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# ALPHA-ADRENERGIC RECEPTOR GENE POLYMORPHISMS AND CARDIOVASCULAR REACTIVITY TO STRESS IN BLACK ADOLESCENTS AND YOUNG ADULTS

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## Abstract

Cardiovascular reactivity to stress and α-adrenergic receptor (α-AR) function may contribute to the development of hypertension. As Black Americans have an increased risk of hypertension, we evaluated associations between $\alpha_{1A}$-AR (Arg492Cys), $\alpha_{2A}$-AR (−1291C/G), and $\alpha_{2B}$-AR (Ins/Del301-303) gene variants and cardiovascular reactivity in 500 normotensive Black youth. Heart rate, preejection period, total peripheral resistance, and blood pressure were measured during cold and psychological stress. The Arg492Cys polymorphism in the $\alpha_{1A}$-AR gene was associated with heart rate reactivity to stress, but the association depended on sex. The −1291C/G promoter polymorphism in the $\alpha_{2A}$-AR gene was associated with vascular reactivity to stress; vasoconstriction increased as a linear function of the number of copies of the variant G allele. Thus, specific associations emerged between genetic variations in α-ARs and cardiovascular reactivity in young Blacks.

### Keywords

Alpha-adrenergic receptor; genetics; hemodynamics; impedance cardiography; stress; African Americans

The prevalence of hypertension is greater in Black/African Americans than in other ethnic groups in the United States (Ong, Cheung, Man, Lau, & Lam, 2007). Cardiovascular reactivity to stress may contribute to this increased risk among Blacks (Anderson, 1989). Prior research has shown that cardiovascular responses to cold stress and psychological stress are greater in Blacks than in Whites (Anderson, 1989; Kelsey, Alpert, Patterson, & Barnard, 2000; Murphy, Alpert, & Walker, 1992; Saab et al., 1992; Sherwood, May, Siegel, & Blumenthal, 1995; Stein, Lang, Singh, He, & Wood, 2000; Taherzadeh, Brewster, van Montfrans, & VanBavel, 2010; Treiber et al., 1990). These cardiovascular responses are reliable (Kamarck, 1992; Kelsey, Ornduff, & Alpert, 2007; McGrath & O'Brien, 2001; Swain & Suls, 1996) and heritable (De Geus, Kupper, Boomsma, & Snieder, 2007; Snieder et al., 2002), and predict future elevations in resting blood pressure (BP) and the development of hypertension later in life (Chida & Steptoe, 2010; Kelsey, 2004; Matthews

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et al., 2004; Treiber et al., 2003). The heritability of cardiovascular responses to stress and their association with the development of hypertension indicate that these responses can be used as intermediate phenotypes for investigating genetic contributions to cardiovascular risk. Furthermore, given that cardiovascular reactivity to stress is defined as a change in cardiovascular function evoked by an environmental stressor, genetic studies of cardiovascular reactivity to stress are inherently investigations of gene-environment interaction effects.

Different types of stressors tend to elicit different patterns of cardiovascular reactivity (Kamarck & Lovallo, 2003; Kasprowicz, Manuck, Malkoff, & Krantz, 1990; Obrist, 1981; Saab et al., 1992; Sherwood, Dolan, & Light, 1990). Stressors that involve effortful active coping (e.g., mental arithmetic, video games) tend to elicit stronger cardiac responses, whereas stressors that involve passive coping or cold exposure tend to elicit stronger vasoconstrictive responses. Nonetheless, there are significant individual differences in the magnitude and pattern of cardiovascular responses that cut across different stressors. Regardless of the type of stressor, some individuals are characteristically hyper-reactive whereas others are characteristically hypo-reactive, and some are primarily cardiac reactors whereas others are primarily vascular reactors (Kamarck & Lovallo, 2003; Kasprowicz et al., 1990; Obrist, 1981; Saab et al., 1992; Sherwood, Dolan et al., 1990). These individual differences are an important prerequisite for using cardiovascular reactivity as a marker for hypertensive risk because only a portion of the population develops hypertension.

The sympathetic nervous system (SNS) plays a key role in regulating BP and cardiovascular reactivity to stress. The SNS response to stress includes the release of norepinephrine (NE) and epinephrine (EPI), which stimulate α- and β-adrenergic receptors (ARs) in the heart and vasculature to alter cardiovascular function and BP. Several lines of evidence implicate excessive adrenergic activation in the pathophysiology of hypertension, including altered AR function in borderline and established hypertension (Michel, Brodde, & Insel, 1990; Sherwood & Hinderliter, 1993), the effectiveness of AR antagonists as antihypertensive medications (Flamenbaum et al., 1985; Veelken & Schmieder, 1996), and increased adrenergic reactivity to stress in hypertensive or high-risk normotensive subjects (Julius & Nesbitt, 1996; Noll et al., 1996; Sherwood, Hinderliter, & Light, 1995; Stein et al., 2000; Taherzadeh et al., 2010). Consistent with this evidence and with the elevated risk of hypertension in Blacks, research has shown that α-adrenergic vasoconstrictive reactivity tends to be greater in Blacks than in Whites (Kelsey, Alpert, et al., 2000; Saab et al., 1992; Sherwood & Hinderliter, 1993; Sherwood, May, et al., 1995; Stein et al., 2000; Taherzadeh et al., 2010; Treiber et al., 1990). Therefore, we evaluated associations between α-AR gene polymorphisms and cardiovascular reactivity to stress in Black adolescents and young adults. We focused on this young population with an increased risk for developing hypertension because our aim was to identify genetic variation associated with the initiation and evolution of the disease, rather than with the sustained or secondary effects of established hypertension.

Six distinct α-AR subtypes have been identified in the central nervous system and the SNS (Dorn, 2010; Flordellis, Manolis, Scheinin, & Paris, 2004; Kanagy, 2005; Kirstein & Insel, 2004; Michelotti, Price, & Schwinn, 2000; Vargas & Gorman, 1995), including three $\alpha_1$-AR subtypes ($\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$) and three $\alpha_2$-AR subtypes ($\alpha_{2A}$, $\alpha_{2B}$, and $\alpha_{2C}$). The three $\alpha_1$-AR subtypes regulate BP, peripheral vasoconstriction, and aspects of cardiac function (Kirstein & Insel, 2004; Michelotti et al., 2000; Vargas & Gorman, 1995). The $\alpha_{1A}$-AR is the predominant $\alpha_1$-AR subtype involved in cardiovascular regulation and vasoconstrictive reactivity to stress (Michelotti et al., 2000; Vargas & Gorman, 1995). The $\alpha_{2A}$-AR occurs primarily at presynaptic sites on central and peripheral sympathetic nerves where it inhibits the release of NE, thereby reducing peripheral vasoconstriction and BP (Flordellis et al.,

2004; Kanagy, 2005; Kirstein & Insel, 2004). The $\alpha_{2B}$-AR occurs primarily at postsynaptic arterial sites where it regulates vasoconstriction and BP (Flordellis et al., 2004; Kanagy, 2005; Kirstein & Insel, 2004). The $\alpha_{2C}$-AR is located postsynaptically in cutaneous arterioles where it promotes vasoconstriction, and presynaptically on peripheral sympathetic nerves where it reduces the release of NE (Flordellis et al., 2004; Kanagy, 2005; Kirstein & Insel, 2004); it is thought to play a lesser role in hemodynamic regulation than the other $\alpha_2$-AR subtypes (Flordellis et al., 2004). Moreover, the presynaptic inhibitory effects of the $\alpha_{2A}$-AR are most pronounced at high NE concentrations, whereas those of the $\alpha_{2C}$-AR are most apparent at low NE concentrations; thus, the $\alpha_{2A}$-AR is likely to be more important than the $\alpha_{2C}$-AR in modulating NE release and cardiovascular function during stress (Hein, Altman, & Kobilka, 1999). Hence, the $\alpha_{1A}$-AR, the $\alpha_{2A}$-AR, and the $\alpha_{2B}$-AR are the main α-ARs involved in hemodynamic regulation, especially during stress.

Several genome-wide association studies have implicated single nucleotide polymorphisms (SNPs) at or near α-AR genes in blood pressure and electrocardiographic variation (Newton-Cheh et al., 2007; Sober et al., 2009; Wang et al., 2009). Genetic variation is present in the $\alpha_{1A}$-AR, the $\alpha_{2A}$-AR, and the $\alpha_{2B}$-AR, and may contribute to variability in cardiovascular reactivity to stress. Relatively well-characterized, common polymorphisms in these α-AR genes have been identified in individuals of African descent (Dorn, 2010; Flordellis et al., 2004; Kirstein & Insel, 2004; Small, McGraw, & Liggett, 2003), including: (a) a nonsynonymous SNP in the $\alpha_{1A}$-AR gene (*ADRA1A*, located in chromosomal region 8p21.2), resulting in either arginine (Arg) or cysteine (Cys) at codon 492 (Arg492Cys, also known as Arg347Cys, rs1048101); (b) a promoter SNP (−1291C/G, rs1800544) in the $\alpha_{2A}$-AR gene (*ADRA2A*, located in 10q25.2); (c) an insertion/deletion polymorphism in the $\alpha_{2B}$-AR gene (*ADRA2B*, located in 2q11.2), resulting in the presence or absence of 3 glutamic acids at amino acid positions 301–303 (Ins/Del301-303, rs29000568) in the third intracellular loop of the receptor. These three polymorphisms have received considerable attention in the biomedical literature because of their common occurrence and reported associations with cardiovascular, other physiological, or pharmacological variability (Dorn 2010; Fava et al., 2009; Flordellis et al., 2004; Freitas, Pereira, Floriano, Mill, & Krieger, 2008; Gu et al., 2006; Heinonen et al., 2002; Iacoviello et al., 2006; Jiang et al., 2005; Kirstein & Insel, 2004; Kurnik et al., 2006; Ohlin, Berglund, Nilsson, & Melander, 2007; Rana et al., 2007; Rosmond, Bouchard, & Björntorp, 2002; Rosskopf & Michel, 2008; Small, Brown, Forbes, & Liggett, 2001; Small et al., 2003; Snapir et al., 2003; Suzuki et al., 2003; Ueno et al., 2006; Vasudevan, Ismail, Stanslas, Shamsudin, & Ali, 2008; Zhang et al., 2005). Importantly, there are marked population-specific differences in allelic frequencies of these polymorphisms, with allelic frequencies in Blacks differing from those in Whites (Belfer et al., 2005; Dorn, 2010; Flordellis et al., 2004; Kirstein & Insel, 2004; Rosskopf & Michel, 2008; Small et al., 2003). Previous research on the effects of variation in these receptors has largely focused on Whites, but it is critically important to understand how these variants affect hypertensive risk in Blacks.

To address this need, we evaluated associations between these three genetic polymorphisms and cardiovascular reactivity to stress in healthy, normotensive Black adolescents and young adults. Only two previous studies have evaluated genetic variation in α-ARs and cardiovascular reactivity to stress (Wu, Snieder, & de Geus, 2010). McCaffery and colleagues (McCaffery, Pogue-Geil, Ferrell, Petro, & Manuck, 2002) studied polymorphisms in the $\alpha_{1B}$-AR and the $\alpha_{2A}$-AR in association with BP and heart rate (HR) reactivity to mental challenge in young European-American men and women ($N$ = 309). They found no significant associations between genetic variants in these receptors and cardiovascular reactivity. Kurnik et al. (2008) studied associations between a common $\alpha_{2C}$-AR deletion polymorphism and BP and HR reactivity to the cold pressor test in a sample of Blacks ($n$ = 40) and Whites ($n$ = 39). They found that the deletion variant in the $\alpha_{2C}$-AR

gene was associated with increased HR reactivity to cold stress, and that this polymorphism largely accounted for an observed racial difference in HR reactivity. Neither of these studies evaluated genetic associations with measures of cardiovascular reactivity other than BP or HR. Moreover, to date, there has been no study of $\alpha_{1A}$-AR or $\alpha_{2B}$-AR gene polymorphisms and cardiovascular reactivity to stress, nor has there been any study of $\alpha_{2A}$-AR gene polymorphisms and cardiovascular reactivity to stress in Blacks. The present study addressed these gaps in the literature.

There is a growing awareness of the importance of considering sex differences in genetic associations with disease-related traits, especially for diseases such as hypertension with characteristic sex differences in prevalence and age of onset (Mendelsohn & Karas, 2005; Padmanabhan et al., 2010; Rana et al., 2007; Seda et al., 2008; Weiss, Pan, Abney, & Ober, 2006). There are considerable sex differences in α-AR stimulation affecting blood pressure and cardiovascular reactivity (Bowyer, Brown, & Jones, 2001; Freedman, Sabharwal, & Desai, 1987; Kneale, Chowienczyk, Brett, Coltart, & Ritter, 2003; Schmitt, Joyner, Charkoudian, Wallin, & Hart, 2010). Recent studies have reported significant sex-specific associations of $\alpha_{1A}$-AR and $\alpha_{2A}$-AR gene polymorphisms with BP levels (Padmanabhan et al., 2010; Rana et al., 2007) and stress hormones (Haefner et al., 2008). Accordingly, we included both sex and genotype as independent variables in our analyses to determine whether there were any sex differences in associations between α-AR gene polymorphisms and cardiovascular reactivity to stress.

## Methods

### Participants

Normotensive, unrelated Black adolescents and young adults (N = 535; 267 females, 268 males; age 15 to 21 yrs) were recruited from the Memphis, Tennessee area between February 2004 and January 2008. All participants identified themselves as “Black or African-American” in response to a census-style ethnic self-identification question from the Multigroup Ethnic Identity Measure (Phinney, 1992). Potential participants who reported a significant medical condition or the use of any medication that would affect BP or cardiovascular responses were excluded from the study. All participants were asked to refrain from eating for at least 2 h before testing, and from smoking, drinking beverages containing caffeine or alcohol, or taking any medication or drugs for at least 8 h before testing. Informed consent was obtained from each young adult, and assent and informed consent were obtained from each adolescent and a parent or legally authorized representative. The Institutional Review Board of the University of Tennessee Health Science Center approved the protocol, and all procedures conformed to institutional guidelines. Each participant received $100 for participating.

Data from 35 participants were excluded. Three participants were excluded for health reasons (one with significant cardiac arrhythmia, one with hypertensive resting BP, and one with acute gastric distress); six were excluded for failure to comply with pre-experimental instructions; eleven were excluded due to equipment failure; fifteen were excluded because of poor signal quality of cardiovascular recordings. Thus, the final sample included 500 participants (254 females, 246 males).

### Apparatus and Measures

The study was conducted in the cardiovascular laboratory at the University of Tennessee General Clinical Research Center. The laboratory had a central equipment/control room, an adjoining participant testing room (approximately 3× 2 × 2.5 m, maintained at a temperature

of 22°C), and an adjoining cold chamber (approximately 2 × 1.5 × 2.5 m, maintained at a temperature of 9°C to 12°C).

**Psychological Stressors—**Participants completed two mental arithmetic (MA) tasks and a video game (VG) task. For the MA tasks, participants counted backward aloud by steps of 7 from a 4-digit number for 5 min. A 5-min rest period separated the two tasks, and performance during the second task was overtly videotaped to increase evaluative threat. The pre-recorded instructions, procedures, and equipment have been described in detail elsewhere (Kelsey et al., 2007; Kelsey, Soderlund, & Arthur, 2004). For the VG task, participants played three 1-min games of "Centipede" (Atari) on a handheld device (Game Boy, Nintendo; Kelsey et al., 2007). As in previous studies (Kelsey et al., 2007; Murphy et al., 1992), the games progressed through three levels of challenge: for the first game, participants were instructed to see how well they could do; for the second game, they were instructed to try harder and improve their scores; for the third game, they were instructed to try harder and exceed their previous scores to win a monetary reward.

**Cold Stressors—**Participants were exposed to forehead cold pressor (CP) and whole-body cold exposure (CE). For CP, participants sat upright in a comfortable chair with their heads tilted back while the experimenter applied a plastic bag containing crushed ice and water (approximately 3°C to 4°C) to their foreheads for 3 min (Kelsey et al., 2007; Treiber et al., 1990). For CE, participants sat upright in a comfortable chair in a cold chamber for 10 min (Kelsey, Alpert, et al., 2000). A refrigerated ventilation system maintained the cold chamber at a temperature of 9°C to 12°C (50% to 60% humidity). Participants were instructed to sit quietly and remain awake, and were observed continuously through an observation window.

**Cardiovascular Measures—**Systolic BP (SBP, mmHg), diastolic BP (DBP, mmHg), and mean arterial pressure (MAP, mmHg) were measured once per minute from the brachial artery region of the non-dominant arm using a cuff of appropriate size and an automated oscillometric BP monitor (model 9300 OscilloMate, CAS Medical Systems, Inc., Branford, CT; Alpert, 1996). Heart rate (HR, bpm), preejection period (PEP, ms), and cardiac output (CO, L/min) were measured continuously with a standard tetrapolar band-electrode system and an HIC-2000 Impedance Cardiograph (model 2000, Bio-Impedance Technology, Inc., Chapel Hill, NC) according to established guidelines (Sherwood, Allen, et al., 1990). Impedance cardiographic data were acquired and scored with commercial software (COP-Win 6.2, Bio-Impedance Technology, Inc.). As in previous studies (Kelsey et al., 1998; Kelsey et al., 2004; Kelsey et al., 2007), HR was measured using the internal electrocardiographic signal from the impedance cardiograph, and PEP was measured as the interval between the peak of the electrocardiographic R-wave and the B-point of the impedance cardiographic dZ/dt waveform. Total peripheral resistance (TPR, dynes/cm$^5$) was derived from concurrent measures of CO and MAP, using the formula TPR = (MAP/CO) × 80 (Sherwood, Allen, et al., 1990). Prior research has established the reliability and validity of these cardiovascular measures (Berntson et al., 1994; Kelsey et al., 1998; Kelsey et al., 2007; Mezzacappa, Kelsey, & Katkin, 1999; Sherwood, Allen, et al., 1990; also see Results below for reliability data).

**Stress Appraisals—**Participants rated their experience of stress immediately after each stressor by answering five stress appraisal questions (Kelsey, Blascovich, et al., 2000): (a) "How stressful was the task you just completed?"; (b) "How threatening was the task you just completed?"; (c) "How demanding was the task you just completed?"; (d) "How well were you able to cope with the task?"; (e) "How well do you think you performed the task?" Each item included an appropriately anchored 7-point Likert-type scale, ranging from "not

at all" to "extremely". The fourth and fifth items were reverse scored and the five items were summed to create a stress appraisal scale for each task (Kelsey, Blascovich, et al., 2000), with scores ranging from 5 (not at all stressful) to 35 (extremely stressful). Our previous research has shown that these stress appraisals are reliable and sensitive to the effects of task repetition and evaluative threat (Kelsey, Blascovich, et al., 2000; Kelsey et al., 2004).

## Experimental Procedure

After informed consent and assent were obtained, each participant provided a buccal swab sample for genetic analysis and then removed garments from the upper body and put on a hospital gown. A standard hospital balance beam scale and stadiometer were used to measure each participant's weight (in kg) and height (in cm) for the calculation of body mass index (BMI, $kg/m^2$). The experimenter administered a battery of psychosocial (data not reported) and demographic questionnaires, and then connected the participant to the cardiovascular recording devices.

Participants sat quietly in a comfortable chair for a 10-min baseline rest period before each stress task; cardiovascular data were recorded during the last 3 min of each baseline period. Previous research has demonstrated that a 10-min rest period is sufficient to establish a stable cardiovascular baseline (Jennings, Kamarck, Stewart, Eddy, & Johnson, 1992; Kelsey et al., 1999; Murphy, Alpert, & Walker, 1994). The MA, VG, and CP tasks were presented in counterbalanced order; cardiovascular data were recorded during each minute of each task. After completing these tasks, participants rested for an additional 10-min baseline period and then were seated in the cold chamber. Cardiovascular measures were recorded during the last 3 min of the baseline period and each min of CE. Following CE, the cardiovascular recording devices were removed and any remaining questions by the participant and/or parent were answered.

## DNA Extraction

Buccal swabs were stored at −20°C until extraction. DNA was extracted from the buccal swabs using the Epicentre MasterAmp Buccal Swab DNA Extraction kit (MB79015, Epicentre Biotechnologies, Madison, WI) and stored at −80°C.

## SNP Genotyping Assays

All genotypic analysis was performed blind with respect to phenotypic data. All assays included DNA samples with known genotypes as controls. At least 10% of the samples were analyzed a second time for each polymorphism and each gave an identical result, thus confirming reproducibility. For the $\alpha_{1A}$- and $\alpha_{2A}$-AR SNPs, DNA samples were initially genotyped using PCR amplification and gel electrophoresis, but as new technology became available during the course of the study DNA samples were subsequently genotyped using Taqman SNP Genotyping assays (Applied Biosystems, Foster City, CA). For the $\alpha_{2B}$-AR polymorphism, all DNA samples were genotyped by PCR amplification and gel electrophoresis. For assays using PCR amplification and gel electrophoresis, two researchers independently assessed the results from the analyses and assigned genotypes. For the $\alpha_{1A}$- and $\alpha_{2A}$-AR SNPs, a subset of samples was assayed using both methods and the results were identical for each SNP, thus verifying the equivalence of the methods.

**Assay of the $\alpha_{1A}$-AR Polymorphism—**The $\alpha_{1A}$-AR SNP (rs1048101) resulting in an Arg or Cys at amino acid position 492 was assayed using either the Taqman Assay ($N$ = 195) or by PCR amplification and restriction digestion ($N$ = 305) as described previously (Shibata et al., 1996). There were two genotyping failures, leaving $N$ = 498 for this SNP.

**Assay of the $\alpha_{2A}$-AR Polymorphism—**The $\alpha_{2A}$-AR SNP at nucleotide position −1291 (rs1800544) was assayed using either the Taqman Assay (*N* = 194) or PCR amplification and restriction digestion (*N* = 306), as reported previously with some modification (Lario et al., 1997). Briefly, the following primers were used for the PCR: 5′TCACACCGGAGGTTACTTCCCTCG3′ and 5′GAGACTTAAAGAGGGAGCCCG3′. The conditions for PCR were: 94°C for 5 min and then 35 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 1 min. The PCR products were digested with *Msp*I and analyzed by electrophoresis on a 3% Nusieve gel stained with ethidium bromide.

**Assay of the $\alpha_{2B}$-AR Polymorphism—**The insertion/deletion polymorphism resulting in 9 or 12 Glu residues in the $\alpha_{2B}$-AR (rs29000568) was assayed using a strategy similar to that reported previously, which examined the size of the PCR product after amplification of the region containing the polymorphism (Small et al., 2001). Briefly, the following primers were used for the PCR: 5′GCATCTCCAGAGGATGAAGC3′ and 5′AGACACTGGCACTGCCTGG3′. The conditions for PCR were: 95°C for 2 min and then 35 cycles of 95°C for 15 sec, 60°C for 30 sec, and 72°C for 45 sec. The sizes of the PCR products were determined by electrophoresis on a 10% polyacrylamide gel. There were five genotyping failures, leaving *N* = 495 for this SNP.

## Data Processing and Analysis

The genetic data were analyzed using SAS/GENETICS v.9.2 (SAS inc., Cary, NC) and the genhwi routine of StataSE 9 (StataCorp, College Station, TX; Cleves, 1999). The three polymorphisms were tested for deviation from Hardy-Weinberg equilibrium using both $\chi^2$ and permutation exact tests.

Cardiovascular data were averaged over the last 3 min of each 10-min baseline rest period to compute baseline means, and over all minutes of each stress task period to compute stressor means. Reactivity was evaluated for HR, PEP, TPR, SBP, and DBP by subtracting the means for each pre-stress baseline rest period from the means for each corresponding stress task period. Focusing on these five measures served to minimize the number of statistical tests while still providing broad coverage of adrenergically mediated cardiovascular reactivity.

Measures of cardiovascular reactivity were analyzed in a mixed factorial design using multivariate analysis of covariance (MANCOVA) for repeated measures. The between-subjects factors were sex (2 levels) and genotype (2 or 3 levels, depending on the frequency distribution for each polymorphism, as indicated below), and the within-subjects factor was stress task period (4 levels, one for each stressor). Note that this design is truly multivariate only for tests involving the within-subjects factor (i.e., the main effect of stress task period and interactions involving this factor); tests of the sex and genotype main effects and the sex × genotype interaction effect are actually univariate, as they involve reactivity averaged over all stressors (i.e., the grand mean). Covariates included age, BMI, and two dummy-coded variables for stress task order (MA first and VG first). Age and BMI are commonly used as covariates in genetic studies of cardiovascular function (e.g., Iacoviello et al., 2006; Padmanabhan et al., 2010; Rana et al., 2007) and reactivity (e.g., Kurnik et al., 2008; McCaffery et al., 2002). We included covariates for task order because previous research has shown that prior exposure to similar psychological stressors attenuates subsequent cardiovascular reactivity to stress (Kelsey et al., 1999; Kelsey, Blascovich, et al., 2000; Kelsey et al., 2004). Comparable analyses were conducted for pre-stress baseline levels, using a sex x genotype x baseline period mixed factorial design.

A family-wise Type I error rate of 5% was preserved for these analyses by using a modified hierarchical Bonferroni adjustment procedure (Simes, 1986). Adjusting for significance tests

over five cardiovascular measures and three SNPs resulted in an initial adjusted α = .00333, with subsequent α-levels increasing by integer multiples of two (i.e., α = .00667) through fifteen (i.e., α = .05). Significant interaction effects were evaluated further in simple effects analyses using MANCOVA techniques. Effect sizes were estimated using partial eta squared ($\eta^2$).

The biological role of each of the α-AR polymorphisms was assessed through bioinformatics data mining using the UCSC Genome Browser (Fujita et al., 2011), SNPedia (by M. Cariaso, unpublished; http://www.snpedia.com/), PharmGKB (Thorn, Klein, & Altman, 2010), IHOP (Hoffman & Valencia, 2004), and Varietas (Paananen, Ciszek, & Wong, 2010) online databases and resources, and by searches of the biomedical literature in PubMed.

## Results

Table 1 presents means and standard deviations (SD) for age, BMI, overall cardiovascular baseline levels (i.e., mean levels over all baseline rest periods), overall cardiovascular reactivity scores (i.e., mean changes over all stress task periods), and overall stress appraisal scores for males and females. The cardiovascular baseline and reactivity measures were highly stable, as indicated by intraclass correlation coefficients (ICCs) based on an absolute agreement definition (Shrout & Fleiss, 1979); these ICCs ranged from .98 to .99 for resting levels over all baseline minutes and from .84 to .89 for reactivity scores over all stress task minutes. The 5-item stress appraisal scales also were reliable, with internal consistency coefficients (Cronbach's α) ranging from .65 to .80. All measures of overall cardiovascular reactivity differed significantly from baseline and overall stress appraisals exceeded the minimum level for both sexes, all $p < .0005$.

There were no significant sex differences in age or BMI. Preliminary tests for sex differences in overall cardiovascular baseline levels and overall cardiovascular reactivity were conducted using analysis of covariance with sex as the between-subjects factor and age, BMI, and stress task order as covariates. As expected, males had lower resting HR, longer resting PEP, and higher resting SBP as compared to females, all $p < .0005$. Significant covariate effects on baseline levels occurred for age (higher TPR, SBP, and DBP levels with increasing age, all $p < .03$), BMI (longer PEP and higher SBP and DBP levels with increasing BMI, all $p < .03$), and task order (higher SBP levels with MA first, $p < .01$). During stress, males showed larger overall increases in TPR, SBP, and DBP, and smaller overall increases in HR, as compared to females, all $p < .008$. Significant covariate effects on reactivity occurred for age (greater increases in HR and SBP, and smaller increases in TPR with increasing age, all $p < .05$) and task order (greater increases in HR and SBP with VG first, both $p < .03$). Similar analyses of stress appraisals showed higher overall ratings by females than males, $p < .02$. The only significant covariate effect for stress appraisals involved task order (higher stress appraisals with either MA or VG first, both $p < .008$).

### Genetic Association Analyses

The genotype counts and minor allele frequencies of the three α-AR polymorphisms are shown in Table 2. All of the polymorphisms were in Hardy-Weinberg equilibrium, $p = .29$ to .77. Given the genotype frequency distributions, association analyses for the insertion/deletion polymorphism in *ADRA2B* compared homozygotes for the major allele to carriers of the minor allele, whereas analyses for the Arg492Cys SNP in *ADRA1A* and the promoter SNP in *ADRA2A* compared all three genotypes. There were no significant associations between any of the α-AR polymorphisms and cardiovascular baseline levels, or between the $\alpha_{2B}$-AR polymorphism and cardiovascular reactivity to stress. However, there were significant associations between the $\alpha_{1A}$-AR and $\alpha_{2A}$-AR SNPs and specific measures of cardiovascular reactivity to stress (Table 3).[1]

The Arg492Cys SNP in the $\alpha_{1A}$-AR was associated with overall HR reactivity, although the association depended on sex, as indicated by a significant sex x genotype interaction effect, $F(2, 488) = 6.46$, MSE = 44.52, $p < .002$, $\eta^2 = .026$ (Table 3). Figure 1 depicts the means and standard errors (SEM) pertaining to this effect. Simple effects analyses revealed a significant effect of genotype on HR reactivity for males, $F(2, 488) = 5.43$, $p < .005$, $\eta^2 = .022$, but not for females, $F(2, 488) = 2.07$, $p > .12$, $\eta^2 = .008$. Subsequent pairwise comparisons indicated that the effect for males was limited to a significant difference in HR reactivity between those who were homozygous for the Arg492 allele and those who were heterozygous, $p < .002$. However, as indicated in the figure, the sex x genotype interaction effect was primarily attributable to a significant difference in HR reactivity between males and females who were homozygous for the Arg492 allele, $F(1, 488) = 31.17$, $p < .0005$, $\eta^2 = .060$; the overall increase in HR during stress was significantly greater for Arg492/Arg492 females than for males of the same genotype. There were no significant differences in HR reactivity between males and females who carried the other two genotypes (both $p > .20$). The Arg492Cys SNP also tended to be associated with TPR reactivity, as indicated by a marginally significant sex x genotype x stressor interaction effect, Wilks' $\Lambda = .971$, multivariate $F(6, 972) = 2.38$, $p < .028$, $\eta^2 = .014$ (Table 3). This effect arose primarily from a sex x genotype interaction for vasoconstrictive reactivity during CP, $F(2, 488) = 3.71$, $p < .025$, $\eta^2 = .015$; the increase in TPR during CP tended to be relatively diminished in Cys492/Cys492 males (Mean ± SEM = 14.5 ± 39.4 dyne-s/cm$^5$) but relatively enhanced in Cys492/Cys492 females (Mean ± SEM = 103.0 ± 34.6 dyne-s/cm$^5$).

The −1291C/G promoter SNP in the $\alpha_{2A}$-AR was associated with overall TPR reactivity, as indicated by a significant genotype main effect, $F(2, 490) = 6.30$, MSE = 34494.9, $p < .002$, $\eta^2 = .025$ (Table3). Vasoconstrictive reactivity during stress increased as a linear function of the number of copies of the G allele (Table 4), $F(1, 490) = 12.58$, $p < .001$, $\eta^2 = .025$. A marginally significant sex x genotype x stressor interaction effect suggested that this linear allelic association tended to be stronger during MA for males and during CP for females, Wilks' $\Lambda = .974$, multivariate $F(6, 976) = 2.14$, $p < .047$, $\eta^2 = .013$ (Table 3; data not shown). Finally, there was a marginally significant main effect of this promoter SNP on overall DBP reactivity, $F(2, 490) = 3.40$, MSE = 45.83, $p < .034$, $\eta^2 = .014$ (Table 3). Echoing the effect for TPR reactivity, DBP reactivity tended to increase as a linear function of the number of copies of the G allele (Table 4), $F(1, 490) = 6.73$, $p < .01$, $\eta^2 = .014$.

## Secondary Analyses

We conducted secondary analyses of potential confounding factors that might have contributed to the observed genetic associations with cardiovascular reactivity to stress.

**Hormonal Status—**We collected self-reported information from our female participants about the date of the last menses and the use of oral contraceptives (Slauterbeck et al., 2002; Wegienka & Baird, 2005), so we were able to evaluate whether menstrual cycle phase or birth control medication contributed to sex differences in genetic associations with cardiovascular reactivity. Information on the date of the last menses was missing for 32 females, while information on the use of oral contraceptives was missing for one female. Analyses comparing males to females in the follicular phase (cycle days 1 – 14, $n = 113$) versus luteal phase (cycle days 15 – 28, $n = 109$), or comparing males to females not using ($n = 189$) versus using oral contraceptives ($n = 64$), yielded results that were comparable to those of the principal association analyses. Exclusion of all females who were using oral contraceptives yielded a similar pattern of results for the menstrual cycle phase analyses,

[1]Multivariate analysis of variance for repeated measures (MANOVA) without adjustment for the covariates yielded similar results and identical decisions regarding statistical significance.

albeit with reduced statistical power. Thus, it seems unlikely that hormonal status contributed appreciably to the observed sex differences in genetic associations with cardiovascular reactivity.

**Stress appraisals—**There was a significant sex difference in post-task appraisals of stress, so we evaluated whether this difference contributed to sex differences in the genetic associations with cardiovascular reactivity. A series of mixed factorial MANCOVAs paralleling the principal association analyses showed no significant associations between any of the α-AR SNPs and stress appraisals. Furthermore, the results of association analyses for the α-AR SNPs and cardiovascular reactivity with stress appraisals as covariates were virtually identical to those of our principal association analyses. Thus, the sex difference in stress appraisals was unrelated to the associations between the α-AR SNPs and cardiovascular reactivity.

**Compliance with Pre-testing Instructions—**Most of the participants reported that they complied with the pre-testing instructions to refrain from eating (88.0%), drinking beverages containing caffeine (84.3%) or alcohol (99.6%), smoking (96.4%), or taking medications or drugs (95.4%) within the specified time before the testing session. Nevertheless, we evaluated whether noncompliance with pre-testing instructions influenced the observed genetic associations with cardiovascular reactivity. First, we used $\chi^2$ tests to evaluate associations between the α-AR SNPs and compliance with pre-testing instructions. These analyses revealed no significant genetic associations. Second, we evaluated the associations between the α-AR SNPs and cardiovascular reactivity after controlling for compliance with each pre-testing instruction. These analyses yielded results that were essentially the same as those in our principal association analyses, implying that noncompliance with pre-testing instructions was not responsible for the observed genetic associations with cardiovascular reactivity.

## Discussion

We evaluated the effects of common genetic variations in the $\alpha_{1A}$-AR, the $\alpha_{2A}$-AR, and the $\alpha_{2B}$-AR on cardiovascular reactivity to cold and psychological stress in healthy, normotensive Black adolescents and young adults, a population at increased risk for hypertension. Significant associations emerged between a coding SNP (rs1048101) in the $\alpha_{1A}$-AR and HR reactivity and between a promoter SNP (rs1800544) in the $\alpha_{2A}$-AR and TPR reactivity.

The association between the Arg492Cys SNP in the $\alpha_{1A}$-AR and HR reactivity differed between males and females, with a significant association appearing only in males. More importantly, the overall increase in HR during stress was larger for females who were homozygous for the Arg492 allele but smaller for males who were homozygous for that allele. Carriers of the variant Cys492 allele tended to show intermediate increases in HR that were equivalent for both sexes. There also was a marginal association between the Arg492Cys SNP and TPR reactivity that depended on sex and type of stressor. Specifically, the increase in TPR during CP tended to be enhanced in females but diminished in males who were homozygous for the Cys492 allele.

Early studies found no association between the Arg492Cys SNP and hypertension (Xie et al., 1999). More recently, one study reported that the Arg492 allele was associated with hypertension in a Han Chinese population (Gu et al., 2006), whereas other studies reported that the Cys492 allele was associated with DBP and hypertension in a Brazilian cohort (Freitas et al., 2008) and with the therapeutic BP response to irbesartan (an angiotensin II Type 1 receptor antagonist) in Chinese hypertensive patients (Jiang et al., 2005). It should be

noted that the participants in these studies were considerably older (age Mean ± SD = 44.8 ± 10.8 yr to 54.1 ± 7.2 yr) than those in our study. A recent meta-analysis based on four studies of the Arg492Cys SNP and hypertension "detected a significant protective effect" for the Cys492 allele (Kitsios & Zintzaras, 2010, p. 309).

The mechanism linking the Arg492Cys SNP to HR reactivity is unclear. Although this substitution occurs in a potential palmitoylation site which could alter subcellular localization, analysis in cells transfected with the Arg492 or Cys492 variant have shown similar levels of expression, binding affinity, receptor-mediated calcium signaling, and receptor desensitization (Shibata et al., 1996; Dorn, 2010). However, transfected systems may not accurately reflect what occurs with endogenous receptors. Our findings suggest that the Arg492Cys SNP may have some as yet undetected effect on endogenous receptor level or function, or may be in linkage disequilibrium with an as yet unidentified functionally important SNP in the $\alpha_{1A}$-AR gene. Alternatively, the Arg492Cys SNP may interact with a SNP in another gene for a protein involved in AR signaling or regulation of cardiovascular reactivity. Interestingly, with regard to the latter possibility, Rana et al. (2007) found that the Arg492Cys SNP interacted with a SNP in a gene involved in AR signaling (the Gsα subunit gene, GNAS1) to affect DBP levels in White women but not men.

It may seem somewhat surprising that the effect of the $\alpha_{1A}$-AR SNP was stronger for HR reactivity than for vascular or BP reactivity, but the $\alpha_{1A}$-AR plays a role in cardiac control in addition to its role in the regulation of peripheral vasoconstriction (Kirstein & Insel, 2004). A genome-wide association study of electrocardiographic and heart rate variability measures from middle-aged men and women in the Framingham Heart Study identified multiple SNPs in *ADRA1A* that were associated with resting measures of HR and the electrocardiographic P-R interval, and with ambulatory measures of HR variability (Newton-Cheh et al., 2007). Consistent with the Framingham study, two small studies of young European adults have reported associations between the Arg492Cys SNP and electrocardiographic measures. One study found an association between the Arg492Cys SNP and the electrocardiographic P-R interval during EPI infusion; P-R intervals were longer in those who were homozygous for the Cys492 allele than in those who carried the Arg492 allele (Snapir et al., 2003). Another study found an association between the Arg492Cys SNP and 24-hr ambulatory electrocardiographic measures of HR and HR variability; those who were homozygous for the Arg492 allele showed lower HR and higher HR variability than those who carried the Cys492 allele (Iacoviello et al., 2006). Although the nature of the relationship between genetic variation in the $\alpha_{1A}$-AR and electrocardiographic measures is far from clear, these findings together with our current findings suggest that the $\alpha_{1A}$-AR plays a role in electrocardiographic regulation.

Sex differences in genetic associations with cardiovascular reactivity are interesting in light of well-known sex differences in the prevalence and age of onset of hypertension and related cardiovascular diseases (Mendelsohn & Karas, 2005; Padmanabhan et al., 2010; Rana et al., 2007; Seda et al., 2008; Weiss et al., 2006). Sex-specific genetic effects may explain some of the past failures to replicate genetic associations with cardiovascular phenotypes, especially since prior studies have typically focused on only one sex or evaluated sex as a covariate rather than as a moderator of genetic associations (Kelsey, Alpert, Dahmer, Krushkal, & Quasney, 2010; Padmanabhan et al., 2010; Rana et al., 2007). Nevertheless, the reasons for the sex differences that we observed in the associations between the Arg492Cys SNP and cardiovascular reactivity are not readily apparent. Previous studies have found sex differences in HR reactivity similar to those that we observed in this sample (see Table 1), with significantly greater stress-related increases in HR occurring in females than in males (Allen, Stoney, Owens, & Matthews, 1993; Girdler, Turner, Sherwood, & Light, 1990; Saab et al., 1997; Sherwood, May, et al., 1995; Suarez, Saab, Llabre, Kuhn, & Zimmerman,

2004). Evidence indicates that this sex difference in HR reactivity is especially pronounced in Blacks (Saab et al., 1997; Suarez et al., 2004). Our findings suggest that sex differences in HR reactivity in the Black population may be associated with the Arg492Cys SNP (or a closely related, functional genetic variant) in the $\alpha_{1A}$-AR, and thus may be more likely to emerge in studies that include sufficient numbers of males and females who are homozygous for the common Arg492 variant. In contrast, the marginal association between the Arg492Cys SNP and vascular reactivity, which was limited primarily to CP stress, was largely attributable to a difference in TPR reactivity between males and females who were homozygous for the minor Cys492 allele. Given the small number of participants who carried the Cys492/Cys492 genotype, this trend should be interpreted with caution.

The extent to which variations over the menstrual cycle contribute to sex differences in adrenergic and cardiovascular function remains unclear, but differences in adrenergic and cardiovascular reactivity between men and women have been found regardless of female menstrual cycle phase or oral contraceptive use (Bowyer et al., 2001; Freedman et al., 1987; Kneale et al., 2003; Schmitt et al., 2010). Our secondary analyses suggest that neither of these factors contributed appreciably to the sex differences that we observed. Nevertheless, an independent replication of these findings with improved assessment of menstrual cycle phase, either through improved self-report assessment (e.g., information on both the first day of last menses and the cycle length; cf. Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009) or through objective measurement of hormonal status, is necessary before drawing firm conclusions. Our additional secondary analyses of post-task appraisals of stress indicated that the sex differences in genetic associations with cardiovascular reactivity were not attributable to the observed sex difference in stress appraisals.

As expected, the −1291C/G promoter SNP in the $\alpha_{2A}$-AR was associated with vascular reactivity to stress. The overall increase in TPR during stress was linearly related to the number of copies of the G allele. A similar but weaker linear association occurred for DBP reactivity. Thus, the primary impact of the −1291C/G promoter SNP was apparently vascular in nature. There was some indication that the association between the promoter SNP and TPR reactivity tended to be stronger during MA for males and during CP for females, thus underscoring the value of using multiple stressors to assess cardiovascular reactivity.

Although vascular postsynaptic $\alpha_{2A}$-ARs mediate peripheral vasoconstriction in certain vascular beds, the primary role of the $\alpha_{2A}$-AR involves presynaptic feedback inhibition of NE release from central and peripheral adrenergic neurons (Flordellis et al., 2004; Kirstein & Insel, 2004). This sympatho-inhibition by presynaptic $\alpha_{2A}$-ARs results in reductions in both BP and peripheral vasoconstriction, and is more effective at higher concentrations of NE, such as those that occur during stress (Hein et al., 1999). Thus, our novel finding of a positive linear relationship between vascular reactivity and the number of copies of the −1291G allele may reflect diminished presynaptic $\alpha_{2A}$-AR function with the G allele, resulting in decreased inhibition of NE release and increased peripheral vasoconstriction. Although the −1291G allele is the minor allele in Whites, it is the more common allele in Blacks (Flordellis et al., 2004; Kirstein & Insel, 2004). Therefore, the effect of the G allele at the −1291C/G SNP on vascular reactivity may be especially important for hypertensive risk in African Americans.

The −1291C/G promoter SNP in the $\alpha_{2A}$-AR has been associated with aspects of attention deficit/hyperactivity disorder (Polanczyk et al., 2007; Schmitz et al., 2006), body fat accumulation (Garenc et al., 2002), and cortisol response and serum glucose concentrations (Rosmond et al., 2002). Moreover, Rosmond et al. (2002) reported that DBP was marginally elevated in White men who carried the C allele, whereas Rana et al. (2007) reported that

DBP was elevated in White men who were homozygous for the C allele and in White women who were homozygous for the G allele. Although McCaffery et al. (2002) found no significant associations between the −1291C/G promoter SNP and BP or HR reactivity during psychological stress (math and Stroop tests), their study differed from ours in several important ways: they evaluated a sample of young White adults (−1291G allele frequency = 29%) that was smaller than our sample of Black youth (−1291G allele frequency = 66%); they did not include any form of cold exposure as a stressor; they did not evaluate a specific measure of vascular reactivity. The latter point is particularly important because our findings were considerably stronger for TPR reactivity than for DBP reactivity. Thus, the statistical power to detect an association between the −1291C/G SNP and cardiovascular reactivity was likely lower in their study than in the present study.

The Ins/Del301-303 polymorphism in the $\alpha_{2B}$-AR is located in the region which is important for G protein-coupled receptor kinase (GRK)-mediated phosphorylation and receptor desensitization; accordingly, the presence of the deletion variant is associated with reduced agonist-promoted phosphorylation and a complete loss of receptor desensitization (Flordellis et al., 2004; Kirstein & Insel, 2004; Rosskopf & Michel, 2008; Small et al., 2001; Small et al., 2003). Consequently, we expected to find an association between the deletion variant and increased vascular reactivity to stress. However, there were no significant associations between the Ins/Del301-303 polymorphism and cardiovascular reactivity in our group of young Blacks. Previous studies have reported associations of this polymorphism with BP (Ohlin et al., 2007), and cardiovascular reactivity during physical or pharmacological manipulations (Heinonen et al., 2002; Snapir et al., 2003; Talke et al., 2005; Ueno et al., 2006). However, those studies used cohorts that were predominantly or exclusively White and experimental methods that were markedly different from our approach. In addition, the power to detect an association between the Ins/Del301-303 polymorphism and cardiovascular function in our study might have been lower than it would be in a comparable study with Whites because the frequency of the deletion variant is much lower in Black Americans (reported to be approximately 12% to 21%, which is similar to the 15% found in our sample) than in Whites (approximately 31% to 37%; see Flordellis et al., 2004; Kirstein & Insel, 2004; Rosskopf & Michel, 2008; Small et al., 2003). Muszkat et al. (2005) suggested that other SNPs in *ADRA2B* cause *in vivo* vasoconstriction in response to an $\alpha_2$-AR agonist, but many of those SNPs represent rare alleles and their role at the population level remains to be investigated.

None of the α-AR gene polymorphisms was associated with resting cardiovascular baseline levels in our sample of young Blacks. Previous studies examining α-AR SNPs and cardiovascular baseline measures have yielded conflicting results (Fava et al., 2009; Freitas et al., 2008; Kirstein & Insel, 2004; Kurnik et al., 2006; Newton-Cheh et al., 2007; Rana et al., 2007; Rosmond et al., 2002; Suzuki et al., 2003; Zhang et al., 2005). Some of these studies failed to control adequately for Type I error, and nearly all included participants who were older and from different racial/ethnic groups than those in our study. It is possible that different genetic factors influence resting cardiovascular function over the lifespan, or that environmental factors affecting resting cardiovascular function vary among different racial/ethnic groups. Finally, it should be noted that the timing of the cardiovascular baseline assessments in our study precluded an adequate evaluation of genetic associations with cardiovascular recovery from stress.

## Conclusions

The genetic associations with cardiovascular reactivity to stress that emerged in our study highlight the impact of gene-environment interaction effects on cardiovascular function. The associations that we observed were relatively small, but such modest associations are typical for complex, heterogeneous, polygenic traits and disorders (Shih & O'Connor, 2008). It is

generally assumed that such traits and disorders involve additive and interactive effects of genetic contributions from multiple biological pathways. Indeed, we recently reported significant associations between common β-AR gene polymorphisms and cardiovascular reactivity to stress in this same cohort (Kelsey et al., 2010). Although our findings should be viewed with caution pending replication in independent cohorts, the NCI-NHGRI Working Group on Replication in Association Studies (Chanock et al., 2007) has stated that initial findings from carefully designed studies still provide valuable information. Future studies will be required to determine whether similar effects are observable in other samples of Blacks and in other racial/ethnic populations. Studies of these genetic associations in other racial/ethnic groups may require different sample sizes to ensure adequate statistical power, as there are substantial racial/ethnic differences in the published allele frequencies for α-AR gene polymorphisms (Belfer et al., 2005; Dorn, 2010; Flordellis et al., 2004; Kirstein & Insel, 2004; Rosskopf & Michel, 2008; Small et al., 2003). Further investigation of the associations between variations in the α-AR genes and cardiovascular reactivity will benefit from a detailed analysis of genetic variations in coding and noncoding regions of the α-AR genes, including both common and rare alleles.

Our findings indicate that specific α-AR gene polymorphisms are associated with cardiovascular reactivity to stress in Black adolescents and young adults. Taken together with our recent findings of associations between β-AR gene polymorphisms and cardiovascular reactivity in this sample (Kelsey et al., 2010), it appears that for young Blacks cardiac responses to stress are primarily associated with $\beta_1$-AR and $\alpha_{1A}$-AR gene polymorphisms, whereas vascular and BP responses are primarily associated with $\beta_2$-AR and $\alpha_{2A}$-AR gene polymorphisms. These associations are complex, however, as they differ between males and females in many cases and depend on specific types of stressors in some cases. Given that measures of cardiovascular reactivity qualify as intermediate phenotypes for hypertensive risk, the identification of genetic variations associated with increased cardiovascular reactivity to stress in young Blacks may provide valuable insights into the pathophysiological processes involved in the development of hypertension, and may lead to new and improved methods for early detection and intervention to reduce the risk of hypertension in this vulnerable population.

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## References

Allen MT, Stoney CM, Owens JF, Matthews KA. Hemodynamic adjustments to laboratory stress: The influence of gender and personality. Psychosomatic Medicine. 1993; 55:505–517. [PubMed: 8310111]

Alpert BS. Validation of CAS Model 9010 automated blood pressure monitor: Children/adult and neonatal studies. Blood Pressure Monitoring. 1996; 1:69–73. [PubMed: 10226205]

Anderson NB. Racial differences in stress-induced cardiovascular reactivity and hypertension: Current status and substantive issues. Psychological Bulletin. 1989; 105:89–105. [PubMed: 2648440]

Belfer I, Buzas B, Hipp H, Phillips G, Taubman J, Lorincz I, Goldman D. Haplotype-based analysis of alpha 2A, 2B, and 2C adrenergic receptor genes captures information on common functional loci at each gene. Journal of Human Genetics. 2005; 50:12–20. [PubMed: 15592690]

Berntson GG, Cacioppo JT, Binkley PF, Uchino BN, Quigley KS, Fieldstone A. Autonomic cardiac control III: Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. Psychophysiology. 1994; 31:599–608. [PubMed: 7846220]

Bouma EMC, Riese H, Ormel J, Verhulst FC, Oldehinkel AJ. Adolescents' cortisol responses to awakening and social stress; Effects of gender, menstrual phase and oral contraceptives. The TRAILS study. Psychoneuroendocrinology. 2009; 34:884–893. [PubMed: 19195792]

Bowyer L, Brown MA, Jones M. Vascular reactivity in men and women of reproductive age. American Journal of Obstetrics and Gynecology. 2001; 185:88–96. [PubMed: 11483910]

Chida Y, Steptoe A. Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: A meta-analysis of prospective evidence. Hypertension. 2010; 55:1026–1032. [PubMed: 20194301]

Cleves MA. Hardy-Weinberg equilibrium tests and allele frequency estimation. STATA Technical Bulletin. 1999; 48:34–37.

De Geus EJC, Kupper N, Boomsma DI, Snieder H. Bivariate genetic modeling of cardiovascular stress reactivity: Does stress uncover genetic variance? Psychosomatic Medicine. 2007; 69:356–364. [PubMed: 17510291]

Dorn GW II. Adrenergic signaling polymorphisms and their impact on cardiovascular disease. Physiological Reviews. 2010; 90:1013–1062. [PubMed: 20664078]

Fava C, Montagnana M, Guerriero M, Almgren P, von Wowern F, Minuz P, Melander O. Chromosome 2q12, the ADRA2B I/D polymorphism and metabolic syndrome. Journal of Hypertension. 2009; 27:1794–1803. [PubMed: 19593211]

Flamenbaum W, Weber MA, McMahon FG, Materson BJ, Carr AA, Poland M. Monotherapy with labetalol compared with propranolol: Differential effects by race. Journal of Clinical Hypertension. 1985; 1:56–69. [PubMed: 3915317]

Flordellis C, Manolis AS, Scheinin M, Paris H. Clinical and pharmacological significance of α2-adrenoceptor polymorphisms in cardiovascular diseases. International Journal of Cardiology. 2004; 97:367–372. [PubMed: 15561320]

Freedman RR, Sabharwal SC, Desai N. Sex difference in peripheral vascular adrenergic receptors. Circulation Research. 1987; 61:581–585. [PubMed: 3652401]

Freitas SR, Pereira AC, Floriano MS, Mill JG, Krieger JE. Association of alpha1a-adrenergic receptor polymorphism and blood pressure phenotypes in the Brazilian population. BMC Cardiovascular Disorders. 2008; 8:40–46. [PubMed: 19105822]

Fujita PA, Rhead B, Zweig AS, Hinrichs AS, Karolchik D, Cline MS, Kent WJ. The UCSC Genome Browser database: update 2011. Nucleic Acids Research. 2011; 39:D876–882. Database issue. [PubMed: 20959295]

Garenc C, Pérusse L, Chagnon YC, Rankinen T, Gagnon J, Borecki IB, Bouchard C. The alpha 2-adrenergic receptor gene and body fat content and distribution: the HERITAGE Family Study. Molecular Medicine. 2002; 8:88–94. [PubMed: 12080184]

Girdler SS, Turner JR, Sherwood A, Light KC. Gender differences in blood pressure control during a variety of behavioral stressors. Psychosomatic Medicine. 1990; 52:571–591. [PubMed: 2247562]

Gu D, Ge D, Snieder H, He J, Chen S, Huang J, Qiang B. Association of alpha1A adrenergic receptor gene variants on chromosome 8p21 with human stage 2 hypertension. Journal of Hypertension. 2006; 24:1049–1056. [PubMed: 16685204]

Haefner S, Baghai TC, Schule C, Eser D, Spraul M, Zill P, Bondy B. Impact of gene-gender effects of adrenergic polymorphisms on hypothalamic-pituitary-adrenal axis activity in depressed patients. Neuropsychobiology. 2008; 58:154–162. [PubMed: 19088492]

Hein L, Altman JD, Kobilka BK. Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. Nature. 1999; 402:181–184. [PubMed: 10647009]

Heinonen P, Jartti L, Järvisalo MJ, Pesonen U, Kaprio JA, Rönnemaa T, Scheinin M. Deletion polymorphism in the alpha2B-adrenergic receptor gene is associated with flow-mediated dilatation of the brachial artery. Clinical Sciences. 2002; 103:517–524.

Hoffmann R, Valencia A. A gene network for navigating the literature. Nature Genetics. 2004; 36:664. [PubMed: 15226743]

Iacoviello M, Forleo C, Sorrentino S, Romito R, De Tommasi E, Lucarelli K, Pitzalis MV. Alpha- and beta-adrenergic receptor polymorphisms in hypertensive and normotensive offspring. Journal of Cardiovascular Medicine. 2006; 7:316–321. [PubMed: 16645408]

Jennings JR, Kamarck T, Stewart C, Eddy M, Johnson P. Alternate cardiovascular baseline assessment techniques: Vanilla or resting baseline. Psychophysiology. 1992; 29:742–750. [PubMed: 1461961]

Jiang S, Mao G, Zhang S, Hong X, Tang G, Li Z, Wang X. Individual and joint association of alpha1A-adrenergic receptor Arg347Cys polymorphism and plasma irbesartan concentration with blood pressure therapeutic response in Chinese hypertensive subjects. Clinical Pharmacology & Therapeutics. 2005; 78:239–248. [PubMed: 16153395]

Julius S, Nesbitt S. Sympathetic overactivity in hypertension: A moving target. American Journal of Hypertension. 1996; 9:113S–120S. [PubMed: 8931844]

Kamarck TW. Recent developments in the study of cardiovascular reactivity: Contributions from psychometric theory and social psychology. Psychophysiology. 1992; 29:491–503. [PubMed: 1410179]

Kamarck TW, Lovallo WR. Cardiovascular reactivity to psychological challenge: Conceptual and measurement considerations. Psychosomatic Medicine. 2003; 65:9–21. [PubMed: 12554812]

Kanagy NL. Alpha(2)-adrenergic receptor signaling in hypertension. Clinical Science. 2005; 109:431–437. [PubMed: 16232127]

Kasprowicz AL, Manuck SB, Malkoff SB, Krantz DS. Individual differences in behaviorally evoked cardiovascular response: Temporal stability and hemodynamic patterning. Psychophysiology. 1990; 27:605–619. [PubMed: 2100346]

Kelsey, RM. Heart disease and reactivity. In: Anderson, NB., editor. Encyclopedia of Health and Behavior. Thousand Oaks, CA: SAGE Publications; 2004. p. 510-517.

Kelsey RM, Alpert BS, Dahmer MK, Krushkal J, Quasney MW. Beta-adrenergic receptor gene polymorphisms and cardiovascular reactivity to stress in Black adolescents and young adults. Psychophysiology. 2010; 47:863–873. [PubMed: 20374546]

Kelsey RM, Alpert BS, Patterson SM, Barnard M. Racial differences in hemodynamic responses to environmental thermal stress among adolescents. Circulation. 2000; 101:2284–2289. [PubMed: 10811596]

Kelsey RM, Blascovich J, Leitten CL, Schneider TR, Tomaka J, Wiens S. Cardiovascular reactivity and adaptation to recurrent psychological stress: The moderating effects of evaluative observation. Psychophysiology. 2000; 37:748–756. [PubMed: 11117455]

Kelsey RM, Blascovich J, Tomaka J, Leitten CL, Schneider TR, Wiens S. Cardiovascular reactivity and adaptation to recurrent psychological stress: Effects of prior task exposure. Psychophysiology. 1999; 36:818–831. [PubMed: 10554594]

Kelsey RM, Ornduff SR, Alpert BS. Reliability of cardiovascular reactivity to stress: Internal consistency. Psychophysiology. 2007; 44:216–225. [PubMed: 17343705]

Kelsey RM, Reiff S, Wiens S, Schneider TR, Mezzacappa ES, Guethlein W. The ensemble-averaged impedance cardiogram: An evaluation of scoring methods and interrater reliability. Psychophysiology. 1998; 35:337–340. [PubMed: 9564753]

Kelsey RM, Soderlund K, Arthur CM. Cardiovascular reactivity and adaptation to recurrent psychological stress: Replication and extension. Psychophysiology. 2004; 41:924–934. [PubMed: 15563345]

Kiive E, Kurrikoff T, Maestu J, Harro J. Effect of $\alpha_{2A}$-adrenoceptor C-1291G genotype and maltreatment on hyperactivity and inattention in adolescents. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2010; 34:219–224. [PubMed: 19922756]

Kirstein SL, Insel PA. Autonomic nervous system pharmacogenomics: A progress report. Pharmacological Reviews. 2004; 56:31–52. [PubMed: 15001662]

Kitsios GD, Zintzaras E. Synopsis and data synthesis of genetic association studies in hypertension for the adrenergic receptors family genes: the CUMAGAS-HYPERT database. American Journal of Hypertension. 2010; 23:305–313. [PubMed: 20044737]

Kneale BJ, Chowienczyk PJ, Brett SE, Coltart DJ, Ritter JM. Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. Journal of the American College of Cardiology. 2003; 36:1233–1238. [PubMed: 11028476]

Kurnik D, Friedman EA, Muszkat M, Sofowora GG, Xie HG, Dupont WD, Stein CM. Genetic variants in the $\alpha_{2c}$-adrenoceptor and G-protein contribute to ethnic differences in cardiovascular stress responses. Pharmacogenetics and Genomics. 2008; 18:743–750. [PubMed: 18698227]

Kurnik D, Muszkat M, Li C, Sofowora GG, Solus J, Xie HG, Stein CM. Variations in the alpha2A-adrenergic receptor gene and their functional effects. Clinical Pharmacology & Therapeutics. 2006; 79:173–185. [PubMed: 16513442]

Lario S, Calls J, Cases A, Oriola J, Torras A, Rivera F. Short report on DNA marker at candidate locus. Clinical Genetics. 1997; 51:129–130. [PubMed: 9112004]

Maestu J, Allik J, Merenaak L, Eensoo D, Parik J, Ceidebaum T, Harro J. Associations between an alpha 2a adrenergic receptor gene polymorphism and adolescent personality. American Journal of Medical Genetics Part B (Neuropsychiatric Genetics). 2008; 147B:418–423.

Matthews KA, Katholi CR, McCreath H, Whooley MA, Williams DR, Zhu S, Markovitz JH. Blood pressure reactivity to psychological stress predicts hypertension in the CARDIA study. Circulation. 2004; 110:74–78. [PubMed: 15210592]

McCaffery JM, Pogue-Geile MF, Ferrell RE, Petro N, Manuck SB. Variability within α- and β-adrenoreceptor genes as a predictor of cardiovascular function at rest and in response to mental challenge. Journal of Hypertension. 2002; 20:1105–1114. [PubMed: 12023679]

McGrath JJ, O'Brien WH. Pediatric impedance cardiography: Temporal stability and intertask consistency. Psychophysiology. 2001; 38:479–484. [PubMed: 11352136]

Mendelsohn ME, Karas RH. Molecular and cellular basis of cardiovascular gender differences. Science. 2005; 308:1583–1587. [PubMed: 15947175]

Mezzacappa ES, Kelsey RM, Katkin ES. The effects of epinephrine administration on impedance cardiographic measures of cardiovascular function. International Journal of Psychophysiology. 1999; 31:189–196. [PubMed: 10076773]

Michel MC, Brodde OE, Insel PA. Peripheral adrenergic receptors in hypertension. Hypertension. 1990; 16:107–120. [PubMed: 2166000]

Michelotti GA, Price DT, Schwinn DA. Alpha 1-adrenergic receptor regulation: basic science and clinical implications. Pharmacology & Therapeutics. 2000; 88:281–309. [PubMed: 11337028]

Murphy JK, Alpert BS, Walker SS. Ethnicity, pressor reactivity, and children's blood pressure: Five years of observations. Hypertension. 1992; 20:327–332. [PubMed: 1516952]

Murphy JK, Alpert BS, Walker SS. When to measure resting values in studies of children's cardiovascular reactivity. Journal of Behavioral Medicine. 1994; 17:501–510. [PubMed: 7877158]

Muszkat M, Kurnik D, Solus J, Sofowora GG, Xie HG, Jiang L, Stein CM. Variation in the $a_{2B}$-adrenergic receptor gene (*ADRA2B*) and its relationship to vascular response *in vivo*. Pharmacogenetics and Genomics. 2005; 15:407–414. [PubMed: 15900214]

Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, Collins FS. NCI-NHGRI Working Group on Replication in Association Studies. Replicating genotype-phenotype associations. Nature. 2007; 447:655–660. [PubMed: 17554299]

Newton-Cheh C, Guo CY, Wang TJ, O'Donnell CJ, Levy D, Larson MG. Genome-wide association study of electrocardiographic and heart rate variability traits: the Framingham Heart Study. BMC Medical Genetics. 2007; 8(Suppl 1):S7. [PubMed: 17903306]

Noll G, Wenzel RR, Schneider M, Oesch V, Binggeli C, Shaw S, Luscher TF. Increased activation of sympathetic nervous system and endothelin by mental stress in normotensive offspring of hypertensive parents. Circulation. 1996; 93:866–869. [PubMed: 8598076]

Obrist, PA. Cardiovascular Psychophysiology: A Perspective. New York: Plenum Press; 1981.

Ohlin B, Berglund G, Nilsson PM, Melander O. Job strain, decision latitude and alpha2B-adrenergic receptor polymorphism significantly interact, and associate with higher blood pressures in men. Journal of Hypertension. 2007; 25:1613–1619. [PubMed: 17620957]

Ong KL, Cheung BMY, Man YB, Lau CP, Lam KSL. Prevalence, awareness, treatment, and control of hypertension among United States adults 1999–2004. Hypertension. 2007; 49:69–75. [PubMed: 17159087]

Padmanabhan S, Menni C, Lee WK, Laing S, Brambilla P, Sega R, Dominiczak AF. The effects of sex and method of blood pressure measurement on genetic associations with blood pressure in the PAMELA study. Journal of Hypertension. 2010; 28:465–477. [PubMed: 20032787]

Paananen J, Ciszek R, Wong G. Varietas: a functional variation database portal. Database (Oxford), 2010. 2010:baq016.

Phinney J. The multigroup ethnic identity measure: A new scale for use with adolescents and young adults from diverse groups. Journal of Adolescent Research. 1992; 7:156–176.

Polanczyk G, Zeni C, Genro JP, Guimarães AP, Roman T, Hutz MH, Rohde LA. Association of the adrenergic alpha2A receptor gene with methylphenidate improvement of inattentive symptoms in children and adolescents with attention-deficit/hyperactivity disorder. Archives of General Psychiatry. 2007; 64:218–224. [PubMed: 17283289]

Rana BK, Insel PA, Payne SH, Abel K, Beutler E, Ziegler MG, O'Connor DT. Population-based sample reveals gene-gender interactions in blood pressure in White Americans. American Journal of Hypertension. 2007; 49:96–106.

Rosmond R, Bouchard C, Björntorp P. A C-1291G polymorphism in the alpha2A-adrenergic receptor gene (ADRA2A) promoter is associated with cortisol escape from dexamethasone and elevated glucose levels. Journal of Internal Medicine. 2002; 251:252–257. [PubMed: 11886485]

Rosskopf D, Michel MC. Pharmacogenomics of G protein-coupled receptor ligands in cardiovascular medicine. Pharmacological Reviews. 2008; 60:513–535. [PubMed: 19074621]

Saab PG, Llabre MM, Hurwitz BE, Frame CA, Reineke LJ, Fins AI, Schneiderman N. Myocardial and peripheral vascular responses to behavioral challenges and their stability in black and white Americans. Psychophysiology. 1992; 29:384–397. [PubMed: 1410171]

Saab PG, Llabre MM, Schneiderman N, Hurwitz BE, McDonald PG, Evans J, Klein B. Influence of ethnicity and gender on cardiovascular responses to active coping and inhibitory-passive coping challenges. Psychosomatic Medicine. 1997; 59:434–446. [PubMed: 9251164]

Schmitt JAM, Joyner MJ, Charkoudian N, Wallin BG, Hart EC. Sex differences in alpha-adrenergic support of blood pressure. Clinical Autonomic Research. 2010; 20:271–275. [PubMed: 20221842]

Schmitz M, Denardin D, Silva TL, Pianca T, Roman T, Hutz MH, Rohde LA. Association between alpha-2a-adrenergic receptor gene and ADHD inattentive type. Biological Psychiatry. 2006; 60:1028–1033. [PubMed: 16806103]

Seda O, Tremblay J, Gaudet D, Brunelle PL, Gurau A, Merlo E, Hamet P. Systematic, genome-wide, sex-specific linkage of cardiovascular traits in French Canadians. Hypertension. 2008; 51:1156–1162. [PubMed: 18259002]

Sherwood A, Allen MT, Fahrenberg J, Kelsey RM, Lovallo WR, van Doornen LJ. Committee report: Methodological guidelines for impedance cardiography. Psychophysiology. 1990; 27:1–23. [PubMed: 2187214]

Sherwood A, Dolan CA, Light KC. Hemodynamics of blood pressure responses during active and passive coping. Psychophysiology. 1990; 27:656–668. [PubMed: 2100351]

Sherwood A, Hinderliter AL. Responsiveness to α- and β-adrenergic receptor agonists: Effects of race in borderline hypertensive compared to normotensive men. American Journal of Hypertension. 1993; 6:630–635. [PubMed: 8398005]

Sherwood A, Hinderliter AL, Light KC. Physiological determinants of hyperreactivity to stress in borderline hypertension. Hypertension. 1995; 25:384–390. [PubMed: 7875764]

Sherwood A, May CW, Siegel WC, Blumenthal JA. Ethnic differences in hemodynamic responses to stress in hypertensive men and women. American Journal of Hypertension. 1995; 8:552–557. [PubMed: 7662238]

Shibata K, Hirawasa A, Moriyama N, Kawabe K, Ogawa S, Tsujimoto G. $\alpha_{1A}$-Adrenoreceptor polymorphism: pharmacological characterization and association with benign prostatic hypertrophy. British Journal of Pharmacology. 1996; 118:1402–1408.

Shih PB, O'Connor DT. Hereditary determinants of human hypertension: strategies in the setting of genetic complexity. Hypertension. 2008; 51:1456–1464. [PubMed: 18413494]

Shrout PE, Fleiss JL. Intraclass correlations: Uses in assessing rater reliability. Psychological Bulletin. 1979; 86:420–428. [PubMed: 18839484]

Simes RJ. An improved Bonferroni procedure for multiple tests of significance. Biometrika. 1986; 73:751–754.

Slauterbeck JR, Fuzie SF, Smith MP, Clark RJ, Xu KT, Starch DW, Hardy DM. The menstrual cycle, sex hormones, and anterior cruciate ligament injury. Journal of Athletic Training. 2002; 37:275–280. [PubMed: 12937585]

Small KM, Brown KM, Forbes SL, Liggett SB. Polymorphic deletion of three intracellular acidic residues of the alpha 2B-adrenergic receptor decreases G protein-coupled receptor kinase-mediated phosphorylation and desensitization. Journal of Biological Chemistry. 2001; 276:4917–4922. [PubMed: 11056163]

Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. Annual Review of Pharmacology and Toxicology. 2003; 43:381–411.

Snapir A, Koskenvuo J, Toikka J, Orho-Melander M, Hinkkas S, Saraste M, Scheinin M. Effects of common polymorphisms in the $\alpha_{1A}$-, $\alpha_{2B}$-, $\beta_1$- and $\beta_2$-adrenoreceptors on haemodynamic responses to adrenaline. Clinical Sciences. 2003; 104:509–520.

Snieder H, Harshfield GA, Barbeau P, Pollock DM, Pollock JS, Treiber FA. Dissecting the genetic architecture of the cardiovascular and renal stress response. Biological Psychology. 2002; 61:73–95. [PubMed: 12385670]

Sõber S, Org E, Kepp K, Juhanson P, Eyheramendy S, Gieger C, Laan M. Targeting 160 candidate genes for blood pressure regulation with a genome-wide genotyping array. PLoS One. 2009; 4:e6034. [PubMed: 19562039]

Stein CM, Lang CC, Singh I, He HB, Wood AJJ. Increased vascular adrenergic vasoconstriction and decreased vasodilation in blacks: Additive mechanisms leading to enhanced vascular reactivity. Hypertension. 2000; 36:945–951. [PubMed: 11116105]

Suarez EC, Saab PG, Llabre M, Kuhn CM, Zimmerman E. Ethnicity, gender, and age effects on adrenoceptors and physiological responses to emotional stress. Psychophysiology. 2004; 40:450–460. [PubMed: 15102131]

Suzuki N, Matsunaga T, Nagasumi K, Yamamura T, Shihara N, Moritani T, Yasuda K. Alpha(2B)-adrenergic receptor deletion polymorphism associates with autonomic nervous system activity in young healthy Japanese. Journal of Clinical Endocrinology & Metabolism. 2003; 88:1184–1187. [PubMed: 12629104]

Swain A, Suls J. Reproducibility of blood pressure and heart rate reactivity: A meta-analysis. Psychophysiology. 1996; 33:162–174. [PubMed: 8851244]

Taherzadeh Z, Brewster LM, van Montfrans GA, VanBavel E. Function and structure of resistance vessels in Black and White people. The Journal of Clinical Hypertension. 2010; 12:431–438. [PubMed: 20591088]

Talke P, Stapelfeldt C, Lobo E, Brown R, Scheinin M, Snapir A. Alpha-2B adrenoceptor polymorphism and peripheral vasoconstriction. Pharmacogenetics and Genomics. 2005; 15:357–363. [PubMed: 15864138]

Thorn CF, Klein TE, Altman RB. Pharmacogenomics and bioinformatics: PharmGKB. Pharmacogenomics. 2010; 11:501–505. [PubMed: 20350130]

Treiber FA, Kamarck T, Schneiderman N, Sheffield D, Kapuku G, Taylor T. Cardiovascular reactivity and development of preclinical and clinical disease states. Psychosomatic Medicine. 2003; 65:46–62. [PubMed: 12554815]

Treiber FA, Musante L, Braden D, Arensman F, Strong WB, Levy M, Leverett S. Racial differences in hemodynamic responses to the cold face stimulus in children and adults. Psychosomatic Medicine. 1990; 52:286–296. [PubMed: 2367620]

Ueno LM, Frazzatto ES, Batalha LT, Trombetta IC, do Socorro Brasileiro M, Irigoyen C, Negrão CE. Alpha2B-adrenergic receptor deletion polymorphism and cardiac autonomic nervous system responses to exercise in obese women. International Journal of Obesity. 2006; 30:214–220. [PubMed: 16247508]

Vargas HM, Gorman AJ. Vascular alpha-1 adrenergic receptor subtypes in the regulation of arterial pressure. Life Sciences. 1995; 57:2291–2308. [PubMed: 7491088]

Vasudevan R, Ismail P, Stanslas J, Shamsudin N, Ali AB. Association of insertion/deletion polymorphism of alpha-adrenoceptor gene in essential hypertension with or without type 2 diabetes mellitus in Malaysian subjects. International Journal of Biological Sciences. 2008; 4:362–367. [PubMed: 18953403]

Veelken R, Schmieder RE. Overview of alpha 1-adrenoceptor antagonism and recent advances in hypertensive therapy. American Journal of Hypertension. 1996; 9:139S–149S. [PubMed: 8931846]

Wang Y, O'Connell JR, McArdle PF, Wade JB, Dorff SE, Shah SJ, Chang YPC. Whole-genome association study identifies STK39 as a hypertension susceptibility gene. Proceedings of the National Academy of Sciences. 2009; 106:226–231.

Wegienka G, Baird DD. A comparison of recalled date of last menstrual period with prospectively recorded dates. Journal of Women's Health. 2005; 14:248–252.

Weiss LA, Pan L, Abney M, Ober C. The sex-specific genetic architecture of quantitative traits in humans. Nature Genetics. 2006; 38:218–222. [PubMed: 16429159]

Wu T, Snieder H, de Geus E. Genetic influences on cardiovascular stress reactivity. Neuroscience and Biobehavioral Reviews. 2010; 35:58–68. [PubMed: 19963006]

Xie HG, Kim RB, Stein CM, Gainer JV, Brown NJ, Wood AJ. Alpha1A-adrenergic receptor polymorphism: association with ethnicity but not essential hypertension. Pharmacogenetics. 1999; 9:651–656. [PubMed: 10591546]

Zhang H, Li X, Huang J, Li Y, Thijs L, Wang Z, Wang JG. Cardiovascular and metabolic phenotypes in relation to the ADRA2B insertion/deletion polymorphism in a Chinese population. Journal of Hypertension. 2005; 23:2201–2207. [PubMed: 16269962]

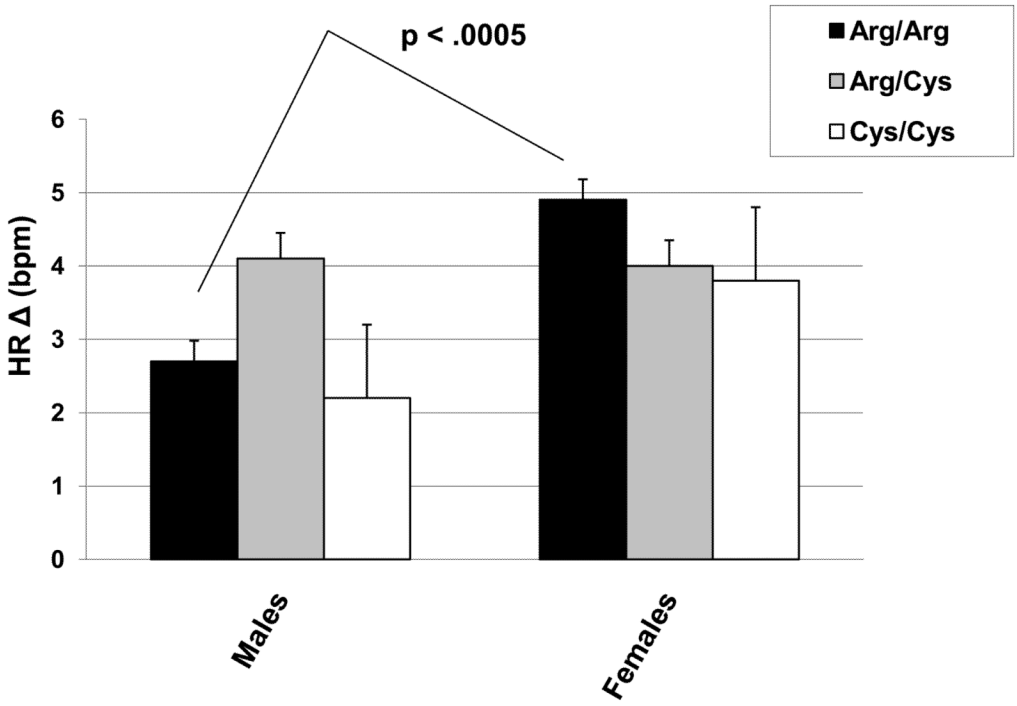


**Figure 1.**
Arg492Cys polymorphism in the $\alpha_{1A}$-adrenergic receptor and overall heart rate reactivity (HRΔ, mean ± SEM) in healthy, young Black males and females (*N* = 498).

## Table 1

Sample Characteristics

| | Male (*n* = 246) | Female (*n* = 254) |
|---|---|---|
| Age (yr) | 17.7 ± 1.9 | 17.9 ± 1.9 |
| BMI (kg/m$^2$) | 25.4 ± 5.5 | 26.4 ± 6.5 |
| Overall Baseline Levels | | |
| HR (bpm)* | 62.6 ± 8.8 | 70.8 ± 8.6 |
| PEP (ms)* | 73.2 ± 9.4 | 69.3 ± 9.3 |
| TPR (dyne-s/cm$^5$) | 1023.7 ± 233.6 | 1025.4 ± 260.3 |
| SBP (mmHg)* | 116.0 ± 9.0 | 109.0 ± 7.5 |
| DBP (mmHg) | 68.5 ± 7.4 | 68.4 ± 6.6 |
| Overall Reactivity (Δ) | | |
| HR Δ (bpm)† | 3.2 ± 3.2 | 4.5 ± 3.6 |
| PEP Δ (ms) | −3.0 ± 3.4 | −2.6 ± 2.9 |
| TPR Δ (dyne-s/cm$^5$)† | 118.6 ± 93.1 | 87.7 ± 94.5 |
| SBP Δ (mmHg)† | 7.0 ± 4.1 | 6.2 ± 3.3 |
| DBP Δ (mmHg)† | 6.5 ± 3.6 | 5.2 ± 3.1 |
| Stress Appraisals# | 15.4 ± 4.0 | 16.2 ± 4.1 |

**Note**. Data are shown as mean ± SD. BMI, Body Mass Index; HR, Heart Rate; PEP, Preejection Period; TPR, Total Peripheral Resistance; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure.

* Sex difference, $p < .0005$.

† Sex difference, $p < .008$.

# Sex difference, $p < 0.02$.

## Table 2

Genotype Counts and Minor Allele Frequencies of α-Adrenergic Receptor (α-AR) Gene Polymorphisms in Black Youth

| **Gene Polymorphism** | **Genotype Count** | | | **Minor Allele Frequency (%)** |
|---|---|---|---|---|
| **$\alpha_{1A}$-AR Arg492Cys (rs1048101)** | **Arg492Arg** | **Arg492Cys** | **Cys492Cys** | **Cys** |
| *N* = 498 | 294 | 181 | 23 | 23 |
| Male (*n* = 244) | 148 | 86 | 10 | |
| Female (*n* = 254) | 146 | 95 | 13 | |
| **$\alpha_{2A}$-AR −1291C/G (rs1800544)** | **C/C** | **C/G** | **G/G** | **C** |
| *N* = 500 | 57 | 230 | 213 | 34 |
| Male (*n* = 246) | 32 | 108 | 106 | |
| Female (*n* = 254) | 25 | 122 | 107 | |
| **$\alpha_{2B}$-AR Ins/Del301-303 (rs29000568)** | **Ins/Ins** | **Ins/Del** | **Del/Del** | **Del** |
| *N* = 495 | 363 | 119 | 13 | 15 |
| Male (*n* = 243) | 179 | 55 | 9 | |
| Female (*n* = 252) | 184 | 64 | 4 | |

**Table 3**

Summary of F-tests and Effect Sizes ($\eta^2$) for Genetic Associations with Cardiovascular Reactivity

| | | *ADRA1A* Arg492Cys | | | | *ADRA2A* −1291C/G | | | | *ADRA2B* Ins/Del 301-303 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Measure** | **Source** | ***F*** | **df** | ***p*** | **$\eta^2$** | ***F*** | **df** | ***p*** | **$\eta^2$** | ***F*** | **df** | ***p*** | **$\eta^2$** |
| **Heart Rate** | **Genotype** | 1.05 | 2/488 | .35 | .004 | 0.25 | 2/490 | .78 | .001 | 1.62 | 1/487 | .20 | .003 |
| | **Sex x Genotype** | **6.46** | 2/488 | **.002** | **.026** | 1.96 | 2/490 | .14 | .008 | 0.00 | 1/487 | .99 | .000 |
| | **Genotype x Stressor** | 1.25 | 6/972 | .28 | .008 | 0.25 | 6/976 | .96 | .002 | 2.74 | 3/485 | .043 | .017 |
| | **Sex x Genotype x Stressor** | 0.84 | 6/972 | .54 | .005 | 0.78 | 6/976 | .59 | .005 | 2.24 | 3/485 | .08 | .014 |
| **Preejection Period** | **Genotype** | 0.26 | 2/488 | .77 | .001 | 0.02 | 2/490 | .98 | .000 | 0.77 | 1/487 | .38 | .002 |
| | **Sex x Genotype** | 2.39 | 2/488 | .095 | .010 | 0.43 | 2/490 | .65 | .002 | 1.76 | 1/487 | .19 | .004 |
| | **Genotype x Stressor** | 0.85 | 6/972 | .53 | .005 | 1.57 | 6/976 | .15 | .010 | 0.99 | 3/485 | .40 | .006 |
| | **Sex x Genotype x Stressor** | 0.79 | 6/972 | .58 | .005 | 1.36 | 6/976 | .23 | .008 | 0.19 | 3/485 | .90 | .001 |
| **Total Peripheral Resistance** | **Genotype** | 0.90 | 2/488 | .41 | .004 | **6.30** | **2/490** | **.002** | **.025** | 0.08 | 1/487 | .78 | .000 |
| | **Sex x Genotype** | 0.08 | 2/488 | .92 | .000 | 0.22 | 2/490 | .81 | .001 | 0.01 | 1/487 | .92 | .000 |
| | **Genotype x Stressor** | 0.69 | 6/972 | .66 | .004 | 0.10 | 6/976 | 1.00 | .001 | 1.39 | 3/485 | .25 | .009 |
| | **Sex x Genotype x Stressor** | 2.38 | 6/972 | .028 | .014 | 2.14 | 6/976 | .047 | .013 | 1.38 | 3/485 | .25 | .008 |
| **Systolic Blood Pressure** | **Genotype** | 0.64 | 2/488 | .53 | .003 | 0.56 | 2/490 | .57 | .002 | 2.52 | 1/487 | .11 | .005 |
| | **Sex x Genotype** | 2.47 | 2/488 | .09 | .010 | 0.88 | 2/490 | .41 | .004 | 5.66 | 1/487 | .018 | .011 |
| | **Genotype x Stressor** | 1.34 | 6/972 | .24 | .008 | 0.23 | 6/976 | .97 | .001 | 0.29 | 3/485 | .83 | .002 |
| | **Sex x Genotype x Stressor** | 0.39 | 6/972 | .89 | .002 | 0.52 | 6/976 | .79 | .003 | 1.32 | 3/485 | .27 | .008 |
| **Diastolic Blood Pressure** | **Genotype** | 0.23 | 2/488 | .79 | .001 | 3.40 | 2/490 | .034 | .014 | 0.00 | 1/487 | .98 | .000 |
| | **Sex x Genotype** | 0.30 | 2/488 | .74 | .001 | 0.04 | 2/490 | .96 | .000 | 0.76 | 1/487 | .38 | .002 |
| | **Genotype x Stressor** | 0.33 | 6/972 | .92 | .002 | 0.61 | 6/976 | .72 | .004 | 0.42 | 3/485 | .74 | .003 |
| | **Sex x Genotype x Stressor** | 0.73 | 6/972 | .63 | .004 | 1.77 | 6/976 | .10 | .011 | 0.35 | 3/485 | .79 | .002 |

**Note**. Bold typeface indicates a significant effect.

**Table 4**

Means and Standard Errors (mean ± SEM) for Total Peripheral Resistance (TPRΔ) and Diastolic Blood Pressure (DBPΔ) Reactivity as a Function of the −1291C/G Promoter Polymorphism in the $\alpha_{2A}$-Adrenergic Receptor for healthy Black adolescents and young adults (N = 500)

| Reactivity Measure | −1291C/G Genotype | | | *P* (linear) |
|---|---|---|---|---|
| | C/C | C/G | G/G | |
| **TPR Δ (dyne-s/cm$^5$)** | 74.1 ± 12.4 | 96.7 ± 6.2 | 118.3 ± 6.4 | $p < .001$ |
| **DBP Δ (mmHg)** | 5.0 ± 0.5 | 5.7 ± 0.2 | 6.2 ± 0.2 | $p < .01$ |