Published in final edited form as:

Pharmacogenet Genomics. 2012 April ; 22(4): 254–260. doi:10.1097/FPC.0b013e328350a274.

Catecholamine pathway gene variation is associated with norepinephrine and epinephrine concentrations at rest and exercise

Laxmi V. GHIMIRE^a, Utkarsh KOHLI^a, Chun LI^{b,c}, Gbenga G. SOFOWORA^a, Mordechai MUSZKAT^a, Eitan A. FRIEDMAN^a, Joseph F. SOLUS^d, Alastair J.J. WOOD^a, C. Michael STEIN^a, and Daniel KURNIK^{a,e,f}

^aDepartments of Medicine and Pharmacology, Division of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

^bCenter for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

^cDepartment of Biostatistics, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

^dDivision of Rheumatology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

^eInstitute of Clinical Pharmacology and Toxicology, Sheba Medical Center, Tel Hashomer, Israel

^fSackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

Abstract

Objective—To examine the hypothesis that genetic variation in enzymes and transporters associated with synthesis, storage, release, and metabolism of catecholamines contributes to the interindividual variability in plasma catecholamine concentrations at rest and after exercise.

Methods—We measured plasma NE and epinephrine concentrations at rest and after a standardized exercise protocol in 165 healthy subjects (60% Caucasian, 40% African-American) and examined 29 functional or common variants in 14 genes involved in synthesis, transport, or metabolism of catecholamines. We examined the relationship among genotypes and NE concentrations at rest, and the increase after exercise (Δ NE), by multiple linear regression with adjustment for covariates (age, race, sex, BMI, fitness, and, for Δ NE, resting NE). As a secondary outcome, we performed similar analyses for epinephrine concentrations.

Results—There was large interindividual variability in resting NE (mean, 204±102 pg/mL; range, 39 to 616 pg/mL) and Δ NE (mean, 256±206 pg/mL; range, -97 to 953 pg/mL). Resting NE was significantly associated with variants in 4 genes: *CYP561* ($P<0.001$), *VMAT2* ($P=0.016$), *CHGA* ($P=0.039$), and *PNMT* ($P=0.038$). Δ NE after exercise was associated with 3 variants in *PNMT* ($P=0.041$) and *COMT* ($P=0.033$ and 0.035), and resting and exercise epinephrine concentrations with 2 variants each.

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.

Potential conflicts of interest: None declared

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusions—The findings of this exploratory study suggest that variation in catecholamine pathway genes contributes to the interindividual variability in plasma NE and epinephrine concentrations at rest and after exercise.

Keywords

Sympathetic Nervous System; Catecholamines; Exercise; Genetics; Polymorphism

Introduction

The sympathetic nervous system is an important regulator of physiologic cardiovascular responses at rest and during stress. Increased sympathetic activity plays an important role in the pathophysiology and prognosis of various diseases including hypertension, heart failure, ischemic heart disease, and obstructive sleep apnea [1].

Norepinephrine (NE) is the primary neurotransmitter of cardiovascular sympathetic innervation. In the sympathetic neuron, NE synthesis starts from tyrosine, which is converted to dopamine by tyrosine hydroxylase. The vesicular monoamine transporter (VMAT) then transports dopamine into storage vesicles, where it is further converted to NE by the action of dopamine- β -hydroxylase. Chromogranins are secretory proteins and play important roles in the aggregation, packaging, sorting, and processing of catecholamines in secretory vesicles prior to secretion. In response to sympathetic stimulation, NE is released into the synaptic cleft and acts on post-synaptic adrenergic receptors to mediate sympathetic effects. A small proportion of the NE released into the synaptic cleft reaches the systemic circulation, where plasma NE concentrations can be measured as a marker of sympathetic activation. Monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) catalyze degradation of NE and Epinephrine by deamination and methylation. Epinephrine is produced mainly in the adrenal medulla and functions as a circulating hormone.

Plasma NE and epinephrine concentrations, indirect measures of sympathetic nervous system activity, vary markedly among individuals. Some of this variability is due to environmental and constitutional factors, for example, diet, obesity, and posture [2]. In addition, genetic factors contribute to this variability: heritability studies performed in twins show that genetic factors explain more than half (55%) of the interindividual variability in plasma NE concentrations [3]. However, little is known about the specific genetic variants that contribute to the heritability of plasma NE concentrations or other measures of sympathetic activation. In previous studies, we did not find any association between common genetic variants in α_2 -adrenergic receptors (key regulators of sympathetic tone) [4] or the NE transporter [5] (which clears NE from the synaptic cleft) with plasma NE and Epinephrine concentrations. Recently, Fung et al. [6] studied variants in candidate genes in the catecholamine pathway, for example dopamine β -hydroxylase, monoamine oxidase, and chromogranins, and found that some variants were significantly correlated with hand vein constrictor response to local tyramine infusion (tyramine is an indirect sympathomimetic agent that releases NE from sympathetic nerve terminals). However, the hereditary contribution to interindividual variability in plasma NE concentrations remains unexplained.

Therefore, we examined the hypothesis that genetic variation in enzymes and transporters associated with synthesis, storage, release, and metabolism of catecholamines contributes to the interindividual variability in plasma catecholamine concentrations at rest and after exercise.

Methods

Subjects

The Institutional Review Board of the Vanderbilt University Medical Center approved the study, and all participants gave written informed consent. Most subjects contributed data to a previous study, and details of the study design and methods have been published [7]. Unrelated subjects were eligible for recruitment if they were aged between 18-50 years and had no clinically significant medical illness based on medical history, physical examination, electrocardiogram, and routine laboratory results. To minimize confounding from disease and drug treatment, our subjects were all healthy volunteers from the greater Nashville area in middle Tennessee. Many subjects were recruited through a database of volunteers maintained by the Vanderbilt Clinical Research Center and from advertisements [8]. Subjects reported their average weekly physical activity during three month preceding the study (types, intensity, duration, and frequency), and this information was converted into Metabolic Equivalents using standard charts [9]. A fitness score was defined for each subject as average number of Metabolic Equivalents /week. Subjects reported their ethnicity and that of their parents and grandparents using check-boxes to choose among “Caucasian”, “African-American”, “Hispanic”, “Chinese”, “Japanese”, and “other” (the latter to be specified). Multiple choices were permitted. A subject was assigned to an ethnic group when both parents and at least 3 out of 4 grandparents were of the same ethnicity. Body mass index (BMI) was calculated as weight/height² (kg/m²). Subjects were free of medications for at least two weeks and received a caffeine- and alcohol-free diet (providing 150 mmol of sodium, 70 mmol of potassium, and 600 mmol of calcium daily) for five days before the study.

Protocol

On day 6 of the study diet, between 8:00 and 10:00 am, subjects were admitted to a temperature-controlled room (22-23°C) in the Vanderbilt University Clinical Research Center after an overnight fast. A 20 G intravenous cannula was inserted into an antecubital vein, and a blood sample drawn for DNA extraction. Digitized ECG signals were continuously acquired on a personal computer at 500 MHz using the Windaq software (v. 2.20, Dataq Instruments Inc., Akron, Ohio) before and during exercise. After 30 minutes of supine rest, subjects then slid to an adjacent electronically braked supine bicycle ergometer. The exercise protocol consisted of cycling at a constant rate (60 revolutions/min) at increasing workloads of 25, 50, and 75 Watts for 2 minutes each. Blood samples for the determination of catecholamine concentrations were drawn after the 30-minute rest before exercise and immediately after the completion of exercise.

Selection of candidate genes and genotyping

We identified 14 candidate genes involved in the synthesis, transport, or metabolism of NE by literature search, and evaluated 29 SNPs in these genes (Table 1). We selected these variants because they are relatively common (expected minor allele frequencies >5%) and are located in the coding or promoter regions or were shown to have functional effects in previous studies [10-13]. Genotyping was performed by allelic discrimination with TaqMan 5'-nuclease assays on an ABI 7900 HT instrument (Applied Biosystems, Foster City, CA) using validated TaqMan probes and a 95% quality value threshold and according to the manufacturer's recommended protocols. We only included SNPs with call rates higher than 87.5% for analysis.

Plasma catecholamine determination

Blood was collected into cooled heparinized tubes which were immediately placed on ice until centrifuged at 4°C for 10 minutes at 3,000 rpm. Plasma was harvested and stored in tubes containing 40 µL of reduced glutathione (6%) at –20°C until assayed. Norepinephrine (NE) and epinephrine concentrations were measured by high-performance liquid chromatography using electrochemical detection with dihydroxybenzylamine as internal standard [14].

Data and statistical analysis

We measured resting heart rate after 20 minutes of supine rest from the computerized ECG recordings as the average during a 10 minute-period preceding the exercise test. Exercise heart rate was defined as mean heart rate during the second minute of each exercise step. Data are expressed as mean and 95% confidence interval or standard deviation, or median and interquartile range as appropriate. We used multiple linear regression analysis to examine the effect of genotypes on the primary outcomes, resting NE concentrations and the increase in NE at maximal exercise (Δ NE). Genotypes were coded as number of minor alleles (0-2), and we assumed an additive mode of inheritance. As secondary outcome, we also examined the effect of genotypes on resting plasma epinephrine concentrations and on its increase (Δ epinephrine) after exercise. Analyses were adjusted for potential confounding factors including age, sex, race, fitness score, and BMI and, for outcomes after exercise, also for the corresponding resting value. Residuals were inspected for normality. In addition, we performed analyses separately in each racial group. We tested whether genotype distributions conformed to Hardy-Weinberg Equilibrium using a chi-square test with 1 degree of freedom. All tests were two-tailed. In these hypothesis-generating analyses, we did not adjust for multiple comparisons, and P-values < 0.05 were considered significant. All analyses were performed using the statistical software R (www.r-project.org).

Results

Subject Characteristics

We studied 165 subjects (74 males and 91 females, 99 Caucasians and 66 African-Americans). The mean age was 26.2±6.0 years, and the mean BMI was 25.4±5.2 kg/m².

Non-genetic determinants of norepinephrine

The increase in NE after exercise (Δ NE) was associated with lower BMI ($P=0.014$), African-American race ($P=0.001$), and female sex ($P=0.012$).

Genotyping

We genotyped 29 variants in 14 genes (Table 1) with a mean call rate of 92.9%. Minor allele frequencies were in the expected range, and all genotypes conformed to Hardy-Weinberg equilibrium in each ethnic group.

Genetic variants and plasma norepinephrine concentrations

The mean resting plasma NE concentration was 204±102 pg/mL (range, 39 to 616 pg/mL). SNPs in two genes (*CYB561* and *VMAT2*) were significantly associated with resting NE concentrations before and after adjustment for race, age, sex, BMI, and fitness score (Table 2). In addition, the associations with one variant each from *CHGA* and *PNMT* were statistically significant after adjustment. The genotype effect was strongest for *CYB561* rs2058203; subjects with no variant allele ($n=31$) had a mean resting NE concentration that was 45% higher than that of subjects with 2 variant alleles ($n=41$), while carriers of 1 variant allele had intermediate NE concentrations (Table 2 and Figure). Adding *CYB561* rs2058203

to a regression model that predicted resting NE and included race, age, sex, BMI, and fitness score as independent variables increased the coefficient of determination R^2 , a measure of the intersubject variability explained by the model, from 1.3% to 8.6% ($P < 0.001$).

In a race-specific sensitivity analysis, the genotype effects were largely similar between blacks and whites, but did not reach statistical significance for some associations, likely due to smaller sample sizes.

After exercise, plasma NE concentrations increased by a mean of 256 ± 206 pg/mL (range, -97 to 953), corresponding to a mean increase of 155%. After adjustment for covariates (including resting NE), three SNPs in two genes were significantly associated with Δ NE: *PNMT* rs876493 and *COMT* rs4680 and rs4633 (Table 2). The association of Δ NE with *PNMT* rs876493 genotype was strongest, with carriers of 2 variant alleles having a 39% smaller increase in NE compared to subjects without a variant allele (Table 2).

Genetic variants and plasma epinephrine concentrations

The mean resting plasma epinephrine concentration was 21 ± 14 pg/mL (range, 2 to 83 pg/mL), and the mean increase in epinephrine after exercise (Δ epinephrine) was 29 ± 36 pg/mL. SNPs in two genes (*ATP6V1B* rs17720303 and *COMT* rs737865) were associated with resting plasma epinephrine concentrations, while three SNPs in two genes (*COMT* rs4680 and rs4633 and *MAOA* rs6323) were associated with Δ epinephrine (Table 3). Among non-genetic covariates, only male sex was associated with higher resting epinephrine concentrations ($P = 0.002$).

Discussion

There is great interindividual variability in plasma NE concentrations, more than half of which has been attributed to genetic factors. In this study, we examined the association of variants in candidate genes involved in synthesis, storage, transport, and metabolism of catecholamines with plasma NE and Epinephrine concentrations at rest and after exercise. We found several variants in candidate genes to be associated with plasma NE concentrations.

Candidate gene variants associated with catecholamine concentrations

Among the genetic variants examined, resting plasma NE concentrations were most strongly associated with a variant in cytochrome b561 (*CYB561*). *CYB561* is a transmembrane protein located in the membrane of neuropeptide secretory vesicles in neuroendocrine tissues. Functioning as an electron channel, it shuttles electrons between the cytoplasm and the neurosecretory vesicles to maintain the intravesicular redox state essential for catecholamine synthesis [15]. There is little information about functional effects of genetic variants in *CYB561*. Healthy subjects heterozygous for *CYB561* rs2058203 had less tyramine-induced vasoconstriction in the dorsal hand vein model (suggesting less NE release) compared to homozygotes of either the minor or major allele, but the lack of a gene-dose effect makes the interpretation of that finding difficult [6]. In our study, the minor allele of *CYB561* rs2058203 was associated with lower resting NE concentrations, and subjects without a variant allele had approximately 45% higher mean NE concentrations compared to subjects with 2 variant alleles.

Phenylethanolamine N-methyltransferase (PNMT) catalyzes the methylation of NE to Epinephrine, the last step of catecholamine biosynthesis, and is highly expressed in the adrenal medulla and the left atrium of the heart [16]. A haplotype defined by two common genetic variants in the 5'-untranslated region, rs3764351 and rs876493, was associated *in vitro* with 30% reduced enzyme activity [17], and these variants were inconsistently

associated with hypertension in some ethnic groups [18, 19]. In a recent study of the association of *PNMT* variants with catecholamine plasma concentrations, two carriers of a rare intron variant [III(280) G/A] had lower epinephrine plasma concentrations at rest and during exercise, while no *PNMT* variant was associated with plasma NE [11]. In our study, in a larger cohort, after adjustment for potential confounders the 5'-untranslated region *PNMT* variant rs876493 was associated with higher resting NE concentrations (a finding of borderline statistical significance) and a 39% smaller increase during exercise ($P=0.041$). The contribution of *PNMT* rs876493 genotype to intersubject variability in Δ NE was modest: Adding *PNMT* rs876493 genotype to a regression model that predicted Δ NE and included resting NE, race, age, sex, BMI, and fitness score as independent variables increased the coefficient of determination R^2 from 9.9% to 11.9%. Interestingly, in a previous study, subjects homozygous for this variant had lower urinary epinephrine excretion compared to other genotypes [20]. In the present study, we did not find any association between *PNMT* rs876493 and plasma epinephrine concentrations at rest or exercise.

The vesicular monoamine transporter (VMAT) transfers neurotransmitters from the cytosol of presynaptic neurons into synaptic vesicles, where they are stored for subsequent release, and is therefore an important regulator of intraneuronal and synaptic monoamine concentration. Pharmacological blockade of VMAT2 (e.g., by reserpine) results in NE depletion in synaptic vesicles and the synaptic cleft and significantly reduces plasma NE concentrations [21], blood pressure, and heart rate. *VMAT2* genetic variants have been variably associated with psychiatric diseases [22], but there is little information on their cardiovascular effects. In our study, compared to non-carriers, carriers of one and two *VMAT2* rs363227 variants had 20% and 32% higher resting plasma NE concentrations, respectively, suggesting increased function. Interestingly, Δ NE after exercise was approximately 51% higher and Δ Epinephrine 107% higher in carriers of 2 variant alleles ($n=9$) compared to carriers of 0 ($n=113$) or 1 ($n=36$) variant, but these associations were not statistically significant, likely because of the small number of subjects homozygous for the variant allele.

Chromogranin A (*CHGA*) is an essential component of secretory vesicles of the adrenal medulla and preganglionic sympathetic axons and is co-released together with catecholamines into the synaptic cleft. *CHGA* variants have been associated with blood pressure and hypertension [23-27]. In our study, *CHGA* rs9658655 was weakly associated with higher resting NE concentration without a clear gene-dose effect, and none of the other outcomes was associated with this variant.

Catechol-O-methyltransferase (COMT) catalyzes the methylation of catecholamines, the first step in their biodegradation. Thus, decreased COMT activity would be expected to result in catecholamine accumulation. *COMT* variants and haplotypes are associated with changes in enzyme expression and activity [28, 29], and the rs4680 Val158Met variant was associated with higher heart rate and blood pressure in various cohorts [30, 31]. In our study, two SNPs in linkage disequilibrium (rs4680 and rs4633) were associated with higher Δ NE and Δ epinephrine after exercise: Homozygous variant carriers had a 57-70% higher Δ epinephrine and a 22-24% higher Δ NE compared to subjects without variant alleles. Indeed, the non-synonymous rs4680 variant, resulting in the Val158Met substitution, was previously associated with lower COMT activity [28], providing a plausible biological mechanism for our findings (greater increase of plasma catecholamine concentrations after exercise stress). Interestingly, resting catecholamine plasma concentrations were not associated with these *COMT* variants. It is likely that functional differences in *COMT* rs4680 and rs4633 are more pronounced in conditions of increased catecholamine release, e.g. exercise.

Although several candidate gene variants were associated with plasma catecholamine concentrations, the genetic variants contributed modestly to the large interindividual variability. Thus, *CYB561* rs2058203, the variant with the strongest association with resting NE, increased the coefficient of determination R^2 from 1.3% to 8.6%. Our study protocol was standardized to minimize the influence of other non-genetic factors previously associated with plasma catecholamine concentrations, e.g., postural changes [32], mental stress and pain [33], diurnal variation [34], and salt intake [35].

Our study has several limitations. Our outcomes – plasma catecholamine concentrations – are frequently studied laboratory markers of sympathetic activity with well-defined interindividual variability and heritability, but their relation to clinical outcomes in healthy subjects is unclear, and they are not perfect measures of neuronal catecholamine production and synaptic release [36]. The sample size was large for a translational study, but for uncommon variants, some genotype groups were small. Similarly, for some genetic variants, our study did not have sufficient statistical power for separate race-specific analyses; however, effect size estimates were largely comparable between blacks and whites. Moreover, combined analyses of genetically diverse ethnic subgroups may be helpful in identifying truly causal variants rather than markers merely associated with causal variants by linkage disequilibrium [37]. We considered only common candidate gene variants, since our goal was to determine the role of widely distributed genetic factors on the population variability in plasma catecholamine concentrations. Using tagSNPs for each candidate gene would have provided a better representation of genetic variability in the genes of interest, but also divided our cohort into small, ethnicity-dependent haplotype groups with insufficient power for statistical analyses. Similarly, a systematic approach, such as a genome-wide association study, is likely to identify more genetic determinants of plasma catecholamine concentrations but requires a larger sample size. Additionally, our study was exploratory, and we did not adjust for multiple comparisons in the statistical analysis.

In conclusion, we found associations between plasma catecholamine concentrations and several candidate gene variants in the catecholamine pathway. A systematic search for genetic and non-genetic factors contributing to the interindividual variability in plasma catecholamine concentrations and studies examining the effect of the identified genetic variants on other markers of sympathetic activation, e.g. heart rate, will be of interest.

Acknowledgments

The Vanderbilt DNA Resource Core and Vanderbilt University Center for Human Genetics Research provided technical assistance for this work.

Sources of Funding: This study was supported by Vanderbilt CTSA grant 1 UL 1 RR024975 from the National Center for Research Resources and P01 HL56693 from the National Institutes of Health. Dr. Stein is the recipient of the Dan May Chair in Medicine.

References

1. Charkoudian N, Rabbitts JA. Sympathetic neural mechanisms in human cardiovascular health and disease. *Mayo Clin Proc.* 2009; 84:822–830. [PubMed: 19720780]
2. Tentolouris N, Liatis S, Katsilambros N. Sympathetic system activity in obesity and metabolic syndrome. *Ann N Y Acad Sci.* 2006; 1083:129–152. [PubMed: 17148737]
3. Zhang L, Rao F, Wessel J, Kennedy BP, Rana BK, Taupenot L, et al. Functional allelic heterogeneity and pleiotropy of a repeat polymorphism in tyrosine hydroxylase: prediction of catecholamines and response to stress in twins. *Physiol Genomics.* 2004; 19:277–291. [PubMed: 15367723]

4. Kurnik D, Muszkat M, Friedman EA, Sofowora GG, Diedrich A, Xie HG, et al. Effect of the alpha2C-adrenoreceptor deletion322-325 variant on sympathetic activity and cardiovascular measures in healthy subjects. *J Hypertens.* 2007; 25:763–771. [PubMed: 17351367]
5. Kohli U, Hahn M, English B, Sofowora G, Li C, Blakely R, et al. Genetic Variation in the Presynaptic Norepinephrine Transporter is Associated with Blood Pressure Responses to Exercise in Healthy Humans. *Pharmacogenetics and Genomics.* 2011; 21:171–8. [PubMed: 21412203]
6. Fung MM, Nguyen C, Mehtani P, Salem RM, Perez B, Thomas B, et al. Genetic variation within adrenergic pathways determines in vivo effects of presynaptic stimulation in humans. *Circulation.* 2008; 117:517–525. [PubMed: 18180394]
7. Kurnik D, Li C, Sofowora GG, Friedman EA, Muszkat M, Xie HG, et al. Beta-1-adrenoceptor genetic variants and ethnicity independently affect response to beta-blockade. *Pharmacogenet Genomics.* 2008; 18:895–902. [PubMed: 18794726]
8. Harris PA, Lane L, Biaggioni I. Clinical research subject recruitment: the Volunteer for Vanderbilt Research Program www.volunteer.mc.vanderbilt.edu. *J Am Med Inform Assoc.* 2005; 12:608–613. www.volunteer.mc.vanderbilt.edu [PubMed: 16049231]
9. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc.* 2000; 32:S498–S504. [PubMed: 10993420]
10. Rao F, Zhang L, Wessel J, Zhang K, Wen G, Kennedy BP, et al. Adrenergic polymorphism and the human stress response. *Ann N Y Acad Sci.* 2008; 1148:282–296. [PubMed: 19120120]
11. Ji Y, Snyder EM, Fridley BL, Salavaggione OE, Moon I, Batzler A, et al. Human phenylethanolamine N-methyltransferase genetic polymorphisms and exercise-induced epinephrine release. *Physiol Genomics.* 2008; 33:323–332. [PubMed: 18349382]
12. Ingelsson E, Larson MG, Vasani RS, O'Donnell CJ, Yin X, Hirschhorn JN, et al. Heritability, linkage, and genetic associations of exercise treadmill test responses. *Circulation.* 2007; 115:2917–2924. [PubMed: 17548724]
13. Yamano E, Isowa T, Nakano Y, Matsuda F, Hashimoto-Tamaoki T, Ohira H, et al. Association study between reward dependence temperament and a polymorphism in the phenylethanolamine N-methyltransferase gene in a Japanese female population. *Compr Psychiatry.* 2008; 49:503–507. [PubMed: 18702937]
14. 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]
15. Hook V, Metz-Boutigue MH. Protein trafficking to chromaffin granules and proteolytic processing within regulated secretory vesicles of neuroendocrine chromaffin cells. *Ann N Y Acad Sci.* 2002; 971:397–405. [PubMed: 12438158]
16. Haavik J, Blau N, Thony B. Mutations in human monoamine-related neurotransmitter pathway genes. *Hum Mutat.* 2008; 29:891–902. [PubMed: 18444257]
17. Ji Y, Salavaggione OE, Wang L, Adjei AA, Eckloff B, Wieben ED, et al. Human phenylethanolamine N-methyltransferase pharmacogenomics: gene re-sequencing and functional genomics. *J Neurochem.* 2005; 95:1766–1776. [PubMed: 16277617]
18. Kepp K, Juhanson P, Kozich V, Ots M, Viigimaa M, Laan M. Resequencing PNMT in European hypertensive and normotensive individuals: no common susceptibility variants for hypertension and purifying selection on intron 1. *BMC Med Genet.* 2007; 8:47. [PubMed: 17645789]
19. Cui J, Zhou X, Chazaro I, DeStefano AL, Manolis AJ, Baldwin CT, et al. Association of polymorphisms in the promoter region of the PNMT gene with essential hypertension in African Americans but not in whites. *Am J Hypertens.* 2003; 16:859–863. [PubMed: 14553966]
20. Alam A, O'Connor DT, Perianayagam MC, Kolyada AY, Chen Y, Rao F, et al. Phenylethanolamine N-methyltransferase gene polymorphisms and adverse outcomes in acute kidney injury. *Nephron Clin Pract.* 2010; 114:c253–c259. [PubMed: 20090367]
21. Estan L, Senard JM, Tran MA, Montastruc JL, Berlan M. Reserpine induces vascular alpha 2-adrenergic supersensitivity and platelet alpha 2-adrenoceptor up-regulation in dog. *Br J Pharmacol.* 1990; 101:329–336. [PubMed: 2175232]

22. Christiansen L, Tan Q, Iachina M, Bathum L, Kruse TA, McGue M, et al. Candidate gene polymorphisms in the serotonergic pathway: influence on depression symptomatology in an elderly population. *Biol Psychiatry*. 2007; 61:223–230. [PubMed: 16806099]
23. Hsiao RJ, Parmer RJ, Takiyuddin MA, O'Connor DT. Chromogranin A storage and secretion: sensitivity and specificity for the diagnosis of pheochromocytoma. *Medicine (Baltimore)*. 1991; 70:33–45. [PubMed: 1988765]
24. O'Connor DT. Plasma chromogranin A. Initial studies in human hypertension. *Hypertension*. 1985; 7(3 Pt 2):I76–I79. [PubMed: 3997234]
25. Takiyuddin MA, Cervenka JH, Hsiao RJ, Barbosa JA, Parmer RJ, O'Connor DT. Chromogranin A. Storage and release in hypertension. *Hypertension*. 1990; 15:237–246. [PubMed: 2406199]
26. Takiyuddin MA, Parmer RJ, Kailasam MT, Cervenka JH, Kennedy B, Ziegler MG, et al. Chromogranin A in human hypertension. Influence of heredity. *Hypertension*. 1995; 26:213–220. [PubMed: 7607727]
27. O'Connor DT, Takiyuddin MA, Printz MP, Dinh TQ, Barbosa JA, Rozansky DJ, et al. Catecholamine storage vesicle protein expression in genetic hypertension. *Blood Press*. 1999; 8:285–295. [PubMed: 10803489]
28. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics*. 1996; 6:243–250. [PubMed: 8807664]
29. Nackley AG, Shabalina SA, Tchivileva IE, Satterfield K, Korchynskiy O, Makarov SS, et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science*. 2006; 314:1930–1933. [PubMed: 17185601]
30. Stewart SH, Oroszi G, Randall PK, Anton RF. COMT genotype influences the effect of alcohol on blood pressure: results from the COMBINE study. *Am J Hypertens*. 2009; 22:87–91. [PubMed: 19023276]
31. Annerbrink K, Westberg L, Nilsson S, Rosmond R, Holm G, Eriksson E. Catechol O-methyltransferase val158-met polymorphism is associated with abdominal obesity and blood pressure in men. *Metabolism*. 2008; 57:708–711. [PubMed: 18442637]
32. Tulen JH, Boomsma F, Man in 't Veld AJ. Cardiovascular control and plasma catecholamines during rest and mental stress: effects of posture. *Clin Sci (Lond)*. 1999; 96:567–576. [PubMed: 10334962]
33. 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; 18:743–750. [PubMed: 18698227]
34. Prinz PN, Halter J, Benedetti C, Raskind M. Circadian variation of plasma catecholamines in young and old men: relation to rapid eye movement and slow wave sleep. *J Clin Endocrinol Metab*. 1979; 49:300–304. [PubMed: 222795]
35. Dishy V, Sofowora GG, Imamura H, Nishimi Y, Xie HG, Wood AJ, et al. Nitric oxide production decreases after salt loading but is not related to blood pressure changes or nitric oxide-mediated vascular responses. *J Hypertens*. 2003; 21:153–157. [PubMed: 12544447]
36. Hjemdahl P. Plasma catecholamines as markers for sympatho-adrenal activity in human primary hypertension. *Pharmacol Toxicol*. 1988; 63(Suppl 1):27–31. [PubMed: 3059342]
37. Zaitlen N, Pasaniuc B, Gur T, Ziv E, Halperin E. Leveraging genetic variability across populations for the identification of causal variants. *Am J Hum Genet*. 2010; 86:23–33. [PubMed: 20085711]

Figure. Resting plasma norepinephrine concentrations in *CYB561* rs2058203 genotypes (n=157) Horizontal lines denote medians, whiskers the interquartile ranges. *CYB561* genotype differences in resting plasma norepinephrine concentrations were significant before and after adjustment for potential confounders ($P<0.001$).

Table 1

Minor allele frequencies (MAF) in Caucasian and African-American subjects for 29 variants in genes related to catecholamine synthesis, metabolism, transport, and function.

Gene name	rs number	MAF	
		Caucasians (%)	African Americans (%)
<i>Tyrosine hydroxylase (TH)</i>	rs10770141	42.0	69.1
	rs10840490	11.6	16.4
	rs10770140	41.5	58.0
	rs11042962	45.0	13.6
	rs6356	36.7	12.3
<i>Dopamine β-hydroxylase (DBH)</i>	rs1611115	21.5	13.8
	rs739398	50.0	20.0
<i>Cytochrome b-561 (CYB561)</i>	rs2058203	46.0	60.8
<i>Chromogranin A (CHGA)</i>	rs7610	19.8	8.8
	rs9658655	8.8	17.6
	rs9658634	23.1	18.1
	rs875395	48.0	38.0
<i>Chromogranin B (CHGB)</i>	rs236155	43.2	61.5
	rs2821	31.0	28.1
	rs236153	42.6	62.0
<i>Vesicular monoamine transporter 1 (VMAT1, SLC18A1)</i>	rs1497020	24.2	8.4
<i>Vesicular monoamine transporter 2 (VMAT2, SLC18A2)</i>	rs363227	9.0	25.2
<i>Vacuolar ATP synthase catalytic α-subunit (ATP6V1A)</i>	rs12636577	30.1	28.3
<i>Vacuolar ATP synthase catalytic β-subunit (ATP6V1B1)</i>	rs17720303	17.2	10.2
<i>Monoamine oxidase A (MAOA)</i>	rs6323	20.2	19.5
<i>Monoamine oxidase B (MAOB)</i>	rs1799836	48.6	29.4
<i>Flavin containing monooxygenase 3 (FMO3)</i>	rs2266782	40.8	46.7

Gene name	rs number	MAF	
		Caucasians (%)	African Americans (%)
<i>Catechol-O-methyltransferase (COMT)</i>	rs4680	47.7	33.3
	rs165815	13.3	40.2
	rs4633	43.4	22.1
	rs4818	43.1	20.3
	rs737865	29.8	17.1
<i>Phenylethanolamine N-methyltransferase (PNMT)</i>	rs3764351	37.1	69.7
	rs876493	30.0	70.0

Table 2

Genetic variants associated with resting plasma norepinephrine (NE) concentrations and its increase during exercise (Δ NE).

Gene	Variant	Number of variant alleles			<i>P</i> -value, unadj.	<i>P</i> -value, adj.
		0	1	2		
Resting NE, pg/mL	<i>Cytochrome b-561 (CYB561)</i>	258±115 (n=31)	192±101 (n=85)	178±70 (n=41)	<0.001	<0.001
	<i>Vesicular monoamine transporter 2 (VMAT2=SLC18A2)</i>	191±91 (n=113)	230±122 (n=36)	253±130 (n=9)	0.014	0.016
	<i>Chromogranin A (CHGA)</i>	192±98 (n=122)	235±89 (n=31)	203±121 (n=5)	0.08	0.039
	<i>Phenylethanolamine N-methyltransferase (PNMT)</i>	188±91 (n=59)	211±102 (n=68)	216±122 (n=33)	0.17	0.038
	<i>Phenylethanolamine N-methyltransferase (PNMT)</i>	297±216 (n=59)	254±192 (n=68)	180±174 (n=33)	0.004	0.041
Exercise Δ NE, pg/mL	<i>Catechol-O-methyltransferase (COMT)</i>	255±202 (n=68)	222±178 (n=63)	312±234 (n=28)	0.37	0.035
		233±201 (n=62)	256±201 (n=72)	287±238 (n=24)	0.24	0.033

Genotype effects were adjusted for age, sex, race, BMI and fitness score, and, for the outcome Δ NE, additionally for resting NE concentration, in multiple linear regression analysis, assuming an additive mode of inheritance.

Table 3

Genetic variants associated with resting plasma epinephrine concentrations and its increase during exercise (Δ Epinephrine).

Gene	Variant	Number of variant alleles			P-value, unadj.	P-value, adj.
		0	1	2		
Resting Epinephrine, pg/mL	<i>Vacuolar ATP synthase catalytic β-subunit (ATP6V1B)</i>	rs17720303	19 $\pm$ 12 (n=112)	20 $\pm$ 11 (n=39)	37 $\pm$ 22 (n=8)	0.004
	<i>Catechol-O-methyltransferase (COMT)</i>	rs737865	21 $\pm$ 14 (n=82)	19 $\pm$ 10 (n=59)	15 $\pm$ 7 (n=16)	0.014
Exercise Δ Epinephrine, pg/mL	<i>Catechol-O-methyltransferase (COMT)</i>	rs4680	28 $\pm$ 33 (n=68)	25 $\pm$ 34 (n=63)	45 $\pm$ 43 (n=28)	0.021
		rs4633	23 $\pm$ 23 (n=62)	32 $\pm$ 42 (n=72)	39 $\pm$ 39 (n=24)	0.006
	<i>Monamine oxidase A (MAOA)</i>	rs6323	27 $\pm$ 28 (n=112)	31 $\pm$ 49 (n=26)	44 $\pm$ 47 (n=25)	0.036

Genotype effects were adjusted for age, sex, race, BMI and fitness score, and, for the outcome Δ Epinephrine, additionally for resting Epinephrine concentration, in multiple linear regression analysis, assuming an additive mode of inheritance.