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Genetic variants in the α_{2c} -adrenoceptor and G-protein contribute to ethnic differences in cardiovascular stress responses

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Abstract

Objectives: Cardiovascular responses to stressors are regulated by sympathetic activity, increased in black Americans, and associated with future cardiovascular morbidity. Our aim was to determine whether two functional variants in genes regulating sympathetic activity, a deletion in the α_{2c} -adrenergic receptor (*ADRA2C* del322-325) and a G-protein β_3 subunit variant (*GNB3* G825T), affect cardiovascular responses to physiologic stressors and contribute to their ethnic differences.

Methods: We measured heart rate and blood pressure responses to a cold pressor test (CPT) in 79 healthy subjects (40 blacks, 39 whites), aged 25.7 ± 5.3 years, and determined genotypes for the *ADRA2C* and *GNB3* variants. We examined the response variables (increase in heart rate and blood pressure) in multiple linear regression analyses adjusting first for baseline measures, ethnicity, and other covariates, and then additionally for genotypes.

Results: Black subjects had a greater heart rate response to CPT than whites (mean difference, 9.9 bpm; 95% confidence interval (CI), 4.1 to 15.6; $P=0.001$). Both the *ADRA2C* del/del (15.8 bpm; 95% CI, 8.0 to 23.7; $P<0.001$) and *GNB3* T/T genotypes (6.8 bpm; 95% CI, 0.9 to 12.7; $P=0.026$) were associated with greater heart rate response. After adjusting for genotypes, the ethnic difference was abrogated (1.3 bpm; 95% CI, -5.4 to 8.0; $P=0.70$), suggesting that the genetic variants contributed substantially to ethnic differences.

Conclusions: Variation in genes that regulate sympathetic activity affects hemodynamic stress responses and contributes to their ethnic differences. This study elucidates how genetic factors may in part explain ethnic differences in cardiovascular regulation.

Keywords

Cardiovascular physiology; Stress; Ethnic groups; Genetics; Receptors, Adrenergic, alpha-2; G-protein beta3 subunit

Introduction

The sympathetic nervous system regulates many physiologic responses, particularly those related to stress, and enhanced cardiovascular responses to physiologic and psychological stressors in young, healthy subjects predict long-term adverse cardiovascular outcomes [1].

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Sympathetic activity is also increased in cardiovascular diseases such as heart failure, hypertension, arrhythmias, and sudden death, often before clinical manifestations of disease [2]. Thus, altered regulation of sympathetic activity plays a role in the pathogenesis of cardiovascular disease [2,3].

A major regulator of sympathetic activity is the α_2 -adrenergic receptor (α_2 -AR) [4]. Since sympathetic activity is highly heritable [5,6], and since many diseases associated with increased sympathetic activity have a genetic component, interest has focused on the specific role of α_2 -AR receptor subtypes (α_{2A} , α_{2B} and α_{2C}) and their genetic variations in the regulation of sympathetic response [7,8].

The α_{2C} -AR on the presynaptic nerve terminal is part of a negative feedback system that inhibits further release of norepinephrine. This regulatory function is particularly important when sympathetic activity is increased [9]. Presynaptic α_{2C} -ARs may also inhibit secretion of epinephrine from the adrenal gland [10], and postsynaptic receptors induce vasoconstriction [11]. Genetic variation in the α_{2C} -AR may contribute to interindividual differences in autonomic response.

A common 12 base pair deletion in the coding region of the α_{2C} -AR gene (*ADRA2C*) results in the deletion of four amino acids (del322–325) that results in markedly decreased agonist-mediated responses *in vitro* [12]. Thus, this less functional variant would be expected to result in increased sympathetic activity *in vivo* and increased risk for diseases associated with sympathetic activation. Indeed, one study reported an increased risk of systolic heart failure in African-Americans homozygous for the *ADRA2C* del322–325 variant [13], although a large bi-ethnic population study did not find an association between the deletion variant and imaging markers of systolic dysfunction [14]. The effect of the del322–325 variant on stress responses has not been studied.

Adrenergic receptors couple to heterotrimeric guanine-nucleotide-binding proteins (G proteins) to mediate intracellular signal transduction. A C825T polymorphism in the gene encoding the G protein β_3 -subunit (*GNB3*) results in enhanced G protein signalling *in vitro* [15]. Thus, genetic variation in *GNB3* would be expected to contribute to variability in sympathetic responses to stress [16], but this has not been examined.

Although cardiovascular responses to physiologic and mental stressors have often been noted to be greater in African-Americans than Caucasians, the reasons for this enhanced response are not known [17]. Because the *ADRA2C* del322–325 and the *GNB3* C825T variants are much more common in black Americans than whites (approximately 10-fold and 2.5-fold, respectively), we hypothesized that this could contribute to ethnic differences in cardiovascular stress responses. To test this hypothesis, we evaluated the role of the *ADRA2C* del322–325 and *GNB3* C825T variants in sympathetic responses to the cold pressor test, a stimulus that results in a profound increase in sympathetic activity [18], and the contribution of these genetic variants to ethnic differences in responses.

Methods

Subjects

The Institutional Review Board of Vanderbilt University Medical Center approved the study protocol, and subjects gave written informed consent. Eighty subjects were studied: 72 (39 white and 33 black subjects) recruited by advertisement and from a volunteer database [19], and 8 black subjects with known *ADRA2C* del genotype. Four subjects homozygous for the deletion allele (Del/Del) and 4 homozygous for the insertion allele (Ins/Ins) were recruited to

increase the number of individuals with these less common genotypes. All subjects also contributed data to a previous study [20].

African-American and Caucasian American subjects were eligible to participate if they were unrelated residents of middle Tennessee, between 18-45 years of age, and had no clinically significant abnormality based on medical history, physical examination, and routine laboratory testing. Family history of hypertension was determined by self-report. 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 of 4 grandparents were of the same ethnicity. Body mass index (kg/m^2) was calculated as weight divided by height² (kg/m^2). Subjects were free of medications and dietary supplements for at least 2 weeks and received an alcohol- and caffeine-free diet (providing daily 150 mmol of sodium, 70 mmol of potassium, and 600 mmol of calcium) for 4 days prior to the study.

Resting measures

Studies were performed on the morning (8:00-10:00 am) of study day 5 in a temperature-controlled room (22°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. After 30 minutes, during which subjects rested comfortably supine, blood pressure and heart rate were measured at the left brachial artery by a semi-automated device (Dinamap MPS; GE Medical Systems, Waukesha, WI, USA), with a cuff size appropriate for the arm circumference [21]. To allow habituation, readings were repeated 3-4 times, and the last 2 recordings, obtained one minute apart after 30 minutes of rest, were averaged. Then, a venous blood sample (15 mL) was drawn from the uncuffed arm for determination of plasma catecholamine concentrations and for DNA analysis.

Cold pressor test

All preparations for the cold pressor test were performed only after the resting measures had been obtained in order to minimize confounding through anticipation. With the subject remaining supine, the left foot was fully immersed up to the ankle in a tub filled with ice water (4°C) for 2 minutes. Two subsequent readings of blood pressure and heart rate were taken with the semi-automated device, starting at approximately 15 and 45 seconds after foot immersion. At 1 minute, an additional blood sample (10 mL) was taken for determination of plasma catecholamine concentrations.

Genotyping

DNA for all 79 subjects that completed the study was available for genotyping. After amplification of DNA fragments by polymerase chain reaction (PCR), the *ADRA2C* del322-325 variant was genotyped by DNA fragment analysis and confirmed by direct sequencing of 10 randomly selected samples. DNA fragment analysis was performed as described previously [22]. In short, a fluorescently labeled forward primer (5'-6-FAM-AGACGGACGAGAGCAGCGCA-3') and a reverse primer (5'-AGGCCTCGCGCAGATGCCGTACA-3') were used 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.

Genotyping for the *GNB3* C825T polymorphism (rs5443) was performed by allelic discrimination with TaqMan 5'-nuclease assays [23] on an ABI 7900 HT real-time PCR system (Applied Biosystems, Foster City, CA) using validated TaqMan probes. Genotypes were generated using a 95% quality value threshold. To ensure quality control, we included samples

of known genotype with each genotyping run. Additionally, in a subset of 8 subjects, genotyping by PCR followed by detection of restriction fragment length polymorphisms, as previously described [24], gave concordant results. Genotyping success rate was 100% for the *ADRA2C* Del322-325 and 98.8% for the *GNB3* C825T variant, yielding genotypes in 79 and 78 subjects, respectively.

Plasma catecholamine determination

Blood was collected into cooled heparinized tubes which were immediately placed on ice until centrifuged at 4°C for 10 minutes at 3,000 rpm. Plasma was harvested and stored in tubes containing 40 µL of reduced glutathione (6%) at -20°C until assayed. Norepinephrine and epinephrine concentrations were measured by high-performance liquid chromatography using electrochemical detection with dihydroxybenzylamine as internal standard [25].

Statistical analysis

Data are expressed as mean and standard deviation or 95% confidence interval (CI). Analyses were performed using the highest heart rate and blood pressure measurement obtained during the cold pressor test. Comparisons of demographic data and outcomes in the two ethnic groups and among genotypes were performed by Chi-square test, independent t-test, and one-way analysis of variance, or Mann Whitney U and Kruskal-Wallis test if the normality assumption was not met. Allele distribution was tested for deviations from Hardy-Weinberg equilibrium with the use of a chi-square test with 1 degree of freedom. Previous studies [13,26] reported a recessive mode of inheritance for the functional effects of the *ADRA2C* deletion, and we therefore grouped homozygous and heterozygous insertion carriers and compared them to deletion homozygotes. In preliminary analyses we confirmed that, for both genes, heterozygous and homozygous carriers of the “wild-type” alleles (*ADRA2C* Ins322-325 and *GNB3* C825, respectively) did not differ significantly in their responses, whereas genotypic differences existed between homozygous carriers of variant alleles and the two other genotype groups. Multiple linear regression models were used to assess effects of *ADRA2C* del322-325 and *GNB3* C825T genotypes on systolic and diastolic blood pressure, heart rate, and plasma catecholamine concentrations at rest and on the change in these parameters during cold pressor testing, both before and after adjustment for potential confounding factors. These included ethnicity, sex, body mass index (BMI), family history of hypertension, age, and the resting measurement of the response variable. Since preselecting some subjects to enrich homozygous genotypes could have affected our results, we also performed analyses after excluding them. All tests were two-tailed, and a P-value of <0.05 was considered statistically significant. Analyses were performed with the statistical software SPSS (SPSS v.14.0, SPSS Inc., Chicago, IL, USA) and STATA 9.1 (StataCorp, College Station, TX, USA).

Results

Subject characteristics

Eighty subjects (39 whites and 41 blacks) were studied. One black subject, pre-selected for *ADRA2C* del/del genotype, could not complete the cold pressor test and was excluded from analysis. The demographic characteristics for the remaining 79 subjects are shown in Table 1. Blacks had a higher body mass index than whites (mean difference, 2.3 kg/m²; 95% CI, 0.6 to 4.0; P=0.009).

Genetic analysis

Allele frequencies for the *ADRA2C* deletion were within the expected range and differed significantly among ethnic groups (3.8% for white and 46.3% for black subjects; P<0.001). The *ADRA2C* genotype distribution (Ins/Ins: 92.3% and 35.0%; Ins/Del: 7.7% and 37.5%; and

Del/Del: 0.0% and 27.5% for whites and blacks, respectively) was consistent with Hardy-Weinberg equilibrium in whites ($\chi^2=0.06$, $P=0.81$) and blacks ($\chi^2=2.42$, $P=0.12$). For *GNB3*, frequencies of the T allele (39.5% and 85.0% for white and black subjects, respectively) and genotype distribution (CC: 39.5% and 2.5%; CT: 42.1% and 25.0%; TT: 18.4% and 72.5% for whites and blacks, respectively) were in the expected range, conformed with Hardy-Weinberg equilibrium ($P=0.46$ and $P=0.74$ for blacks and whites, respectively), and differed significantly between the two ethnic groups ($P<0.001$).

Resting cardiovascular measures

Resting cardiovascular measures in the two ethnic groups are shown in Table 1. Compared to whites, blacks had higher systolic blood pressure (mean difference, 5.4 mmHg; 95% CI, 1.5 to 9.4; $P=0.008$). In multiple linear regression models including sex, ethnicity, age, family history of hypertension, body mass index, and *ADRA2C* and *GNB3* genotypes as covariates, higher resting systolic blood pressure was associated with male sex ($P<0.001$) and black ethnicity ($P=0.017$), and higher plasma norepinephrine concentrations were associated with female sex ($P=0.015$) and age ($P=0.019$). *ADRA2C* and *GNB3* genotypes were not significantly associated with any of the dependent resting measures (systolic and diastolic blood pressure, heart rate, and catecholamine concentrations).

Responses to cold pressor test: Unadjusted analyses

All cardiovascular measures and plasma catecholamine concentrations increased significantly during the cold pressor test (all P -values <0.002). Black subjects had a significantly greater unadjusted increase in heart rate than whites (mean difference, 8.4 bpm; 95% CI, 3.2 to 13.6; $P=0.002$; Table 2 and Figure 1a). There were no ethnic differences in the unadjusted rise in blood pressure or plasma catecholamine concentrations (Table 2).

Additionally, *ADRA2C* and *GNB3* genotypes affected responses (Table 2, Figure 1b). Compared to carriers of the insertion allele (Ins/Ins and Ins/Del genotypes), subjects homozygous for the *ADRA2C* 322-325 deletion (Del/Del) had a greater increase in heart rate ($P=0.004$) and systolic blood pressure ($P=0.016$), and a trend to greater increase in diastolic blood pressure ($P=0.058$). Similarly, subjects homozygous for the *GNB3* T variant (T/T) had a greater unadjusted increase in heart rate than carriers of the C allele (C/C and C/T genotypes) ($P=0.003$; Table 2, Figure 1b).

One black female subject, who was homozygous for the *ADRA2C* deletion and *GNB3* T allele, had an extreme rise in plasma epinephrine concentrations during cold pressor testing (from 41 ng/mL at baseline to 347 ng/mL), representing an increase more than 18 times the interquartile range of the increase for all subjects (0-17 ng/mL). Increases in blood pressure, heart rate, and norepinephrine concentrations for this individual were between the 80th and 95th percentile of the range of the responses in the whole cohort. The marked epinephrine response to the cold pressor test in this individual was reproducible and thus not spurious. The analyses of epinephrine responses excluded this extreme outlier.

Heart rate responses: Adjusted analyses

Ethnicity

After adjustment for the resting heart rate, age, sex, BMI, and family history of hypertension, ethnicity remained strongly associated with the heart rate response to cold pressor testing (mean difference between blacks and whites, 9.9 bpm [95% CI, 4.1 to 15.6], $P=0.001$; Figure 2).

Genotype

When genotypes were added to the model, *ADRA2C* (del/del) ($P<0.001$) and *GNB3* (T/T) ($P=0.026$) were significantly associated with a greater increase in heart rate (Table 3). Testing for gene-gene interaction showed that the effects of both genes were largely independent of each other ($P=0.84$). A post-hoc sensitivity analysis restricted to black subjects only, in whom these genetic variants are much more common than in whites, yielded essentially equivalent results, although the association of *GNB3* with the increase in heart rate was of borderline statistical significance ($P=0.056$). *ADRA2C* and *GNB3* variants contributed substantially to the ethnic differences in heart rate response: After inclusion of genotypes in the multiple linear regression model, the strong association between ethnicity and heart rate response was abrogated (mean difference, 1.3 bpm [95% CI, -5.4 to 8.0 bpm]; $P=0.70$; Table 3; Figure 2). Moreover, there were no ethnic differences within subgroups with identical *ADRA2C* and *GNB3* genotypes ($P>0.30$; Figure 1b). Excluding the 7 black subjects pre-selected for their *ADRA2C* genotype yielded essentially unchanged results.

Blood pressure responses: Adjusted analyses

Ethnicity

After adjustment for baseline blood pressure, sex, age, BMI, and family history of hypertension, the mean systolic blood pressure response was 5.5 mmHg (95% CI, -1.0 to 12.1) greater in blacks than whites ($P=0.097$).

Genotype

After adding *ADRA2C* and *GNB3* genotypes to the model, the *ADRA2C* (del/del) genotype was associated with a greater systolic blood pressure response ($P=0.051$; Table 3). Additionally, the trend toward ethnic differences in systolic blood pressure response was no longer present (Table 3), suggesting that it was in part accounted for by *ADRA2C* genotype. For the diastolic blood pressure response, after adjustment for all covariates, there were no significant ethnic differences. Subjects with the *ADRA2C* del/del genotype increased diastolic blood pressure by a mean of 6.2 mmHg (95% CI, -0.8 to 13.2) more than subjects carrying the insertion allele ($P=0.082$).

Catecholamine responses: Adjusted analyses

After adjustment for all non-genetic covariates, black subjects had a greater increase in epinephrine (mean difference, 8.8 pg/mL [95% CI, 0.3 to 19.7]; $P=0.044$). After additional adjustment for *ADRA2C* and *GNB3* genotypes, this ethnic difference was essentially unaffected (mean difference, 10.0 pg/mL; 95% CI, 1.0 to 16.6 ; $P=0.027$). After excluding the 7 pre-selected black subjects, the adjusted ethnic difference was attenuated (mean difference, 7.1 pg/mL; 95% CI, -2.5 to 16.8 ; $P=0.145$). Neither *ADRA2C* genotype ($P=0.68$) nor *GNB3* genotype ($P=0.34$) were associated with the epinephrine response. Similarly, genotypes were not associated with norepinephrine response ($P=0.77$ and $P=0.78$ for *ADRA2C* and *GNB3*, respectively), and there were no ethnic differences ($P=0.84$).

Discussion

This study is the first to identify genetic variants that contribute to ethnic differences in cardiovascular stress response to the cold pressor test. Our main findings are that homozygosity for variants in *ADRA2C* and *GNB3*, key genes in the regulation of sympathetic signaling, is strongly associated with heart rate response to the cold pressor test, and contributes substantially to the ethnic differences observed. Moreover, there was a similar, but less pronounced *ADRA2C* genotype effect on blood pressure responses. Our findings suggest that

these genetic variants contribute to enhanced cardiovascular stress responses in young, healthy subjects and raise the possibility that such variants may play a role in both the increased cardiovascular morbidity associated with such enhanced stress responses and with the interethnic differences seen in these responses and morbidity.

Cardiovascular responses to acute physical and mental stressors have been studied extensively, because they offer mechanistic and prognostic information beyond that provided by resting measures [27]. For example, enhanced blood pressure and heart rate responses to stress tests in young, healthy subjects were associated with subsequent higher resting heart rate, blood pressure, and hypertension [1,28]. Thus, increased cardiovascular reactivity may not only define an intermediate phenotype predicting future cardiovascular disease but also reveal an underlying mechanism for the pathogenesis of adverse outcomes [29].

A classic experimental cardiovascular stressor is the cold pressor test; it elicits profound hemodynamic responses, greater than other physiological or psychological stress tests [1]. Most studies in bi-ethnic populations have found greater hemodynamic responses to the cold pressor test in young, healthy black subjects compared to whites [1,18,30-32]. Genetic contributions to these ethnic differences have not been elucidated. In our study, performed under controlled conditions including a standardized intake of salt, black subjects had a greater heart rate response, and a non-significant trend to higher systolic blood pressure response, in agreement with many previous studies [31,32]. Furthermore, ethnic differences were largely accounted for by variants in *ADRA2C* and *GNB3*. Our study population was deliberately enriched with black subjects homozygous for either *ADRA2C* allele in order to better define the effect of this genetic variation on stress responses. Thus, given the contribution of this variant to cold pressor responses, our study was more likely to detect ethnic and genotypic differences than a study of randomly selected individuals. However, after excluding the preselected subjects from the analysis, similar findings were observed among the randomly selected individuals.

Cardiovascular responses to stress are heritable [33-36]. Therefore, genetic variation may contribute to interindividual and ethnic differences in cardiovascular reactivity. We studied two genes that encode for key regulators of sympathetic activity, the α_2C -AR and G-protein β_3 -subunit. These genes have common variants (*ADRA2C* del322-325 and *GNB3* C825T, respectively) with functional effects, and the variant alleles are much more common in black Americans than whites. Little is known about the effects of the del322-325 variant of *ADRA2C*.

Previously, we found no differences in resting plasma catecholamine concentrations, heart rate variability, and, as also reported in a large population study [37], no differences in resting blood pressure and heart rate among *ADRA2C* del322-325 genotypes [20]. In contrast, a study of 29 black subjects found significantly higher resting mean arterial pressure and catecholamine spillover in subjects homozygous for the *ADRA2C* deletion [26]. Since genetic variants affecting sympathetic activity may contribute differentially to resting cardiovascular measures and stress responses [36], the effect of the deletion variant may be more apparent in states of heightened adrenergic tone.

Concordant with this notion, in the present study subjects homozygous for the *ADRA2C* deletion had a 2-fold greater increase in heart rate, and a directionally similar systolic blood pressure response, but no differences in baseline measures. Importantly, these results remained significant after adjusting for a number of potential confounders. This finding is also concordant with findings in transgenic mice with targeted deletions of *ADRA2C*. At baseline, these animals do not have apparent abnormalities of adrenergic regulation, but in an

experimental model of congestive heart failure they have higher plasma catecholamine concentrations, more severe disease, and a higher mortality [9].

The α_2 C-AR signals through G-proteins, and therefore genetic variation in the signalling pathway could amplify or mute the effects of *ADRA2C* variants. The C825T polymorphism of *GNB3* is the most important functional variant shown to affect G protein signaling *in vivo*. Based on *in vitro* data, we expected the more active *GNB3* genotype (TT) to be associated with greater α_2 C-AR activity and therefore attenuated stress responses. However, we observed the opposite: the TT genotype, independent of *ADRA2C* genotype, was associated with a significantly greater heart rate response. These findings are in line with another study in which the *GNB3* TT genotype was associated with greater sympathetic activation upon orthostatic challenge [16]. The mechanisms for the pleiotropic effects associated with the *GNB3* T allele variant are poorly understood [38], and may include the association of the β_3 -subunit with multiple other G-protein coupled receptors involved in cardiovascular control [39,40], receptor cross-talk [41], or other direct effects of $\beta\gamma$ dimers on cell function [42].

In our study, genotype effects on heart rate and blood pressure responses were not accompanied by corresponding differences in plasma catecholamines that would have explained the enhanced cardiovascular responsiveness in carriers of the more responsive genotypes. However, the antecubital venous plasma catecholamine concentration reflects the combined effect of systemic and local (forearm) release (spillover) and clearance. Thus, it is an indirect marker of sympathetic activity especially during dynamic changes in blood pressure and organ perfusion such as occur during the cold pressor test. Additionally, sympathetic responses are regulated in an organ-specific fashion [2,43], and thus global measures of norepinephrine release may not provide information about cardiac release, for example. Organ-specific regional spill-over studies that adjust for clearance would be a more specific way to assess sympathetic tone in a given organ; such techniques, however, are unsuitable for larger studies due to their invasive nature and the use of radioactive tracers.

The rise in plasma epinephrine concentrations was 2-fold higher in subjects homozygous for the *ADRA2C* deletion variant, but this difference was not statistically significant in view of the high interindividual variability. Of note, in mice, the α_2 C-adrenergic receptor regulates epinephrine release from the adrenal gland, but there is conflicting information about the presence of α_2 C-adrenergic receptors in the human adrenal gland [44,45]. Further studies of the effect of genetic variation in *ADRA2C* and *GNB3* on organ-specific norepinephrine release and adrenal epinephrine secretion under conditions of stress will be of interest.

In conclusion, in this study, the *ADRA2C* del322-325 and *GNB3* C825T variants were associated with enhanced hemodynamic responses to the cold pressor test, substantially accounting for the greater heart rate response in black subjects. Large longitudinal studies are necessary to examine whether these and other genetic variants are part of a common genetic basis linking stress test hyper-responsiveness in young adults to cardiovascular morbidity in later life.

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Figure 1.

Figure 1a: Ethnic difference in heart rate response to cold pressor test. Boxes represent interquartile ranges, error bars the ranges for the unadjusted rise in heart rate during cold pressor testing. Black subjects had a higher unadjusted heart rate response than whites ($P=0.002$).

Figure 1b: The interaction between the *ADRA2C* and *GNB3* genotypes and ethnicity on heart rate response during cold pressor test. Subjects were grouped by homozygosity for the variant alleles (D/D and TT for *ADRA2C* and *GNB3*, respectively). I=Insertion, D=Deletion. Boxes represent interquartile ranges, error bars the ranges. The left panel shows the unadjusted responses for the whole group, the right panel for whites (white bars) and blacks (grey bars) separately. There were no white subjects homozygous for the *ADRA2C* deletion. Heart rate response differed significantly among genotype groups ($P<0.001$). Within genotype groups with bi-ethnic representation, there was no ethnic difference ($P>0.30$).

Table 1

Demographic characteristics and resting cardiovascular measures.

	White subjects	Black Subjects	P-value
n	39	40	
Female (%)	17 (43.6%)	19 (47.5%)	0.73
Age (years)	26.0 ± 5.0	25.5 ± 5.6	0.65
Height (m)	1.72 ± 0.08	1.73 ± 0.11	0.69
BMI (kg/m ²)	24.4 ± 3.5	26.6 ± 4.1	0.009
Positive family history of hypertension [n (%)]	15 (38.5%)	20 (50%)	0.30
Heart rate (bpm)	63.7 ± 10.1	64.2 ± 9.1	0.82
Systolic blood pressure (mmHg)	109.4 ± 8.9	114.8 ± 8.7	0.008
Diastolic blood pressure (mmHg)	64.8 ± 4.7	67.2 ± 7.8	0.10
Plasma norepinephrine (pg/mL)	249.8 ± 142.5	232.9 ± 131.6	0.59
Plasma epinephrine (pg/mL)	17.7 ± 20.0	24.5 ± 14.7	0.10

Continuous variables are represented as mean ± standard deviation. P-values are for ethnic comparisons (independent t-test / Chi-Square test).

Table 2
Unadjusted response variables according to ethnicity and *ADRA2C* del322-325 and *GNB3* genotypes

	Ethnicity			ADRA2C del322-325 genotype			GNB3 C/T genotype		
	White subjects	Black Subjects	P-value	Ins/Ins or Ins/Del	Del/Del	P-value	CC or CT	TT	P-value
Δ Heart rate (bpm)	11.6± 8.4	20.0 ± 14.0	0.002	13.4 ± 9.7	30.9 ± 15.8	0.004	12.1 ± 9.5	20.4 ± 13.8	0.003
Δ Systolic BP (mmHg)	18.2 ± 9.2	22.2 ± 15.2	0.16	18.8 ± 12.1	28.6 ± 13.3	0.016	18.1 ± 11.4	22.8 ± 13.8	0.10
Δ Diastolic BP (mmHg)	14.1 ± 7.3	15.3 ± 11.3	0.56	13.9 ± 9.2	19.7 ± 10.0	0.058	13.6 ± 8.8	15.8 ± 10.2	0.29
Δ Plasma norepinephrine (pg/mL)	100.5 ± 109.5	76.2 ± 80.6	0.28	91.5 ± 101.8	71.3 ± 55.8	0.35	97.8 ± 107.8	76.4 ± 81.9	0.35
Δ Plasma epinephrine (pg/mL)	6.7 ± 12.3	13.9 ± 18.0	0.13	8.9 ± 14.3	19.7 ± 21.9	0.22	9.8 ± 16.5	10.7 ± 14.9	0.74

P-values refer to the comparison between white and black subjects or the comparison between the genotype groups.

Table 3

Adjusted responses of heart rate and systolic blood pressure to cold pressor testing

	Parameter	Coefficient	95% confidence interval	P-value
Heart rate response	Male sex	-0.8	-6.2 to 4.5	0.75
	Black ethnicity	1.3	-5.4 to 8.0	0.70
	Age	0.1	-0.4 to 0.5	0.83
	Body Mass Index	-0.3	-1.0 to 0.4	0.40
	Positive FH of hypertension	-1.0	-6.0 to 4.1	0.70
	Baseline heart rate	-0.1	-0.4 to 0.1	0.29
	<i>ADRA2C</i> del/del genotype	15.8	8.0 to 23.7	<0.001
	<i>GNB3</i> T/T genotype	6.8	0.9 to 12.7	0.026
Systolic blood pressure response	Male sex	2.7	-4.5 to 10.0	0.45
	Black ethnicity	-0.7	-8.8 to 7.5	0.87
	Age	0.5	0.0 to 1.1	0.065
	Body Mass Index	-0.3	-1.1 to 0.5	0.46
	Positive FH of hypertension	1.8	-4.1 to 7.7	0.55
	Baseline systolic blood pressure	0	-0.4 to 0.4	0.85
	<i>ADRA2C</i> del/del genotype	9.1	0.0 to 18.2	0.051
	<i>GNB3</i> T/T genotype	5.7	-1.2 to 12.7	0.10

Listed are covariates and multiple linear regression coefficients, their confidence intervals, and P-values for two response variables (changes from baseline in heart rate and systolic blood pressure). FH=family history; *ADRA2C* del/del genotype=homozygous *ADRA2C* 322-325 deletion genotype (coded as 1) compared to Ins/Ins and Ins/del genotype combined (coded as 0); *GNB3* T/T genotype=homozygous *GNB3* 825 T/T genotype (coded as 1), compared to C/C and C/T combined (coded as 0). Sex and ethnicity were coded as 0/1 for female/male and white/black, respectively, so that the correlation coefficients reflect sex and ethnic differences, respectively.