HHS Public Access

Author manuscript

Pharmacogenomics J. Author manuscript; available in PMC 2017 July 24.

Published in final edited form as:

Pharmacogenomics J. 2017 July ; 17(4): 366–371. doi:10.1038/tpj.2016.29.

GENETIC VARIATION IN THE ALPHA$_{1B}$ - ADRENERGIC RECEPTOR AND VASCULAR RESPONSE

Abiodun Adefurin[1,*], **Laxmi V. Ghimire**[1,*], **Utkarsh Kohli**[1], **Mordechai Muszkat**[1], **Gbenga G. Sofowora**[1], **Chun Li**[2], **Rebecca T. Levinson**[3], **Sachin Y. Paranjape**[1], **C. Michael Stein**[1], and **Daniel Kurnik**[1,4]

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

[2]Department of Biostatistics, Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

[3]Vanderbilt Genetics Institute, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

[4]Clinical Pharmacology Unit, Rambam Medical Center, Haifa, Israel; Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel

Abstract

α_{1B}- adrenergic receptors contribute to vasoconstriction in humans. We tested the hypothesis that variation in the *ADRA1B* gene contributes to interindividual variability and ethnic differences in adrenergic vasoconstriction. We measured dorsal hand vein responses to increasing doses of phenylephrine in 64 Caucasians and 41 African-Americans and genotyped 34 *ADRA1B* variants. We validated findings in another model of catecholamine-induced vasoconstriction, the increase in mean arterial pressure (ΔMAP) during a cold pressor test (CPT). One *ADRA1B* variant, rs10070745, present in 14 African-American heterozygotes but not in Caucasians, was associated with a lower phenylephrine ED_{50} (geometric mean [95% CI], 144 [69–299] ng/ml) compared to 27 African-American non-carriers (208 [130–334] ng/ml; P=0.015) and contributed to the ethnic differences in ED_{50}. The same variant was also associated with a greater ΔMAP during CPT (P=0.008). In conclusion, *ADRA1B* rs10070745 was significantly associated with vasoconstrictor responses after adrenergic stimulation and contributed to the ethnic difference in phenylephrine sensitivity.

Address for correspondence: Daniel Kurnik, Division of Clinical Pharmacology, 542 RRB, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. Phone: +1-615-936-3420, Fax: +1-615-936-2746. daniel.kurnik@vanderbilt.edu.

*Both authors contributed equally to this work

Conflict of Interest: The authors declare no conflict of interest.

Introduction

Alpha$_{1A}$ adrenergic receptors (α_{1A}-ARs) are the prime mediators of vasoconstriction induced by activation of the sympathetic nervous system and thus play an important role in blood pressure regulation. Among the three α_1-AR subtypes (α_{1A}-, α_{1B}-, and α_{1D}-AR), the α_{1A}-AR appears to be the principal mediator of physiological vasoconstriction,[1,2] while the α_{1B}- and α_{1D}-AR also contribute, as demonstrated in various human and animal models.[3,4]

The association between genetic variants in the α_{1A}-AR gene (*ADRA1A*) with outcomes such as vasoconstriction and blood pressure has been studied in a number of experimental models. In a human hand vein model, variation in *ADRA1A* did not explain the large interindividual variability or the ethnic differences in venoconstrictor responses to the infusion of the selective α_1-AR agonist, phenylephrine.[5–7] Also, *ADRA1A* variation was not associated with the rise in blood pressure after sympathetic stimulation in the setting of experimental stress.[8–10]

However, a recent study in 1614 Nigerians examining common variants in candidate genes of 28 different pathways implicated in the regulation of blood pressure found the strongest association between blood pressure and hypertension with variants in the alpha$_1$-adrenergic pathway.[11] Among the α_1- AR- pathway genes, variants in the α_{1B}-AR were most significantly associated with blood pressure and hypertension, while variants in the α_{1A}-AR were not, suggesting that genetic variants in the α_{1B}-AR, rather than in the α_{1A}-AR subtype, may contribute to interindividual variability in regulation of vascular tone and blood pressure control.

α_{1B}-ARs contribute to vasoconstriction in animal studies, possibly by mediating smooth muscle contraction directly and also by regulating the expression and function of other adrenergic receptors.[3,12–14] Little is known about the effects of *ADRA1B* variation on vascular responses in humans. An early study found no association between phenylephrine-mediated vasoconstriction and four infrequent *ADRA1B* coding variants in 45 subjects with and without hypertension.[15] Subsequently, the genetic architecture of *ADRA1B* was explored systematically in various ethnic groups, showing great interindividual and interethnic variability.[16]

We therefore set out to define the association between *ADRA1B* genotypes and vascular sensitivity to vasoconstriction induced by an α_1-agonist using two experimental models: local venous responses to phenylephrine in the dorsal hand vein, and the increase in blood pressure during the cold pressor test (CPT), reflecting the systemic cardiovascular response to acute sympathetic activation. Previous studies showed ethnic differences in these responses, with African-Americans having a greater blood pressure increase after cold pressor stress and greater sensitivity to phenylephrine-induced vasoconstriction compared to Caucasians, suggesting that genetic factors may contribute to these responses.[5,17,18] Thus, we tested the hypothesis that variation in *ADRA1B* contributes to interindividual and ethnic differences in agonist-mediated venoconstriction and in the stress-induced increase in blood pressure following a CPT.

METHODS

Subjects

We studied dorsal hand vein responses in 105 healthy normotensive Caucasians and African-Americans aged 18–45 years. Details of the study procedures and analyses of other genes were published previously.[5,7] 57 of the 105 subjects also participated in a second study that included a CPT. Pregnant females were excluded, and subjects took no medications for at least 2 weeks, abstained from alcohol and caffeine for at least 5 days, and received a diet containing 150 mmol/day of sodium, 70 mmol/day of potassium, and 600 mmol/day of calcium for at least 4 days prior to each study day. The Institutional Review Board of Vanderbilt University Medical Center approved the study protocols, and all subjects provided written informed consent.

Venous response to phenylephrine

Venous responses were measured in a dorsal hand vein with a linear variable differential transformer (LVDT) as previously described.[5] In summary, a 24-gauge intravenous cannula was inserted into a suitable right dorsal hand vein and kept patent with saline solution infused at a flow rate of 0.4 mL/min. A LVDT (MHR 100; Shaevitz Engineering, Pennsaken, NJ) was mounted on the dorsum of the subject's hand. A second intravenous cannula was inserted for blood sampling into the antecubital vein of the contralateral arm. After 30 minutes of saline infusion, a blood sample was taken for the determination of baseline plasma catecholamines and for genotyping. We determined the baseline vein diameter while a sphygmomanometer cuff around the upper arm was inflated to 50 mm Hg to induce venous filling. After 3 stable baseline measurements, we assessed vein constriction in response to increasing doses of phenylephrine, an α_1-AR agonist. Phenylephrine (Elkins-Sinn, Cherry Hill, NJ) was infused through the cannula with a syringe infusion pump (Harvard Apparatus, Holliston, MA) at increasing dose rates (range, 12–12,000 ng/min). The infusion at each dose rate lasted 7 minutes, and the vein diameter was measured during the last two minutes of the infusion. The total flow rate through the vein was kept constant at 0.4 mL/min throughout the various phenylephrine dilutions. Heart rate and blood pressure were continuously monitored with a bedside cardiac monitor (Dinamap MPS; Johnson and Johnson Medical, Tampa, FL).

Analysis of hand vein response to phenylephrine

Venoconstriction was expressed as the percentage reduction in vein diameter from average baseline measurements, plotted against increasing doses of phenylephrine in individual semi-logarithm dose–response graphs and analyzed using a sigmoid dose–response model with variable slope (GraphPad Prism 4.03, GraphPad, La Jolla, CA). We determined the phenylephrine dose that produced 50% of maximal constriction (ED_{50}, representing sensitivity to the drug) and also calculated the maximal venoconstriction (E_{max}, representing maximum response) for each subject. Analyses were performed by a single investigator unaware of the subject's genotype.

Cold Pressor Test

Cold pressor tests were performed as previously described.[18,19] All preparations for the CPT were performed only after the resting measurements had been obtained and after 30 minutes supine rest in order to minimize confounding through anticipation. With the subject in a supine position, the left foot was fully immersed up to the ankle for 2 minutes in a tub filled with a slurry of ice and water (4°C). Two readings of blood pressure and heart rate were taken with the semi-automated device (Dinamap MPS; Johnson and Johnson Medical, Tampa, FL), starting at approximately 15 and 45 seconds after foot immersion. At 1 minute, a blood sample (10 mL) was taken for determination of plasma catecholamine concentrations.

Determination of plasma catecholamine concentrations

Blood was collected into cooled heparinized tubes that were placed on ice until centrifuged at 4°C for 10 minutes at 3000 rpm. Plasma was separated and stored at −20°C in previously cooled tubes containing 40μL of reduced glutathione (6%) until assayed. Norepinephrine and epinephrine concentrations were measured by high-performance liquid chromatography using electrochemical detection with dihydroxybenzylamine as internal standard.[20]

Genotyping

We genotyped 34 *ADRA1B* SNPs listed in Supplementary Table S1. We selected 24 tagSNPs for *ADRA1B* (chromosome 5, position 159269 – 159343kb) from the Hapmap project using Haploview 4.2 software,[21] using data based on Utah residents with Northern and Western ancestry (CEU) and on subjects of African ancestry in southwest USA (ASW). We excluded individuals from the Hapmap project with more than 5% missing genotypes and SNPs with minor allele frequencies (MAF) less than 5%. Two-marker haplotypes were used to tag SNPs in strong linkage disequilibrium (LD), defined as r^2 >0.8. Furthermore, we included five previously published tag SNPs that were not captured in the Haploview tagging,[16] and five additional SNPs previously associated with clinical outcomes (Supplementary Table S1). Genotyping was performed using the Sequenom platform (MassArray, San Diego, CA). For quality control, we included negative and positive controls with each genotyping run. Quality-control procedures included examination of marker and sample genotyping efficiency, allele-frequency calculations, and tests of Hardy-Weinberg equilibrium (HWE).

Statistical Analysis

The cohort was a convenience sample consisting of participants in previous studies, and the sample size was not based on *a priori* calculation.[5,6,22] The primary outcome for the hand vein study was drug sensitivity (expressed as ED_{50}), and drug efficacy (E_{max}) was the secondary outcome. ED_{50} values were not normally distributed and were therefore log transformed and expressed as geometric means with 95% confidence intervals (CIs). The primary outcome for the cold pressor test was the change in mean arterial blood pressure (ΔMAP).

We compared the outcomes among genotypes in a single marker analysis first in each ethnic group separately and then in the combined cohort. We then adjusted for potential

confounders that were associated with the outcomes in our previous studies using the same endpoints.[7] For the hand vein study, we adjusted for sex, BMI, resting norepinephrine concentrations and, for analyses of the combined cohort, ethnicity, using a multiple linear regression model. The secondary outcome, phenylephrine efficacy (E_{max}), was compared among genotypes using the non-parametric Kruskal-Wallis test. SNPs that were nominally significant in the hand vein study for either ED_{50} or E_{max} were then tested for association with blood pressure response during the CPT for validation. For the CPT, we adjusted for age, sex, BMI, and baseline mean arterial blood pressure, and additionally for ethnicity in analyses of the combined cohort.

For all genetic analyses, we assumed an additive genetic model, coding the genotypes according to the number of variant alleles (0–2). We used PLINK software (v. 1.07) to assess overall differences in the outcomes among the genotypes.[23] Other statistical analyses were performed using SPSS software (v. 21, IBM® SPSS® Inc., Chicago, IL). All analyses were two-tailed, and a P-value < 0.05 was considered significant; permutation tests were performed to ensure that the empirical p-value of all SNPs was also significant at the 0.05 threshold.[24]

RESULTS

Genotyping

Minor allele frequencies for 34 *ADRA1B* SNPs in 105 subject were in the expected range, and all genotypes conformed to Hardy-Weinberg equilibrium (Supplemental Table S1). We did not identify any carriers of the rs10070745, rs7736470 and rs876529 variant in Caucasians.

Hand vein study

Subjects and outcomes—African-Americans (n=41) had a higher BMI (P = 0.044), diastolic blood pressure (P = 0.034) and a higher resting heart rate (P = 0.004) than Caucasians (n=64; Table 1). There was wide interindividual variability in response to phenylephrine, with the range of ED_{50} spanning three log units (11 to 5442 ng/min; geometric mean, 260 ng/min; 95% CI, 202 to 335 ng/min; Table 1), and the E_{max} ranging from 13.7% to 100% (median, 87%; IQR, 76% to 97%; Table 1). As previously reported in this cohort, African-Americans had a lower ED_{50} (i.e., greater sensitivity; adjusted P = 0.006) and a trend to a higher E_{max} compared to Caucasians (P = 0.079).[7]

ADRA1B variants and phenylephrine response—Among the 34 *ADRA1B* variants, one variant (rs10070745) was associated with the primary outcome, phenylephrine sensitivity (Table 2). The rs10070745 variant, present in 14 African-American heterozygotes but not in Caucasians, was associated with lower ED_{50} (β-coefficient=−0.47; 95% CI, −0.84 to −0.10; adjusted P = 0.015, Figure 1). This variant also contributed to the ethnic differences in ED_{50}: the effect of ethnicity on ED_{50} (β = −0.29; 95% CI, −0.59 to −0.10, P = 0.006)]) was weakened and no longer statistically significant after adding the rs10070745 genotype to the adjusted model in the combined cohort (β = −0.20; 95% CI, −0.54 to 0.05, P = 0.11).

The secondary outcome, phenylephrine E_{max}, was marginally associated with three variants (Table 3). The rs952037 variant was associated with higher E_{max} (higher efficacy) in the combined group (P = 0.041). The rs7737796 variant showed a non-significant trend to decreased E_{max} in both ethnic groups, which was statistically significant in the combined group (P=0.018, Table 3). The, rs17057303 showed a borderline association with lower E_{max} in African-Americans (P=0.044, Table 3).

Cold Pressor Test (CPT)

Subjects and outcomes—Of the 105 subjects that completed the hand vein study, 57 also participated in the CPT study. The baseline demographics of this subgroup were similar to those of the whole group (Table 1). Following CPT, there was a significant increase in systolic blood pressure (ΔSBP), diastolic blood pressure (ΔDBP) and mean arterial pressure (ΔMAP; all P < 0.001), without ethnic differences in these responses (all P >0.22).

***ADRA1B* variants and blood pressure response to Cold Pressor Test—**Of the four SNPs that were significantly associated with outcomes in the hand vein test, two SNPs (rs10070745 and rs17057303) were also associated with ΔMAP during the CPT (Table 4). The eight African-American subjects carrying the rs10070745 minor allele, the only variant associated with the primary outcome in the hand vein model (greater sensitivity to phenylephrine), had a 29% greater mean ΔMAP (22 mmHg; 95% CI, 14 to 30 mmHg) compared to the 13 African-American non-carriers (mean ΔMAP, 17 mmHg; 95% CI, 11 to 23 mmHg; P = 0.008, Table 4, Figure 2).

Furthermore, rs17057303, which was associated with a lower E_{max} in the hand vein study among African-Americans, also showed a trend to a lower ΔMAP following the CPT among African-Americans and in the combined cohort (Table 4). In the combined cohort, the four heterozygotes had a 44% lower mean ΔMAP (10 mmHg; 95% CI, 2 to 19 mmHg) compared to the 53 non-carriers (18 mmHg; 95% CI, 16 to 21 mmHg; P = 0.050).

DISCUSSION

The major new finding of the study is that genetic variation in *ADRA1B* affects phenylephrine-mediated venoconstriction and blood pressure changes following CPT. One variant, rs10070745, present only in African-Americans, was associated with both phenylephrine-mediated venoconstriction and blood pressure increase during the CPT.

Vascular α_{1B}-ARs are expressed in arterial and venous beds.[1,4,25] The α_{1B}-AR appears to mediate vasoconstriction by directly activating the Gq signaling pathway, leading to increased intracellular calcium through generation of the second messengers, inositol (1,4,5)-triphosphate and diacylglycerol, and by regulating the expression and function of other adrenergic receptors, especially α_{1D}-ARs.[3,26,27] Animal studies using α_{1B}-AR knockout mice revealed that the blood pressure response to phenylephrine was decreased by 45% compared to the wild-type, suggesting that α_{1B}-ARs play an important role in vascular smooth muscle contraction and blood pressure changes in response to an α_1-AR agonist.[12]

ADRA1B consists of two exons separated by a 20-kb intron and is located in a locus containing important candidate genes for blood pressure regulation.[28] Genome-wide analyses have generally not found an association between *ADRA1B* and hypertension or resting blood pressure measurements.[11,29] On the other hand, a recent candidate gene study in a Nigerian population found the strongest association between both blood pressure and hypertension with variants in *ADRA1B*.[11] However, resting blood pressure is a phenotype affected by many factors. Therefore, to more specifically address the functional effects of *ADRA1B* variants, we used two models of adrenergically mediated vasoconstriction, the dorsal hand vein response to phenylephrine and the increase in blood pressure in response to sympathetic activation induced by a cold stimulus.

Vascular studies performed in the human dorsal hand vein provide several advantages. Most important, low doses of agonist that have minimal systemic effects are infused, and thus measures of vascular sensitivity can be obtained *in vivo* without the reflex responses that accompany systemic infusions of vasoactive drugs. Many investigators reported great inter-individual variation in phenylephrine-mediated venoconstriction; much of this variability is thought to be genetic.[30,31] However, the genetic determinants of variability in α_1-AR-mediated vascular responses have not been elucidated.

We previously reported increased sensitivity (lower ED_{50}) for venous and arterial α_1-AR-mediated vasoconstriction in African-Americans compared to Caucasians[5,7]. However, α_2-AR mediated venoconstriction was similar in the two groups, suggesting that ethnic differences in α_1-ARs and their proximal signal transduction pathway (e.g. coupling, phospholipase C activation, calcium release from sarcoplasmatic reticulum) could explain ethnic differences in α_1-AR-mediated vasoconstriction.[32] Based on this premise, we previously studied *ADRA1A* variants and found that they did not explain the ethnic differences in phenylephrine sensitivity, although two *ADRA1A* variants explained some of the inter-individual variation.[7] In the present study the *ADRA1B* variant, rs10070745, was associated with phenylephrine sensitivity and also contributed to the difference observed among the two ethnic groups.

Dorsal hand vein responses represent a challenging phenotype that makes replication in a second cohort unfeasible. Thus, we examined associations of candidate variants identified in the hand vein model in a second vascular phenotype in which α_1-AR-mediated vasoconstriction plays a role. The CPT causes substantial sympathetic activation and vasoconstriction leading to an increase in blood pressure. Concordant with increased sensitivity to phenylephrine in the dorsal hand vein, subjects with rs10070745 had a greater rise in mean arterial pressure following the CPT, suggesting greater vasoconstriction. The finding that the same variant is associated with two related vasoconstrictor phenotypes supports the validity of the findings.

In keeping with published data, the intronic rs10070745 variant was present in 17% of African Americans but not in Caucasians. We did not find a report of an association of this variant with any biological function. However, in the candidate gene study in a large Nigerian population that implicated α_{1B}-AR pathway variants with blood pressure phenotype, *ADRA1B* rs10070745 was one of the variants associated with hypertension

(Personal communication with the author, Nicholas Reder)). This variant is in linkage disequilibrium with several other intronic SNPs, and the mechanism of its association with enhanced vascular responses is unclear.

There are several limitations to our study. Phenylephrine is not selective for the α_{1B}-AR subtype, but acts as an agonist also at α_{1A}-ARs and α_{1D}-ARs. However, there is no selective α_{1B}-AR agonist available for use in humans, and phenylephrine is not known to have affinity for other adrenergic receptors in the human vascular beds. Our findings were derived using the dorsal hand vein model and may therefore not automatically be extrapolated to other venous or arterial vascular beds. However, we previously found similar ethnic differences in α_1-AR-mediated vasoconstriction in both venous and arterial vascular beds, suggesting that responsiveness in the dorsal hand vein model may also reflect that in arterial vascular beds. It will be interesting to study the effects of the *ADRA1B* variants on arterial vasoconstriction. However, these studies are invasive and therefore difficult to conduct. Furthermore, although our sample size for the dorsal hand vein model study was fairly large for a translational study, some genotype groups were small, and we did not account for the multiple comparisons required by the large number of *ADRA1B* variants in the first study. Finally, our sample was not large enough to include homozygotes for the rs10070745 variant. Thus, our findings regarding the association of the rs10070745 variant with responsiveness to phenylephrine and cold pressor test are hypothesis-generating and need to be validated in other populations.

In conclusion, we found that the *ADRA1B* rs10070745 variant, present in African-Americans only, was associated with increased sensitivity to phenylephrine-mediated venoconstriction, contributed to the ethnic differences in hand vein response, and was associated with higher blood pressure responses to a CPT. Further studies exploring the association of this variant with blood pressure phenotypes and hemodynamic responsiveness in black populations, and in particular in subjects homozygous for the variant, will be of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by Vanderbilt CTSA grant 1 UL1 TR000445 from the National Center for Research Resources and P01 HL56693 from the National Institutes of Health. Dr. A. Adefurin's research reported in this publication was supported by the National Institute of General Medical Science of the National Institutes of Health under award number T32 GM007569. Dr. Stein is the recipient of the Dan May Chair in Medicine.

References

1. Rudner XL, Berkowitz DE, Booth JV, Funk BL, Cozart KL, D'Amico EB, et al. Subtype specific regulation of human vascular alpha(1)-adrenergic receptors by vessel bed and age. Circulation. 1999; 100(23):2336–2343. [PubMed: 10587338]
2. Leech CJ, Faber JE. Different alpha-adrenoceptor subtypes mediate constriction of arterioles and venules. Am J Physiol. 1996; 270:H710–H722. [PubMed: 8779849]

3. Docherty JR. Subtypes of functional alpha1-adrenoceptor. Cell Mol Life Sci. 2010; 67:405–417. [PubMed: 19862476]
4. Yan M, Sun J, Bird PI, Liu DL, Grigg M, Lim YL. Alpha1A- and alpha1B-adrenoceptors are the major subtypes in human saphenous vein. Life Sci. 2001; 68:1191–1198. [PubMed: 11228103]
5. Adefurin A, Ghimire LV, Kohli U, Muszkat M, Sofowora GG, Paranjape SY, et al. Blacks have a greater sensitivity to alpha1-adrenoceptor-mediated venoconstriction compared with Whites. Hypertension. 2013; 61(4):915–920. [PubMed: 23399717]
6. Sofowora GG, Dishy V, Landau R, Xie HG, Prasad HC, Byrne DW, et al. Alpha 1A-adrenergic receptor polymorphism and vascular response. Clin Pharmacol Ther. 2004; 75(6):539–545. [PubMed: 15179408]
7. Adefurin A, Ghimire LV, Kohli U, Muszkat M, Sofowora GG, Li C, et al. Genetic variation in the alpha-adrenergic receptor and phenylephrine-mediated venoconstriction. Pharmacogenomics J. 2014
8. Kelsey RM, Alpert BS, Dahmer MK, Krushkal J, Quasney MW. Alpha-adrenergic receptor gene polymorphisms and cardiovascular reactivity to stress in Black adolescents and young adults. Psychophysiology. 2012; 49:401–412. [PubMed: 22091949]
9. Yang X, Gu D, He J, Hixson JE, Rao DC, Lu F, et al. Genome-wide linkage and regional association study of blood pressure response to the cold pressor test in Han Chinese: the genetic epidemiology network of salt sensitivity study. Circ Cardiovasc Genet. 2014; 7(4):521–528. [PubMed: 25028485]
10. He J, Kelly TN, Zhao Q, Li H, Huang J, Wang L, et al. Genome-wide association study identifies 8 novel loci associated with blood pressure responses to interventions in Han Chinese. Circ Cardiovasc Genet. 2013; 6(6):598–607. [PubMed: 24165912]
11. Reder NP, Tayo BO, Salako B, Ogunniyi A, Adeyemo A, Rotimi C, et al. Adrenergic alpha-1 pathway is associated with hypertension among Nigerians in a pathway-focused analysis. PLoS One. 2012; 7(5):e37145. [PubMed: 22615923]
12. Cavalli A, Lattion AL, Hummler E, Nenniger M, Pedrazzini T, Aubert JF, et al. Decreased blood pressure response in mice deficient of the alpha1b-adrenergic receptor. Proc Natl Acad Sci U S A. 1997; 94(21):11589–11594. [PubMed: 9326654]
13. Tanoue A, Koshimizu TA, Tsujimoto G. Transgenic studies of alpha(1)-adrenergic receptor subtype function. Life Sci. 2002; 71:2207–2215. [PubMed: 12215368]
14. Smith KM, Macmillan JB, McGrath JC. Investigation of alpha1-adrenoceptor subtypes mediating vasoconstriction in rabbit cutaneous resistance arteries. Br J Pharmacol. 1997; 122:825–832. [PubMed: 9384497]
15. Buscher R, Herrmann V, Ring KM, Kailasam MT, O'Connor DT, Parmer RJ, et al. Variability in phenylephrine response and essential hypertension: a search for human alpha(1B)-adrenergic receptor polymorphisms. J Pharmacol Exp Ther. 1999; 291(2):793–798. [PubMed: 10525102]
16. Buzas B, Belfer I, Hipp H, Lorincz I, Evans C, Phillips G, et al. Haplotype block and superblock structures of the alpha1-adrenergic receptor genes reveal echoes from the chromosomal past. Mol Genet Genomics. 2004; 272(5):519–529. [PubMed: 15503142]
17. Stein CM, Lang CC, Singh I, He HB, Wood AJ. Increased vascular adrenergic vasoconstriction and decreased vasodilation in blacks. Additive mechanisms leading to enhanced vascular reactivity. Hypertension. 2000; 36:945–951. [PubMed: 11116105]
18. Kurnik D, Friedman EA, Muszkat M, Sofowora GG, Xie HG, Dupont WD, et al. Genetic variants in the alpha2C-adrenoceptor and G-protein contribute to ethnic differences in cardiovascular stress responses. Pharmacogenet Genomics. 2008; 18(9):743–750. [PubMed: 18698227]
19. Kohli U, Muszkat M, Sofowora GG, Harris PA, Friedman EA, Dupont WD, et al. Effects of variation in the human alpha2A- and alpha2C-adrenoceptor genes on cognitive tasks and pain perception. Eur J Pain. 2010; 14(2):154–159. [PubMed: 19423370]
20. He HB, Deegan RJ, Wood M, Wood AJ. Optimization of high-performance liquid chromatographic assay for catecholamines. Determination of optimal mobile phase composition and elimination of species-dependent differences in extraction recovery of 3,4-dihydroxybenzylamine. J Chromatogr. 1992; 574:213–218. [PubMed: 1618952]
21. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

22. Muszkat M, Kurnik D, Sofowora GG, Wood AJ, Stein CM. Independent regulation of alpha1 and alpha2 adrenergic receptor-mediated vasoconstriction in vivo. J Hypertens. 2011; 29:251–256. [PubMed: 21045729]

23. Purcell SNB, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, et al. PLINK: a toolset for whole-genome association and population-based linkage analysis. American Journal of Human Genetics. 2007; 81:559–575. [PubMed: 17701901]

24. Sham PC, Purcell SM. Statistical power and significance testing in large-scale genetic studies. Nat Rev Genet. 2014; 15:335–346. [PubMed: 24739678]

25. Giessler C, Wangemann T, Silber RE, Dhein S, Brodde OE. Noradrenaline-induced contraction of human saphenous vein and human internal mammary artery: involvement of different alpha-adrenoceptor subtypes. Naunyn Schmiedebergs Arch Pharmacol. 2002; 366:104–109. [PubMed: 12122495]

26. Theroux TL, Esbenshade TA, Peavy RD, Minneman KP. Coupling efficiencies of human alpha 1-adrenergic receptor subtypes: titration of receptor density and responsiveness with inducible and repressible expression vectors. Mol Pharmacol. 1996; 50:1376–1387. [PubMed: 8913370]

27. Hosoda C, Koshimizu TA, Tanoue A, Nasa Y, Oikawa R, Tomabechi T, et al. Two alpha1-adrenergic receptor subtypes regulating the vasopressor response have differential roles in blood pressure regulation. Mol Pharmacol. 2005; 67(3):912–922. [PubMed: 15598970]

28. Flordellis C, Paris H, Karabinis A, Lymperopoulos A. Pharmacogenomics of adrenoceptors. Pharmacogenomics. 2004; 5:803–817. [PubMed: 15469404]

29. Krushkal J, Xiong M, Ferrell R, Sing CF, Turner ST, Boerwinkle E. Linkage and association of adrenergic and dopamine receptor genes in the distal portion of the long arm of chromosome 5 with systolic blood pressure variation. Hum Mol Genet. 1998; 7:1379–1383. [PubMed: 9700190]

30. Gupta A, Carruthers SG. Familial studies of heritability of alpha1-adrenergic receptor responsiveness in superficial veins. Clin Pharmacol Ther. 1997; 62:322–326. [PubMed: 9333108]

31. Luthra A, Borkowski KR, Carruthers SG. Genetic aspects of variability in superficial vein responsiveness to norepinephrine. Clin Pharmacol Ther. 1991; 49:355–361. [PubMed: 2015725]

32. Muszkat M, Sofowora GG, Wood AJ, Stein CM. Alpha2-adrenergic receptor-induced vascular constriction in blacks and whites. Hypertension. 2004; 43:31–35. [PubMed: 14656950]

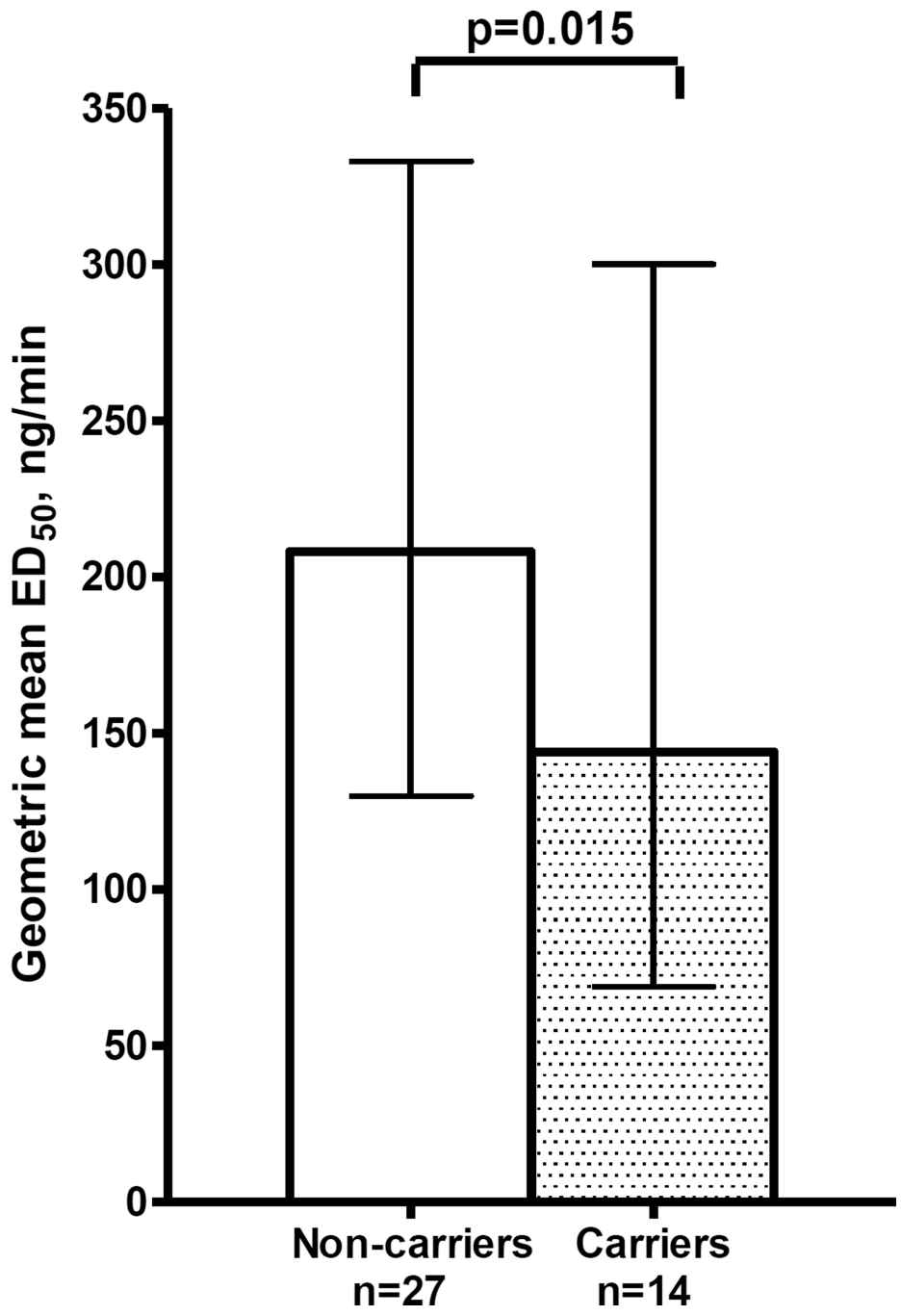

Figure 1.

ADRA1B rs10070745 association with sensitivity to phenylephrine (ED_{50}) in African-Americans

The columns show geometric means, the error bars the 95% confidence intervals. Carriers of the variant allele had a significantly lower geometric mean ED_{50} (P=0.015).

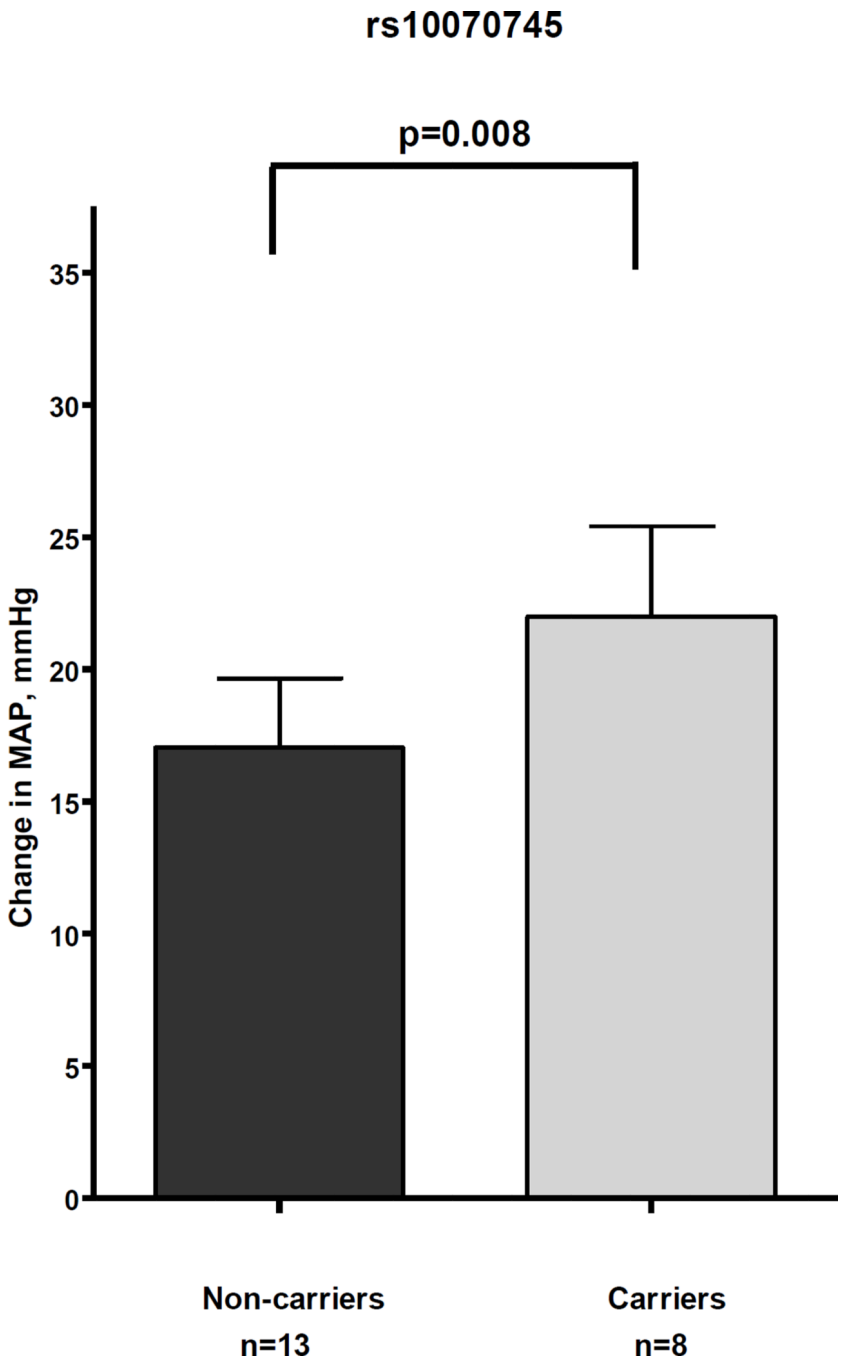

Figure 2.

Association between rs10070745 and increase in mean arterial pressure (ΔMAP) during the cold pressor test.

The columns show the mean, the error bars the standard error of mean. Carriers of the variant alleles had significantly higher mean ΔMAP after CPT (P=0.008).

Table 1

Demographic and cardiovascular measurements in subjects who performed the hand vein study (n=105) and the subgroup that also participated in the cold pressor test (n=57).

Characteristics	**Hand vein study n = 105**	**Cold pressor test n = 57**
Age, years	27.3± 7.2	26.4± 5.8
Female sex, n (%)	47 (44.8)	23 (40)
Caucasians, n (%)	64 (61)	36 (63)
BMI, kg/m^2 *	25.3 ± 4.5	24.8 ± 4.1
Resting SBP, mmHg	111.6 ± 11.5	115.5 ± 10.6
Resting DBP, mmHg	62.4 ± 8.3	64.5± 6.3
Resting MAP, mmHg	72.1 ± 6.6	81.5 ± 6.8
Resting HR, bpm	59.9 ± 8.3	64.0 ± 7.1
Baseline Plasma norepinephrine, pg/ml	167.0 ± 64.5	193.6 ± 111.8
Baseline Plasma epinephrine, pg/ml	19.5 ± 13.8	19.7± 13.3
Phenylephrine ED_{50}, ng/min, Geometric Mean (95%CI)	260 (202 – 335)	306 (213 – 440)
Phenylephrine E_{max}, %, Median (IQR)	87 (76 – 97)	82 (72 – 95)
ΔMAP after CPT, mmHg		17.9± 9.1
ΔHR after CPT, bpm		14.9± 13.4

BMI - body mass index; SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; MAP – Mean Arterial Pressure; HR – Heart rate; bpm -- beats per minute

Table 2

Genetic variants in *ADRA1B* associated with sensitivity to phenylephrine (ED_{50})

SNP	Caucasians			African-Americans			P-value*
	ED_{50}, ng/min, geometric mean (95% CI)			ED_{50}, ng/min, geometric mean (95% CI)			
	Number of variant alleles			Number of variant alleles			
	0	1	2	0	1	2	
rs10070745	325 (234–452) n=64	n=0	n=0	208 (130–334) n=27	144 (69–299) n=14	n=0	0.015

* P value adjusted for sex, BMI and Baseline Norepinephrine

Table 3

Genetic variants of *ADRA1B* associated with maximum response to phenylephrine (E_{max}).

SNP	Caucasians			P-value	African Americans			P-value	All P-value
	E_{max}, % Median (IQR)				E_{max}, %, Median (IQR)				
	Number of variant alleles				Number of variant alleles				
	0	1	2		0	1	2		
rs952037	82 (66 – 93) n= 29	85 (75 – 97) n=31	92 (72 – 100) n=4	0.39	87 (73 – 96) n=11	90 (82–98) n=19	94 (87–100) n=11	0.24	0.041
rs7737796	92 (82–98) n=24	79 (64–88) n=32	82 (66–99) n =8	0.053	95 (86–98) n=10	86 (76–94) n=24	98 (87–100) n=7	0.10	0.018
rs17057303	85 (75 – 96) n=63	61 n=1	n=0	0.20	92 (82 – 99) n=34	85 (64 – 89) n=7	n=0	0.044	0.11

Table 4

Mean arterial blood pressure change (ΔMAP) during the cold pressor test for four *ADRA1B* variants.

SNP	Caucasians				African Americans				All
	ΔMAP, mmHg, Mean (95% CI)			P-value*	ΔMAP, mmHg, Mean (95% CI)			P-value*	P-value*
	Number of variant alleles				Number of variant alleles				
	0	1	2		0	1	2		
rs10070745	17 (14 – 20) n=36	n=0	n=0	NA	17 (11 – 23) n=13	22 (14 –30) n=8	n=0	0.008	NA
rs17057303	18 (14–21) n= 35	8 n=1	n=0	0.38	20 (15 – 25) n=18	11 (−4 – 26) n=3	n=0	0.12	0.050
rs952037	14 (10 – 18) n=20	22 (18 – 27) n =14	13 (−57 – 83) n=2	0.16	17 (12 – 22) n=5	19 (11 – 28) n=11	20 (13 – 28) n = 5	0.75	0.21
rs7737796	15 (9 – 21) n=12	17 (13 – 22) n=19	23 (16 – 30) n=5	0.13	19 (14 – 25) n=5	18 (12 – 24) n=13	22 (−23 – 67) n=3	0.47	0.078

*P value adjusted for age, sex, BMI, baseline mean arterial pressure and ethnicity for analysis of the combined group.

NA=not assessed.