ORIGINAL ARTICLE

Genetic variation in the $\alpha_{1A}$-adrenergic receptor and phenylephrine-mediated venoconstriction

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There is large interindividual variability and ethnic differences in phenylephrine-mediated vasoconstriction. We tested the hypothesis that genetic variation in ADRA1A, the $\alpha_{1A}$ adrenergic receptor gene, contributes to the variability and ethnic differences. We measured local dorsal hand vein responses to increasing doses of phenylephrine in 64 Caucasians and 42 African-Americans and genotyped for 32 ADRA1A single nucleotide polymorphisms. The $E_{D50}$ ranged from 11 to 5442 ng min$^{-1}$, and the $E_{max}$ ranged from 13.5–100%. The rs574647 variant was associated with a trend towards lower log$E_{D50}$ in each race and in the combined cohort ($P = 0.008$). In addition, rs1079078 was associated with a trend to higher log$E_{D50}$ in each race and in the combined cohort ($P = 0.011$). Neither variant accounted for the ethnic differences in response. None of the ADRA1A haplotypes was associated with the outcomes. In conclusion, ADRA1A variants do not contribute substantially to the marked interindividual variability or ethnic differences in phenylephrine-mediated vasoconstriction.

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INTRODUCTION

The sympathetic nervous system regulates blood pressure and vascular resistance, and its effects are mediated through adrenergic receptors (AR). Vascular $\alpha_1$- and $\alpha_2$-ARs are expressed in arterial resistance and venous capacitance vessels, where they regulate blood pressure by maintaining vascular tone through vascular smooth muscle contraction.1,2 Vascular $\alpha_1$-ARs appear to be the prime mediators for vascular smooth muscle contraction.3,4 There is large interindividual variability in the vasoconstrictive response of arterial and venous vessels to the infusion of $\alpha$-AR agonists,5–7 much of which is genetically determined.6,7 The two main $\alpha$-AR subgroups, $\alpha_1$-AR and $\alpha_2$-AR, differ in their signal transduction pathways (coupling to the $G_q$ or $G_i$ subtypes of G-proteins, respectively), but share a common final effector pathway (myosin light chain kinase activation, myosin phosphorylation and cross bridging with actin filaments) to mediate vascular smooth muscle contraction.1,10,11 Studies finding no correlation between an individual’s sensitivity of venoconstriction in response to a selective $\alpha_1$-AR agonist (phenylephrine) and a selective $\alpha_2$-AR agonist (dexamethasomidine)12,13 which share the distal effector pathway, suggested that an important part of the interindividual variability is likely to be explained by differences in the distinctive proximal $\alpha_1$-AR and $\alpha_2$-AR signaling pathways (for example, the membrane-bound ARs and coupling). In support of this interpretation, we found ethnic differences between whites and blacks in vascular sensitivity to $\alpha_1$-AR agonists5,7 but not to the $\alpha_2$-AR agonist dexamethasomidine.6 Thus, genetic variation in the $\alpha_1$-AR could contribute to the interindividual variability in sensitivity to $\alpha_1$-agonists and to the observed ethnic differences.

Of the three $\alpha_1$-AR subtypes ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$), the $\alpha_{1A}$-AR is the predominant subtype expressed in human vascular smooth muscle.1 There is great genetic variation in ADRA1A, the gene encoding the $\alpha_{1A}$-AR, with distinct differences between whites and blacks in single nucleotide polymorphism (SNP) prevalence and haplotype structure.14 There is little information about the functional consequences of ADRA1A genetic variation on agonist-induced vasoconstriction. In an earlier study, we found that a common ADRA1A non-synonymous SNP (rs1048101) resulting in Arg347Cys, formerly called Arg492Cys, did not affect phenylephrine-mediated venoconstriction using the hand vein model.15 However, the effect of many other ADRA1A SNPs and haplotype blocks on agonist-induced vasoconstriction has not been defined.

We therefore set out to study venous responses to phenylephrine in a large cohort of healthy Caucasian and African-American subjects using the dorsal hand vein model and define the association between vascular sensitivity and ADRA1A genotypes and haplotypes. We chose the dorsal hand vein model since it is a reproducible model for the study of local venous responses to vasoactive substances in vivo that elicits only minimal systemic cardiovascular changes, and is less invasive compared with studies in arterial vessels.6,7,16,17 Our hypothesis was that genetic variation in ADRA1A, represented by 32 SNPs, affects phenylephrine-mediated venoconstriction and contributes to interindividual and ethnic differences in agonist-mediated venoconstriction.

METHODS

Subjects
Between February 2002 and April 2004 and between May 2009 and January 2011, we recruited healthy subjects to participate in phenylephrine hand vein studies. These cohorts contributed data to previous publications,7,12,15 which describe the study population and procedures in detail. For the current study, we included normotensive male and non-pregnant female Caucasians and African-Americans aged 18–45 years who

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took no medications for at least 2 weeks and abstained from alcohol and caffeine for at least 5 days before the study. Each subject received a diet containing 150 mmol per day of sodium, 70 mmol per day of potassium and 600 mmol per day of calcium for at least 4 days prior to the study day. Studies were performed in the morning after an overnight fast in a temperature-controlled room at the Vanderbilt Clinical Research Center. The Institutional Review Board of Vanderbilt University Medical Center approved the study protocol, 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 as previously described. 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 per min. A linear variable differential transformer (MRH 100; Shaeveit Engineering, Pennsaken, NJ, USA) 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 min of saline infusion, a blood sample was taken for the determination of baseline plasma catecholamines and for genotyping. We determined the baseline diameter was measured during the last 2 min of the infusion. The total diameter was measured during the last 2 min of the infusion. The total flow rate through the vein was kept constant at 0.4 ml min⁻¹ 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, USA).

Analysis of hand vein response to phenylephrine
Venoconstriction was expressed as the percentage reduction in vein diameter from baseline measurements, we assessed vein constriction in response to increasing doses of phenylephrine. Phenylephrine (Elkins-Sinn, Cherry Hill, NJ, USA) was infused through the cannula with a syringe infusion pump (Harvard Apparatus, Holliston, MA, USA) at increasing doses (range, 12–12,000 ng min⁻¹). The infusion at each dose rate lasted 7 min, and the vein diameter was measured during the last 2 min of the infusion. The total flow rate through the vein was kept constant at 0.4 ml min⁻¹ 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, USA).

Determination of plasma catecholamine concentrations
Blood was collected into cooled heparinized tubes that were placed on ice until centrifuged at 4°C for 10 min at 3000 r.p.m. Plasma was separated and stored at −20°C in previously cooled tubes containing 40 μl 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.© 2015 Macmillan Publishers Limited The Pharmacogenomics Journal (2015), 310 – 315

Genotyping
We genotyped 32 ADRA1A SNPs, including 23 previously described tagSNPs defining four ADRA1A haplotype blocks14 and 9 additional SNPs common in Caucasians and/or African-Americans (Supplementary Table 1). We genotyped these SNPs using the Sequenom platform (MassArray, San Diego, CA, USA), except for two SNPs (rs13261054 and rs1353446), which were genotyped using allelic discrimination by TaqMan 5′-nuclease assays on an ABI 7900 HT real-time PCR system (Applied Biosystems, Foster City, CA, USA) using validated TaqMan probes. 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.

Statistical analysis
Our cohort was a convenience sample consisting of participants in previous studies,12,15 and the sample size was not based on a priori calculations. Our primary outcome was drug sensitivity (expressed as ED₅₀, and drug efficacy (Eₘₐₓ) was the secondary outcome. ED₅₀ values were not normally distributed and were therefore expressed as geometric means with 95% confidence intervals (CIs) following log-transformation. Other data were expressed as mean and standard deviation (s.d.) or median and interquartile range as appropriate. Genotype distributions were tested for deviation from Hardy–Weinberg equilibrium using a χ²-test with 1 degree of freedom.

We compared the outcome, phenylephrine sensitivity (logED₅₀) among genotypes in a single marker analysis using one-way analysis of variance, first in each ethnic group separately and then in the combined cohort. We then adjusted for potential confounders that were associated with the outcome in our previous analyses (sex, body mass index (BMI), resting norepinephrine, and, for analyses of the combined cohort, ethnicity) using a multiple linear regression model. To explore the contribution of genetic markers to ethnic differences in phenylephrine sensitivity, we performed these regression analyses in the whole cohort combined, with ethnicity as additional covariate, with and without the genetic markers of interest. For all genetic analyses, we assumed an additive genetic model, coding the genotypes according to the number of variant alleles (0–2). In a sensitivity analysis, we also used a dominant model for variants with few homozygous subjects to reduce the influence of potential outliers. The secondary outcome, phenylephrine efficacy (Eₘₐₓ) was compared among genotypes using the non-parametric Kruskal–Wallis test. Race-specific haplotypes were derived with the R package haplo.stats which was also used to assess overall differences in the outcomes among the haplotypes.10,21 Other statistical analyses were performed using SPSS software (v. 21, IBM SPSS Inc., Chicago, IL, USA). All analyses were two-tailed, and a P-value < 0.05 was considered significant.

RESULTS
Demographic parameters and outcomes
Among 116 subjects studied, we excluded 10 (DNA not available or Asian ethnicity), and the final cohort included 106 subjects (64 Caucasians and 42 African-Americans). Table 1 shows

Table 1. Demographic characteristic and outcome measures

| Covariates        | Caucasians, n = 64 | African-Americans, n = 42 | All, n = 106 |
|-------------------|--------------------|---------------------------|-------------|
| Age, years        | 26.9 ± 6.8         | 28.1 ± 8.0                | 27.3 ± 7.3  |
| Female sex, n (%) | 31 (48)            | 18 (43)                   | 49 (46)     |
| Family history of hypertension, n (%) | 25 (39) | 20 (48) | 45 (43) |
| Resting SBP, mmHg | 110.3 ± 11         | 111.1 ± 9.5               | 110.6 ± 10.4|
| Resting DBP, mmHg | 60.7 ± 6.8         | 63.4 ± 8.0                | 61.8 ± 7.4  |
| Resting HR, bpm² | 59.0 ± 8.2         | 63.7 ± 8.0                | 60.9 ± 8.4  |
| Baseline plasma norepinephrine, pg ml⁻¹ | 162.3 ± 58.0 | 179.3 ± 70.8 | 168.9 ± 63.3 |
| Baseline plasma epinephrine, pg ml⁻¹ | 18.5 ± 12.5 | 21.0 ± 15.6 | 19.4 ± 15.7 |
| BMI, kg m⁻²       | 24.3 ± 3.8         | 26.8 ± 5.4                | 25.3 ± 4.6  |
| ED₅₀, pg min⁻¹, mean (95%CI)¹ | 310 (222–434) | 172 (115–256) | 245 (190–318) |
| Eₘₐₓ, %, median (IQR) | 85 (75–95) | 89 (82–98) | 87 (76–96) |

Abbreviations: DBP, diastolic blood pressure; HR, heart rate; IQR, interquartile range; SBP, systolic blood pressure. Data presented as mean ± s.d., except otherwise stated. ¹P-value < 0.05 comparing Caucasians and African-Americans.

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The rs574647 was associated with a trend towards lower logED$_{50}$ for sensitivity to phenylephrine. We did not identify any carrier of the rs1496126 variant. Conformity to Hardy–Weinberg allele frequencies were in the expected range, and all genotypes conformed to Hardy–Weinberg equilibrium in each ethnic group. We did not identify any carrier of the rs1496126 variant.

Genotyping
We genotyped 32 ADRA1A SNPs (Supplementary Table S1). Minor allele frequencies were in the expected range, and all genotypes conformed to Hardy–Weinberg equilibrium in each ethnic group. We did not identify any carrier of the rs1496126 variant.

Genetic variants and sensitivity to phenylephrine
The rs574647 was associated with a trend towards lower logED$_{50}$ both in Caucasians ($\beta = 0.25$; 95% CI, $-0.51$ to $0.02$; adjusted $P = 0.064$) and African-Americans ($\beta = 0.40$; 95% CI, $-0.79$ to $0.02$; adjusted $P = 0.039$), and the statistical evidence for this association was stronger in the combined cohort ($\beta = -0.29$; 95% CI, $-0.50$ to $-0.08$; adjusted $P = 0.008$). Geometric mean ED$_{50}$ was 76% lower in subjects with two minor alleles (ED$_{50} = 69$ ng min$^{-1}$; 95% CI, 10 to 489 ng min$^{-1}$; $n = 5$) compared with those with no minor allele (ED$_{50} = 289$ ng min$^{-1}$; 95% CI, 207–405 ng min$^{-1}$; $n = 57$; Figure 1).

In addition, the rs1079078 variant was associated with a trend to higher logED$_{50}$, both in Caucasians ($\beta = 0.23$; 95% CI, $-0.01$ to $0.47$; adjusted $P = 0.055$) and African-Americans ($\beta = 0.31$; 95% CI, $-0.04$–$0.67$; adjusted $P = 0.082$), and the association was stronger in the combined group ($\beta = 0.25$; 95% CI, $0.06$–$0.44$; adjusted $P = 0.011$). Thus, subjects with two variant alleles ($n = 6$) had a geometric mean ED$_{50}$ of 742 ng min$^{-1}$ (95% CI, 345–1595 ng min$^{-1}$), a 3.9-fold higher value compared with the 67 subjects with no minor allele (192 ng min$^{-1}$; 95% CI, 138–267 ng min$^{-1}$; Figure 2). In a sensitivity analysis, after combining heterozygous and homozygous variant carriers into one group assuming a dominant mode of inheritance, the associations between rs574647 and the rs1079078 and logED$_{50}$ in the whole cohort remained significant (adjusted $P = 0.037$ and 0.030, respectively).

The association between rs13270252 and logED$_{50}$ was in opposite directions in the two ethnic groups and was not significant in the combined group (adjusted $P = 0.69$) (Table 2, Supplementary Table S2). The rs580739 variant was significantly associated with logED$_{50}$ in African-Americans (adjusted $P = 0.005$) but not in Caucasians or in the combined cohort (both $P > 0.10$). Moreover, the functional non-synonymous variant rs1048101, resulting in the Arg347Cys polymorphism, was not associated with logED$_{50}$ in any of the ethnic subgroups or the combined group (all $P > 0.35$).

Genetic variants and efficacy of phenylephrine
None of the 32 ADRA1A SNPs was associated with $E_{\text{max}}$ in the ethnic subgroups, while rs1733700 was marginally associated with lower $E_{\text{max}}$ in the combined group ($P = 0.040$, Table 3; Supplementary Table S3; Supplementary Figure 1).

ADRA1A haplotypes and outcome measures
Based on a previously published ADRA1A haplotype analysis, we divided the ADRA1A gene into four blocks and inferred haplotypes for each block separately in each ethnic group (Supplementary Tables S4). Haplotype frequencies were overall similar to those previously published. Phenotype–haplotype analyses did not provide any evidence for an overall association between outcomes (logED$_{50}$ or $E_{\text{max}}$) and haplotypes in any of the four haplotype blocks (Supplementary Tables S5).

Ethnic difference in ED$_{50}$ and ADRA1A variants
As previously described, geometric mean ED$_{50}$ was 45% lower in African-Americans than in Caucasians (172 ng min$^{-1}$ and 310 ng min$^{-1}$ in African-Americans and Caucasians respectively; adjusted $P = 0.004$; Table 1). Since the four ADRA1A variants associated with logED50 (rs13270252, rs580739, rs574647 and rs1079078) had significantly different prevalences in Caucasians and African-Americans, we examined whether these variants contributed to ethnic differences in ED$_{50}$. In the combined cohort, using a multiple linear regression model without genotypes (with logED$_{50}$ as dependent variable and sex, BMI, ethnicity and resting plasma norepinephrine concentrations as covariates), black ethnicity was associated with lower logED$_{50}$ ($\beta = -0.36$; 95% CI, $-0.61$ to $-0.12$; $P = 0.004$). Adding the four genotypes to the model increased the coefficient of variation $R^2$, a measure of the variation in the outcome explained by the model, from 12.9 to
Our study is the first to systematically examine the effect of the genetic variation in ADRA1A on vascular responses to an α1-AR agonist. Our major findings are that genetic variation in ADRA1A explains only little of the large interindividual variability in phenylephrine-mediated vеноconstriction, and that it does not account for the ethnic differences in α1-AR-mediated vеноconstriction. In addition, our study provided some evidence for an association of two intronic SNPs (rs574647 and rs1079078) with increased and decreased sensitivity to phenylephrine-mediated vеноconstriction, respectively.

Family studies have found large heritability in venous response to α-AR agonists. Veins express different α1-AR subtypes, and the α1A*-AR subtype has the highest receptor density and greatest affinity for α1-AR agonists. However, little is known about the effect of genetic variability in ADRA1A on α1-AR-mediated vеноconstriction. We previously studied the effect of a single functional, non-synonymous ADRA1A SNP (rs1048101, resulting in Arg347Cys), in the hand vein model in 74 subjects (52 Caucasians, 5 African-Americans and 17 other ethnicities), finding no association with phenylephrine-mediated vеноconstriction.

In our current study, with a larger sample size, we confirmed this finding. Moreover, in a systematic approach using 32 SNPs selected to better capture ADRA1A genetic variation, we found that it contributed only little at best to the great variability in phenylephrine-mediated vеноconstriction and to its ethnic differences. Thus, our findings are compatible with previous studies failing to show consistent associations of ADRA1A polymorphisms with more complex phenotypes to which α1-mediated vasoconstriction is thought to contribute, for example, blood pressure response to stress and hypertension.

We found some evidence for an association of two intronic SNPs—rs574647 and rs1079078—with increased and decreased sensitivity to phenylephrine-mediated vеноconstriction, respectively. Subjects with two minor rs574647 variant alleles had a geometric mean ED_{50} that was 76% lower than that of subjects without any variant allele. The rs1079078 variant was more prevalent in Caucasians, and subjects with two variant alleles had a 3.9-fold higher geometric mean ED_{50} (reflecting lower α1A-AR sensitivity to phenylephrine-mediated vеноconstriction) compared with subjects with no variant allele. We are not aware of previous studies showing functional associations of these variants. Moreover, they may be only markers of functional haplotypes rather than SNPs primarily responsible for the observed effect. However, haplotype analysis did not provide evidence for functional effects of the haplotypes defined by these two variants.

We have previously shown increased sensitivity of venous and arterial α1-AR-mediated vеноconstriction in African-Americans compared with Caucasians. In view of the ethnic differences in ADRA1A genetic variation, we therefore examined the hypothesis that genetic variants in α1-AR account for these ethnic differences. However, in the present study, the four variants associated with phenylephrine ED_{50} did not explain the ethnic difference in ED_{50}. Thus, after excluding genetic variation in α1-AR, it remains unclear which factors contribute to the large

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**Table 2. Genetic variants of ADRA1A associated with sensitivity to phenylephrine (ED_{50})**

| SNP            | Caucasians |     | African-Americans |     | All    |     |
|----------------|------------|-----|--------------------|-----|--------|-----|
|                | P-value*   |     | P-value*           |     | P-value* |     |
|                | unadjusted|     | unadjusted         |     | unadjusted |     |
|                | adjusted  |     | adjusted           |     | adjusted |     |
|                | mean (95% CI) |     | mean (95% CI)      |     | mean (95% CI) |     |
|                |            |     |                    |     |          |     |
| rs574647       | 0.022 (0.064) | 214 (131–350) | 0.283 (0.039) | 0.064 (0.008) |
| rs1079078      | 0.030 (0.055) | 139 (85–230) | 0.094 (0.082) | 0.003 (0.011) |
| rs132705252    | 0.108 (0.039) | 186 (112–302) | 0.207 (0.027) | 0.586 (0.686) |
| rs580739       | 0.360 (0.623) | 267 (172–416) | 0.007 (0.005) | 0.131 (0.103) |

**Table 3. Genetic variant of ADRA1A associated with efficacy to phenylephrine (E_{max})**

| SNP            | Caucasians |     | African-Americans |     | All    |     |
|----------------|------------|-----|--------------------|-----|--------|-----|
|                | P-value    |     | P-value            |     | P-value |     |
|                | unadjusted|     | unadjusted         |     | unadjusted |     |
|                | adjusted  |     | adjusted           |     | adjusted |     |
|                | median (IQR) |     | median (IQR)      |     | median (IQR) |     |
|                |            |     |                    |     |          |     |
| rs17337300     | 0.078      | 89 (83–98) | —                   | 0.749 | 0.040 |

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18.4%, but the ethnic difference remained unaffected (β = −0.36; 95% CI −0.64 to −0.08; P = 0.013), suggesting that these genetic variants did not contribute substantially to the greater phenylephrine sensitivity of African-Americans compared with Caucasians.

**DISCUSSION**

Our study is the first to systematically examine the effect of the genetic variation in ADRA1A on vascular responses to an α1-AR agonist. Our major findings are that genetic variation in ADRA1A explains only little of the large interindividual variability in phenylephrine-mediated vеноconstriction, and that it does not account for the ethnic differences in α1-AR-mediated vеноconstriction. In addition, our study provided some evidence for an association of two ADRA1A SNPs (rs574647 and rs1079078) with increased and decreased sensitivity to phenylephrine-mediated vеноconstriction, respectively.

Family studies have found large heritability in venous response to α-AR agonists. Veins express different α1-AR subtypes, and the α1A*-AR subtype has the highest receptor density and greatest affinity for α1-AR agonists. However, little is known about the effect of genetic variability in ADRA1A on α1-AR-mediated vеноconstriction. We previously studied the effect of a single functional, non-synonymous ADRA1A SNP (rs1048101, resulting in Arg347Cys), in the hand vein model in 74 subjects (52 Caucasians, 5 African-Americans and 17 other ethnicities), finding no association with phenylephrine-mediated vеноconstriction. In our current study, with a larger sample size, we confirmed this finding. Moreover, in a systematic approach using 32 SNPs selected to better capture ADRA1A genetic variation, we found that it contributed only little at best to the great variability in phenylephrine-mediated vеноconstriction and to its ethnic differences. Thus, our findings are compatible with previous studies failing to show consistent associations of ADRA1A polymorphisms with more complex phenotypes to which α1-mediated vasoconstriction is thought to contribute, for example, blood pressure response to stress and hypertension.

We found some evidence for an association of two intronic SNPs—rs574647 and rs1079078—with increased and decreased sensitivity to phenylephrine-mediated vеноconstriction, respectively. Subjects with two minor rs574647 variant alleles had a geometric mean ED_{50} that was 76% lower than that of subjects without any variant allele. The rs1079078 variant was more prevalent in Caucasians, and subjects with two variant alleles had a 3.9-fold higher geometric mean ED_{50} (reflecting lower α1A-AR sensitivity to phenylephrine-mediated vеноconstriction) compared with subjects with no variant allele. We are not aware of previous studies showing functional associations of these variants. Moreover, they may be only markers of functional haplotypes rather than SNPs primarily responsible for the observed effect. However, haplotype analysis did not provide evidence for functional effects of the haplotypes defined by these two variants.

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interindividual variability in α1-AR mediated vasoconstriction, and to the ethnic differences in particular. Yet, in view of the strong heritability of venous responsiveness to α-AR agonists, genetic variation in other candidate genes remains a likely cause.6,9

In contrast to phenylephrine-mediated vasoconstriction, we previously did not find ethnic differences in α1-AR-mediated vasoconstriction in the hand vein model,6 suggesting that differences in the proximal signaling pathway specific to the α1-AR pathway account for ethnic differences in phenylephrine sensitivity. Thus, taken together with our current findings, genes encoding proteins involved in the early signal transduction cascade below the α1-AR level, for example, in receptor coupling, would be reasonable candidates for explaining interindividual and ethnic differences in α1-AR mediated responsiveness.

There are several limitations to our study. Phenylephrine is not selective for the α1A-AR subtype, but acts as an agonist also at venous α1D-AR and α1D-AR. However, there is no selective α1A-AR agonist available for use in humans. Moreover, although α1A-AR and α1D-ARs are also expressed in human veins,31,32 α1A-ARs are thought to be the prime mediators of vascular constriction.6,9 Second, our findings were derived using the dorsal hand vein model, and may therefore not automatically be extrapolated to other venous or arterial vascular beds, or to more complex and multifactorial phenotypes such as blood pressure. However, we found similar ethnic differences in α1-AR-mediated vasoconstriction both in venous and arterial vascular beds, suggesting that responsiveness in the dorsal hand vein model may, to a certain degree, also reflect that in arterial vascular beds. Finally, although our sample size was rather large for a translational study, some genotype groups were small, and we could not account for the multiple comparisons required by the large number of ADR1A variants. Thus, we consider our findings regarding the association of the rs574647 and rs1079078 variants with responsiveness to phenylephrine preliminary rather than conclusive. Our findings should be validated in a separate cohort, and much larger cohorts would be necessary for systematic, non-candidate gene-based approaches such as genome-wide association studies.

In conclusion, we found some evidence for an association of sensitivity to phenylephrine-mediated vasoconstriction with two intronic ADR1A SNPs (rs574647 and rs1079078), but overall, genetic variation in ADR1A does not contribute substantially to the large interindividual variation in phenylephrine-mediated vasoconstriction and ethnic differences in response between African-Americans and Caucasians. Future studies exploring additional candidate genes involved in the α1-AR signal transduction pathway (for example, those involved in receptor coupling) or genes encoding other α1-AR subtypes will be of interest.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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