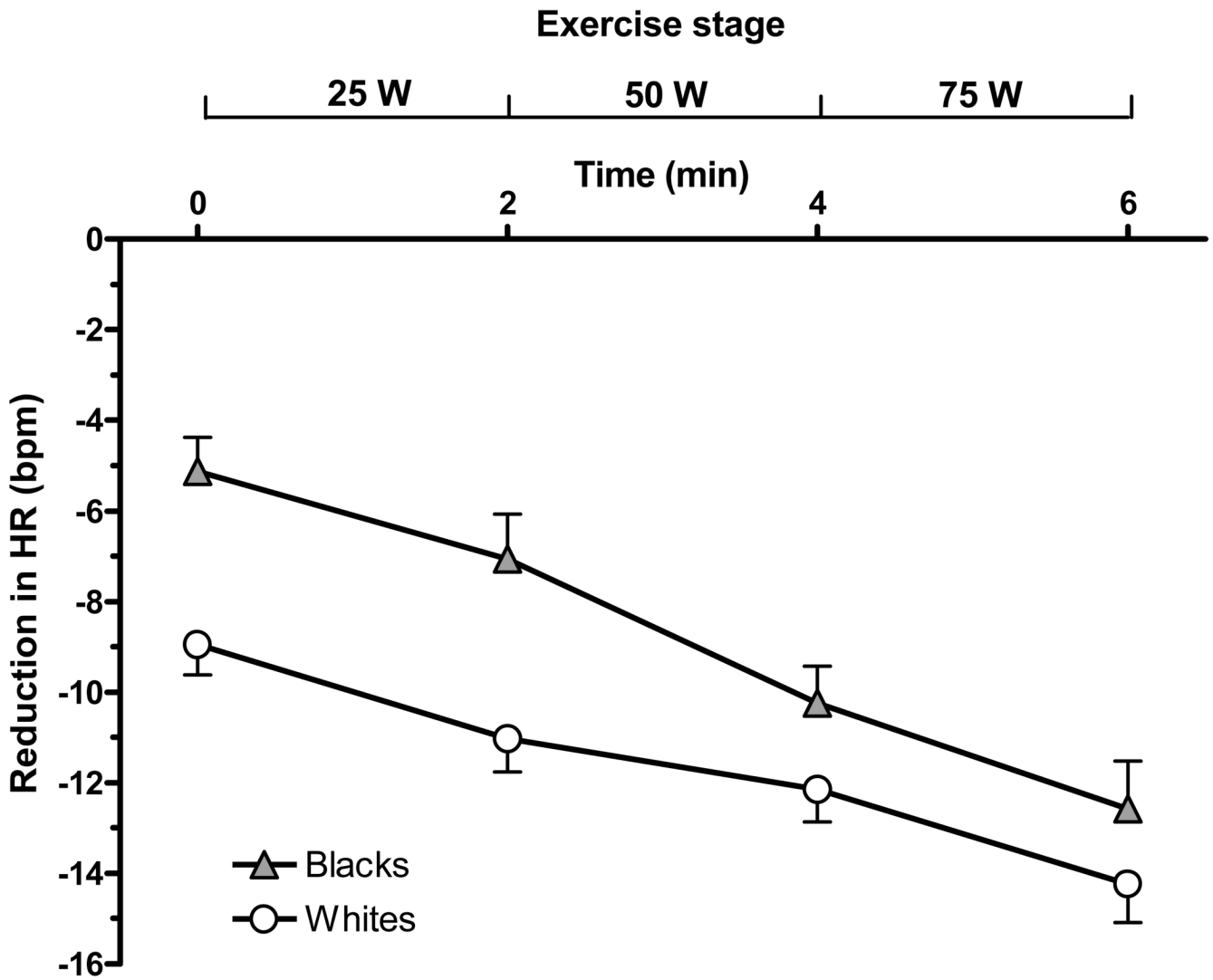
42. Mialet PJ, Rathz DA, Petrashevskaya NN, Hahn HS, Wagoner LE, Schwartz A, et al. Beta 1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. Nat Med 2003;9:1300–1305. [PubMed: 14502278]

43. Terra SG, Hamilton KK, Pauly DF, Lee CR, Patterson JH, Adams KF, et al. Beta1-adrenergic receptor polymorphisms and left ventricular remodeling changes in response to beta-blocker therapy. Pharmacogenet Genomics 2005;15:227–234. [PubMed: 15864115]

44. White HL, de Boer RA, Maqbool A, Greenwood D, van Veldhuisen DJ, Cuthbert R, et al. An evaluation of the beta-1 adrenergic receptor Arg389Gly polymorphism in individuals with heart failure: a MERIT-HF sub-study. Eur J Heart Fail 2003;5:463–468. [PubMed: 12921807]

45. de Groote P, Helbecque N, Lamblin N, Hermant X, Mc FE, Foucher-Hossein C, et al. Association between beta-1 and beta-2 adrenergic receptor gene polymorphisms and the response to beta-blockade in patients with stable congestive heart failure. Pharmacogenet Genomics 2005;15:137–142. [PubMed: 15861037]

46. Reeves PR, McAinsh J, McIntosh DA, Winrow MJ. Metabolism of atenolol in man. Xenobiotica 1978;8:313–320. [PubMed: 27019]



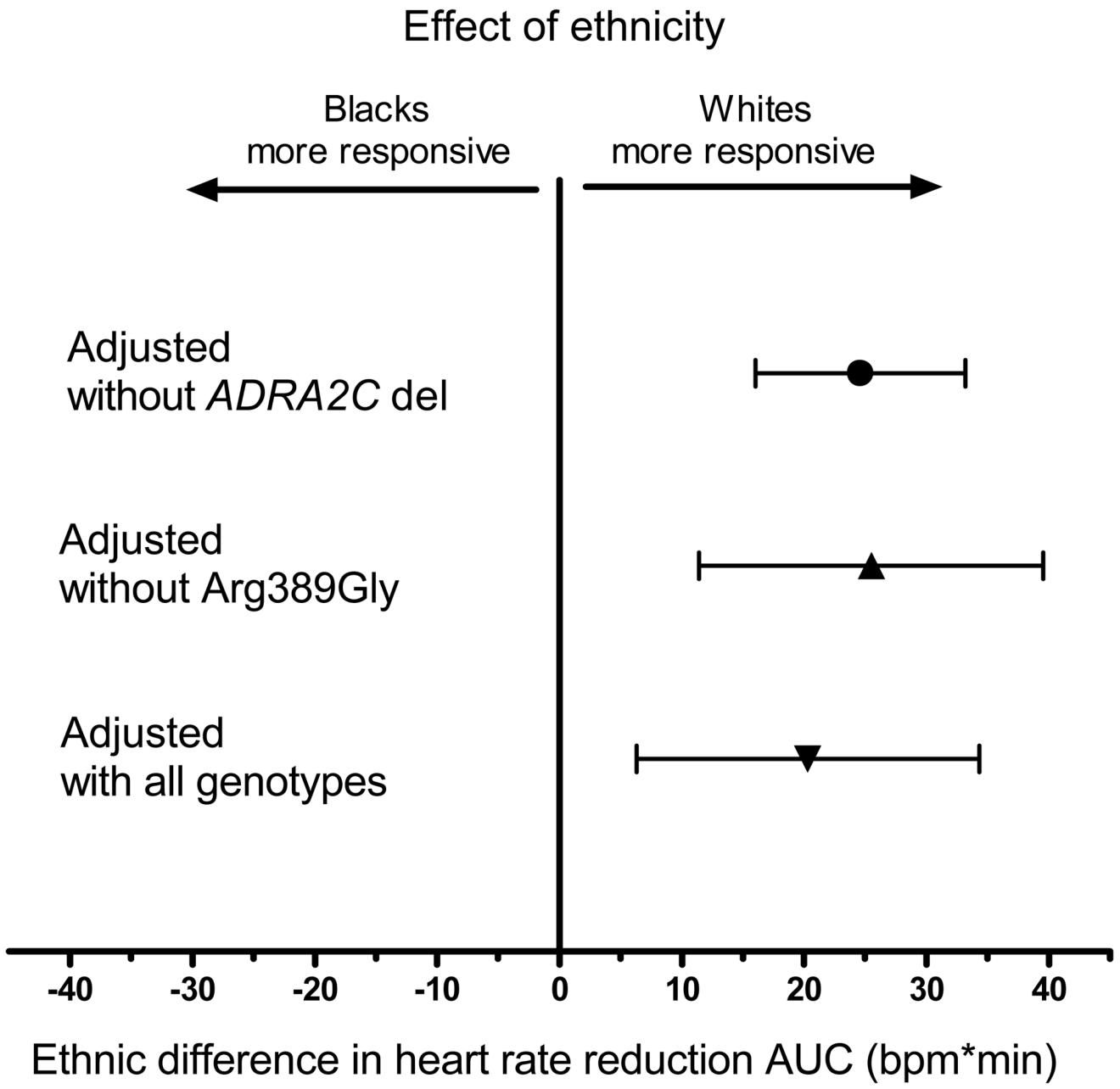
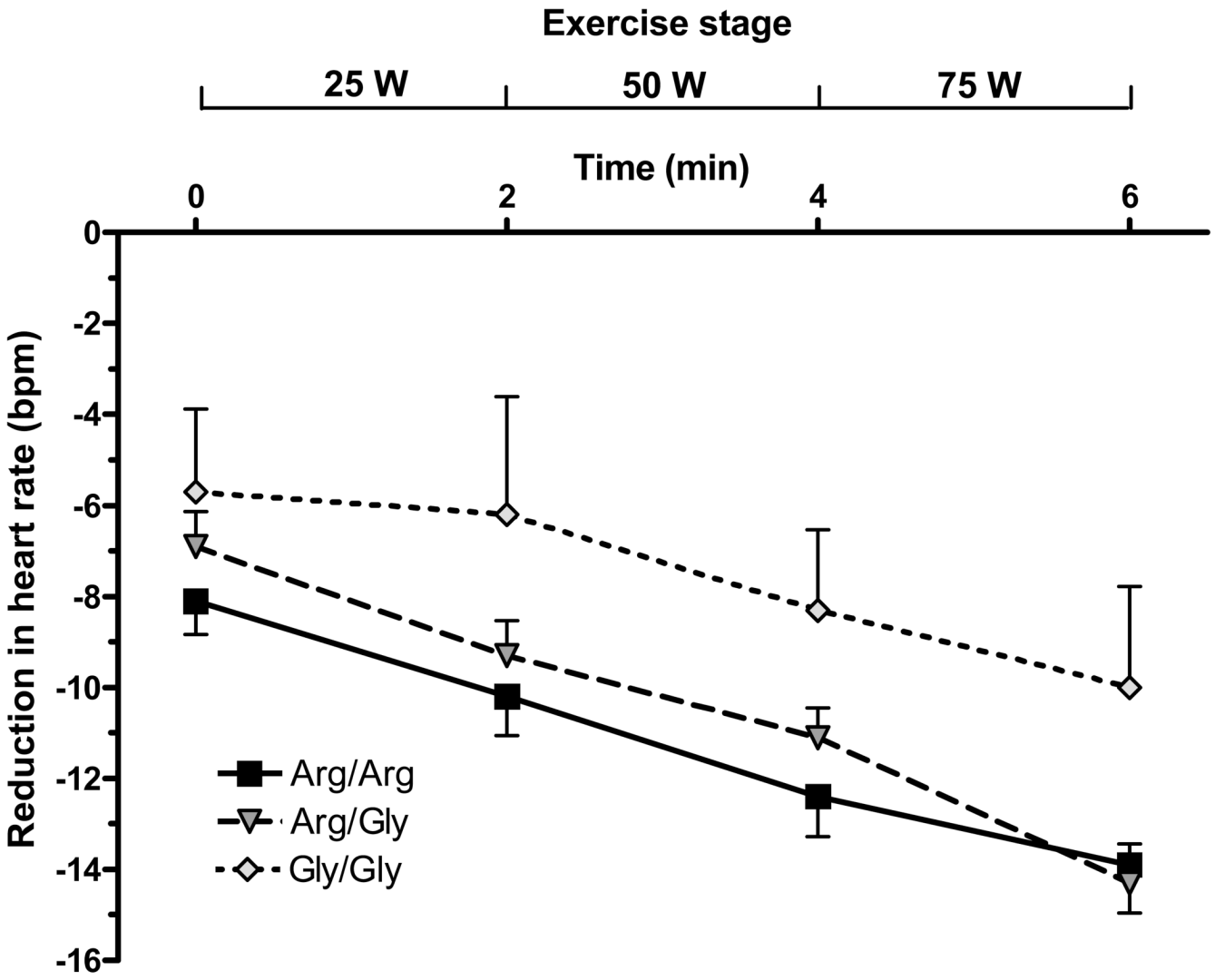


Figure 1.

Figure 1a. Reduction in heart rate over time at rest and during incremental exercise in white and black subjects (n=165) after atenolol. Data points represent the unadjusted mean reduction in heart rate for blacks (triangles) and whites (circles), error bars the standard errors of the mean. The area-under-the-curve was significantly different between white and black subjects (P=0.006)

Figure 1b. Adjusted effect of ethnicity on reduction in heart rate AUC, before and after additional adjustment for *ADRA2C* del322–325 and *ADRB1* Arg389Gly genotypes. The lower point estimate represents the ethnic difference after full adjustment for all non-genetic (pre-atenolol heart rate AUC, age, race, sex, BMI, S-atenolol concentration) and genetic covariates (*ADRB1* Ser49Gly and Arg389Gly and *ADRA2C* Del322–325 genotypes). The

upper and middle point estimates represent the adjusted ethnic difference without adjustment for *ADRA2C* and *ADRB1* Arg389Gly genotypes, respectively. Adjustment for genotypes attenuated the race effect only slightly. Error bars represent 95% confidence intervals.



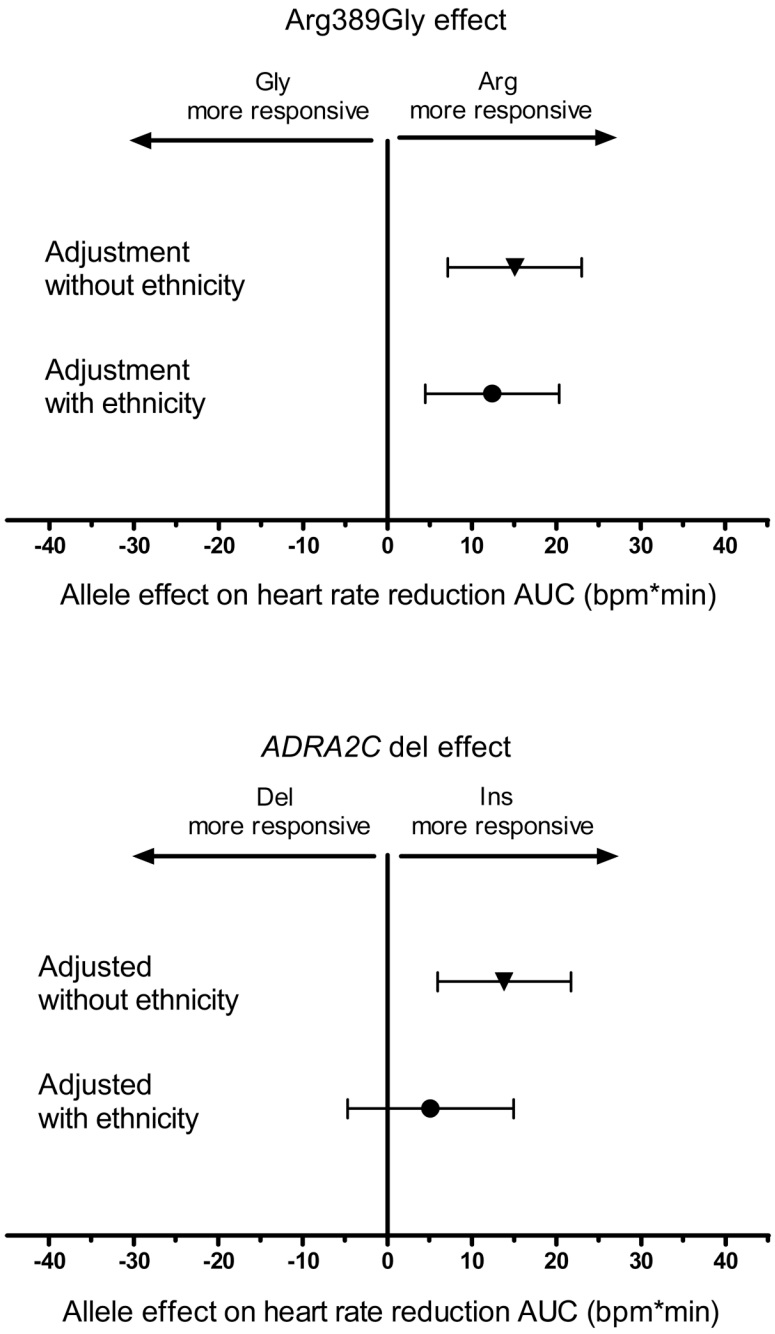


Figure 2.

Figure 2a. Unadjusted reduction in heart rate at rest and incremental exercise in *ADRB1* Arg389Gly genotypes after atenolol. Data points represent the unadjusted mean reduction in heart rate in the three genotype groups, error bars the standard errors of the mean. The area-under-the curve was significantly different among genotypes (P=0.014).

Figure 2b. Adjusted genotype effects on reduction in heart rate AUC, before and after additional adjustment for ethnicity. The upper point estimate in each panel represents the effect of the *ADRB1* Arg389 allele (upper panel) and *ADRA2C* 322–325 insertion allele (lower panel) on the reduction in heart rate AUC after adjustment for all covariates except ethnicity (pre-atenolol heart rate AUC, age, sex, BMI, S-atenolol concentration, *ADRB1* Ser49Gly

genotype, and *ADRA2C* 322–325 genotype [upper panel] or *ADRB1* Arg389Gly genotype [lower panel], respectively). The lower point estimate in each panel represents the allele effects after additional adjustment for ethnicity. Error bars represent 95% confidence intervals. Adjustment for ethnicity attenuated the Arg389 effect only slightly (P-value for allele effect after adjustment, P=0.003), whereas it greatly reduced the *ADRA2C* effect (P=0.31).

Table 1

Demographic and genetic characteristics and resting cardiovascular measures for white and black subjects

		White subjects Black	Black Subjects	P-value
n		92	73	
Female		42 (45.7%)	50 (68.5%)	0.003
Age (years)		27.4 ± 6.5	26.2 ± 6.2	0.24
Body Mass Index (kg/m^2)		24.7 ± 4.2	27.2 ± 6.4	0.005
Heart rate (bpm)		66.8 ± 7.5	67.5 ± 8.2	0.84
Systolic blood pressure (mmHg)		110.2 ± 11.7	112.7 ± 12.2	0.19
Diastolic blood pressure (mmHg)		64.3 ± 7.6	66.3 ± 7.4	0.11
Ser49Gly genotype	Ser/Ser	67 (77.9%)	39 (55.7%)	0.002
	Ser/Gly	19 (22.1%)	26 (37.1%)	
	Gly/Gly	0 (0%)	5 (7.1%)	
	Undetermined	6	3	
Arg389Gly genotype	Arg/Arg	49 (53.3%)	23 (31.9%)	0.024
	Arg/Gly	32 (34.8%)	37 (51.4%)	
	Gly/Gly	11 (12.0%)	12 (16.7%)	
	Undetermined	0	1	
ADRB1 haplotypes	Ser49-Arg389	103 (59.9%)	47 (34.6%)	<0.001
	Ser49-Gly389	50 (29.1%)	56 (41.2%)	
	Gly49-Arg389	19 (11.0%)	32 (23.5%)	
	Gly49-Gly389	0	1 (0.7%)	
	Undetermined	12	10	
ADRA2C genotype	Ins/Ins	79 (94.0%)	21 (30.9%)	<0.001
	Ins/Del	4 (4.8%)	33 (48.5%)	
	Del/Del	1 (1.2%)	14 (20.6%)	
	Undetermined	8	5	

Continuous variables are represented as mean ± SD. P-values are for ethnic comparisons (independent t-test / Chi square / Fisher's exact test).

Table 2

Effect of genotypes, ethnicity, and covariates on reduction in heart rate (at rest and 75 W exercise) and heart rate AUC after atenolol

	Decrease in Heart Rate (bpm)				Decrease in Heart rate-AUC (bpm * min)	
	Rest		Maximum Exercise (75 W)			
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Pre-atenolol heart rate / AUC	0.4 (0.3, 0.5)	<0.001	0.4 (0.4, 0.5)	<0.001	0.4 (0.3, 0.5)	<0.001
Age	0.0 (−0.1, 0.2)	0.59	0.1 (−0.1, 0.3)	0.38	0.3 (−0.5, 1.1)	0.49
Ethnicity	2.7 (0, 5.4)	0.057	4.5 (1.5, 7.5)	0.004	19.9 (6.0, 33.9)	0.006
Sex	1.3 (−0.9, 3.5)	0.22	5.6 (3.0, 8.2)	<0.001	19.1 (7.8, 30.5)	0.001
BMI	0.0 (−0.2, 0.2)	0.70	0.3 (0.0, 0.5)	0.023	0.8 (−0.3, 1.8)	0.15
S-atenolol concentration	0.0 (0.0, 0.1)	0.09	0.1 (0.0, 0.1)	<0.001	0.4 (0.2, 0.5)	<0.001
ADRB1 Arg389 allele	1.1 (−0.5, 2.7)	0.18	2.5 (0.8, 4.2)	0.005	12.4 (4.4, 20.4)	0.003
ADRB1 Ser49 allele	−0.5 (−2.5, 1.5)	0.65	2.4 (0.1, 4.7)	0.039	4.1 (−6.4, 14.5)	0.45
ADRA2C insertion allele	0.9 (−1.1, 2.9)	0.36	−0.7 (−1.4, 2.8)	0.52	5.1 (−4.7, 14.9)	0.31

Regression coefficients for *ADRB1* Arg389 and Ser49 alleles and the *ADRA2C* 322–325 insertion allele as well as other covariates, with their respective 95% confidence intervals (CI) and P-values are shown. Genotypes were coded 0–2 representing the number of Arg 389, Ser49, and *ADRA2C* insertion alleles, and sex and ethnicity were coded 0/1 for female/male and black/white, respectively, so that the correlation coefficients reflect sex and ethnic differences, respectively. AUC=Area-under-the-curve; BMI=body mass index.