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ORIGINAL ARTICLE

Urinary norepinephrine and epinephrine excretion rates are heritable, but not associated with office and ambulatory blood pressure

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Genetic and environmental contributions to urinary excretion rates of norepinephrine ($U_{NE}V$) and epinephrine (U_EV) and their association with blood pressure (BP) were investigated in 91 African American (mean age, 17.3 ± 2.6 years) and 101 European American (mean age, 18.7 ± 3.4 years) mono- and di-zygotic twins. Genetic modeling was performed using Mx software. $U_{NE}V$ (1.9 ± 1.3 μg h^{-1}) and U_EV (0.2 ± 0.2 μg h^{-1}) were highly correlated ($r = 0.81$, $P < 0.001$). Significant heritabilities for $U_{NE}V$ (0.68) and U_EV (0.74) without ethnic and gender effects were observed. The genetic correlation between $U_{NE}V$ and U_EV was 0.86. There was no clear pattern of correlations for $U_{NE}V$ and U_EV with BP measures in European Americans, but African Americans showed some inverse correlations of moderate size. Measurements of $U_{NE}V$ and U_EV provide a viable method for the study of sympathetic tone and are substantially heritable.
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INTRODUCTION

The catecholamines norepinephrine (NE) and epinephrine (E) mediate the early stress response via the sympathetic nervous system,[1,2] but they also have a key role in homeostatic blood pressure (BP) control[3,4] through the activation of adrenergic receptors located on the heart and the blood vessels. It has been suggested that abnormally increased sympathetic function may lead to the development and progression of a hypertensive state[3,5] perhaps initially by influencing transient BP increases to environmental stress.[6,7] Indeed, several studies indicate that this so-called sympathetic overdrive is a hallmark of essential hypertension[8,9] and several mechanisms and consequences have been proposed, with particular emphasis on its role in the development of target organ damage.[10] The substantial dysregulation of sympathetic function in patients with essential hypertension ,as well as in their normotensive offspring, has incited research into susceptibility genes and chromosome loci being associated or linked with sympathetic function and essential hypertension.[11]

As such, identification of genes that influence catecholamine levels (in blood) or excretion rates (in urine) as proxies for sympathetic nervous system activity may improve our insight into the potential role of the sympathetic system in the early stages of essential hypertension. A prerequisite of such gene-finding studies is that heritability of the trait of interest is firmly established. However, very few studies investigated heritability of NE and E levels or excretion rates and none of these examined to what extent genetic and environmental influences on these catecholamines overlap.[5,6,12–14]

In the present study, we have used overnight urinary excretion rates of NE ($U_{NE}V$) and E (U_EV) as measures of basal sympathetic activity. The overnight urine collections were analyzed by radio immune assay (RIA) for NE and E. We aimed to determine the genetic and environmental contributions to $U_{NE}V$ and U_EV and their association using bivariate genetic modeling, and examine the associations of $U_{NE}V$ and U_EV with office and ambulatory BP in 91 African-American and 101 European-American adolescent and young adult twins from the south-eastern USA.

METHODS

Subjects

The present study comprised subjects from the Georgia Cardiovascular Twin Study.[15–17] After excluding 28 individuals who did not have overnight urine volume data, which was used to estimate $U_{NE}V$ and U_EV, participants were 101 European-American and 91 African-American twins (84 pairs and 24 individuals) including monozygotic (MZ) pairs and dizygotic (DZ) pairs

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of the same and the opposite sex (mean age: 18.0 ± 3.1 years; range: 12.0 to 29.2 years). The Medical College of Georgia Institutional Review Board approved the protocol. Written informed parental and subject consents were obtained from each participant family. Zygosity determination and recruitment have been described previously,[17–19] as have been the criteria to classify subjects as European- or African-American.[20] All of the subjects were apparently healthy, based on parental report of the children's medical history. None of the subject used any antihypertensive medication.

Protocol

Eligible twins (that is, healthy with no chronic illness, not on any prescribed medication including contraceptives and not pregnant) were sent instructions on how to collect and bring an overnight urine sample at the start of the testing day, which was used to determine $U_{NE}V$ and U_EV in the current study.[21] Twins recorded the last time they voided before retiring to bed and the time of their morning void, which they brought into the laboratory. The mean (s.d.) collection time for these overnight samples in our study was 7.64 (1.71) h. Twins arrived at the laboratory in the morning between 8:00 and 9:00 AM in pairs. The subjects (and their parents if the twins are <18) were instructed to refrain from consuming foods or beverages (except water), tobacco and alcohol for 11 h before the visit (that is, fasting state) and to refrain from taking non-prescription medications for 2 days before the visit. A minority of twin pairs was scheduled for afternoon visits. These twins were told to refrain from consuming food and beverages (except water), tobacco and alcohol for 5 h before their visit.

Anthropometric measures

Anthropometric measurements were obtained using previously established protocols.[22] Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Body surface area (BSA) was calculated according to the Mosteller formula[23] as the square root of [height (cm) × weight (kg)/3600].

$U_{NE}V$ and U_EV measures

RIA kits (ALPCO, Salem, NH) were used to determine overnight urine concentrations of NE and E. The average intra-assay coefficients of variation for this kit are 4.3 and 9.3% and the inter-assay coefficients of variation are 8.1 and 5.9% for NE and E, respectively. For NE, we tested the performance of the RIA in 10 samples across a wide range of values against the high-performance liquid chromatography method of analysis and found virtually identical results ($r = 0.994$) (Figure 1). Excretion rates of NE ($U_{NE}V$) and E (U_EV) (in μg/h) were calculated as: (concentration × overnight volume)/overnight collection duration.

Office BP recordings

Office systolic BP (SBP) and diastolic BP (DBP) were measured with the Dinamap Vital Signs Monitor (model 1864 SX; Criticon Incorporated, Tampa, FL, USA). BP measurements were taken at the 11th, 13th and 15th minutes during a 15-minute supine relaxation period. The average of the last two readings was used to represent office SBP and DBP values.[20]

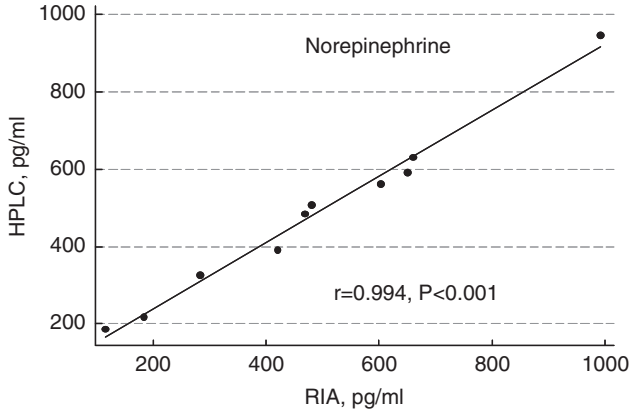

Figure 1 Association of NE concentration determined by RIA and high-performance liquid chromatography ($r = 0.994$).

Ambulatory BP recordings

Our procedures for ambulatory BP recordings have previously been described in detail.[24,25] Briefly, an ambulatory BP monitor was fitted to the non-dominant arm (model 90207, SpaceLabs, Redmond, WA, USA). Measures were obtained every 20 min during the daytime (08:00 to 22:00 h), and every 30 min during the night time (00:00 to 06:00 h). Transitional periods from 06:00 to 08:00 h and 22:00 h to midnight were not included in daytime and night time period. Adequacy of recordings was based on acceptable readings using previously established criteria[24] for $\geqslant 14$ readings over the 14 h designated as daytime and $\geqslant 6$ readings over the 6 h designated as the night time, as suggested by the European Society of Hypertension Working Group on Blood Pressure Monitoring.[26] For the calculation of 24-h mean values (for which transition periods were included), 1-h mean values were first calculated. Subsequently these 1-h mean values were averaged.

Statistical analysis

The major aims of our study were threefold. First, we tested the association of $U_{NE}V$ and U_EV with office and ambulatory BP using correlational analyses. Second, we used univariate model fitting analyses to estimate the relative influence of genetic and environmental factors on individual differences in $U_{NE}V$ and U_EV and investigated gender and ethnicity differences in those variance components. Third, we used a bivariate model including both $U_{NE}V$ and U_EV to estimate the following: (1) the extent to which the phenotypic correlation between $U_{NE}V$ and U_EV can be explained by genetic and/or environmental factors influencing both traits; (2) the extent to which genetic and environmental effects on $U_{NE}V$ are the same or different from those affecting U_EV.

Correlational analyses. Before testing the association of $U_{NE}V$ and U_EV with office and ambulatory BP $U_{NE}V$ and U_EV were log-transformed and adjusted for BSA. BP measures (office SBP/DBP, 24 h SBP/DBP, night time SBP/DBP and daytime SBP/DBP) were adjusted for age and BMI. For both European and African Americans, correlations were calculated overall and in males and females separately. The non-independence between twins was taken into account in calculating the significance of the correlations.

Univariate modeling of twin data. Structural equation modeling was the primary method of analysis. Structural equation modeling is based on the comparison of the variance–covariance matrices in MZ and DZ twin pairs and allows separation of the observed phenotypic variance into its genetic and environmental components: additive (A) or dominant (D) genetic components and common (C) or unique (E) environmental components.[27] We used the ADE model as our initial full model, if correlations among MZ twins substantially exceeded twice those among DZ twins, which would indicate dominance variance; otherwise we used the ACE model.[27] We tested the existence of gender and ethnic differences in the influences of genetic and environmental factors on $U_{NE}V$ and U_EV, as described in detail elsewhere.[28]

Bivariate modeling of twin data. A bivariate Cholesky decomposition was used to model the covariance between $U_{NE}V$ and U_EV.[29] This model allows determination of the extent to which the covariance (or phenotypic correlation, r_p) can be explained by genetic or environmental factors influencing both traits. Genetic and environmental correlations between two traits can be calculated. The genetic correlation (r_g) between two traits gives an indication of the amount of overlap between (sets of) genes influencing those traits. r_g is calculated as the (additive) genetic covariance (COV_A) between two traits divided by the square root of the product of the total genetic variance components (V_A) of each of the traits. The genetic correlation between two traits therefore equals: $r_g = COV_A\ (\text{trait 1, trait 2})/\sqrt{(V_A\text{trait1} * V_A\text{trait2})}$. Common and unique environmental correlations (r_c and r_e, respectively) are calculated in a similar fashion. The genetic and environmental factor loadings and correlations can be used to calculate the proportion of the phenotypic correlation explained by genetic and environmental factors.

The bivariate Cholesky decomposition is shown in Figure 2. Estimates for the path coefficients, that is, the model parameters (for example, a_{11}, c_{11}, e_{11}), are obtained by using a fit function that minimizes the difference between the observed covariance matrix and the expected covariance matrix implied by the model. In a model without dominance effects, the relative contribution of genetic variance to the total variance in U_EV, also known as its heritability, is the effect of the additive genetic factor A, and is obtained as the ratio $a_{11}^2/(a_{11}^2+c_{11}^2+e_{11}^2)$. The heritability of $U_{NE}V$ is the summed effect of the genetic factors A1 and A2, and is obtained as the ratio $(a_{21}^2+a_{22}^2)/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$. The percentage of total $U_{NE}V$ variance caused by genetic effects also influencing U_EV is calculated as $a_{21}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$. Finally, the percentage of total $U_{NE}V$ variance caused by genetic effects that are specific to $U_{NE}V$ is equal to $a_{22}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$.

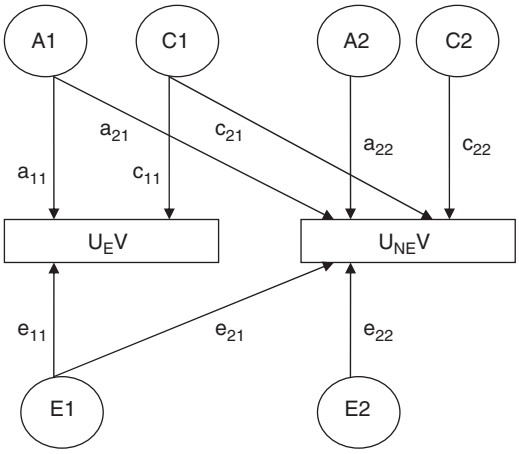

Figure 2 Path diagram for a bivariate model. For clarity only one twin is depicted. A1, A2 = Genetic variance components; C1, C2 = common environmental variance components; E1, E2 = unique environmental variance components; a_{11} through a_{22} = genetic path coefficients (or factor loadings) of which a_{22} represents specific genetic influences on $U_{NE}V$; c_{11} through c_{22} = common environmental path coefficients (or factor loadings) of which c_{22} represents specific common environmental influences on $U_{NE}V$; e_{11} through e_{22} = unique environmental path coefficients (or factor loadings) of which e_{22} represents specific unique environmental influences on $U_{NE}V$. Formula for the different heritability estimates are as follows:

$$h^2 \text{ total } (U_EV) = a_{11}^2/(a_{11}^2+c_{11}^2+e_{11}^2)$$

$$h^2 \text{ total } (U_{NE}V) = (a_{21}^2+a_{22}^2)/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$$

$$h^2 \text{ shared } (U_{NE}V \text{ explained by } U_EV) = a_{21}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$$

$$h^2 \text{ specific } (U_{NE}V) = a_{22}^2/(a_{21}^2+a_{22}^2+c_{21}^2+c_{22}^2+e_{21}^2+e_{22}^2)$$

Before the analysis, $U_{NE}V$ and U_EV were log-transformed to obtain a better approximation of the normal distribution. Mean values of log-transformed $U_{NE}V$ and U_EV were adjusted for age, gender, ethnicity and body size (that is, BSA) before using the residuals in model fitting. Deterioration in model fit, after each term was dropped from the full model, was assessed to determine the significance of variance components A and C (or D). Standard hierarchic χ^2-tests were used to select the best fitting models in combination with Akaike's information criterion (AIC = χ^2-2 df). The model with the lowest AIC reflects the best balance of goodness of fit and parsimony[27]. Effects of gender, ethnicity and their interaction (ethnicity x gender) on mean values were tested while adjusting for age using generalized estimating equations. Generalized estimating equations take the non-independence between twins into account and yields unbiased standard errors and *P*-values.[30] Preliminary analyses and generalized estimating equationss were performed using STATA 10.0 (Stata Corp., College Station, TX, USA). Genetic modeling was carried out with Mx, a computer program specifically designed for the analysis of twin and family data.[31]

RESULTS

Sample and Demographics

Table 1 shows the general characteristics stratified for ethnicity and gender. Males were taller and heavier than females, but the weight difference was larger in European Americans. Compared with their male counterparts, BMI was larger in African-American females but smaller in European-American females. Neither $U_{NE}V$ nor U_EV showed significant effects of ethnicity, gender, age or BSA. None of the traits showed significant differences between MZ and DZ twins.

Cross-trait correlations of office and 24-h, daytime and night-time ambulatory BP with $U_{NE}V$ and U_EV are shown in Table 2. $U_{NE}V$ and U_EV were highly correlated in both European Americans ($r = 0.77$; 0.85 and 0.62 in males and females, respectively) and African Americans ($r = 0.84$; 0.86 and 0.80 in males and females, respectively). There was no clear pattern for correlations of BP measures with either $U_{NE}V$ or U_EV in European Americans. However, in African Americans overall moderately negative correlations were observed, which were especially prominent in African-American females for 24-h and daytime ambulatory BP values.

Table 3 presents the twin correlations for $U_{NE}V$ and U_EV of each zygosity group in European and African Americans as well as in the overall sample. MZ correlations showed consistently higher values than DZ correlations, indicating an important contribution of genetic factors. The DZ correlations of $U_{NE}V$ were less than half of the corresponding MZ correlations, suggesting presence of dominance (D) effects.

Table 1 General characteristics, U_EV and $U_{NE}V$ of 101 European Americans and 91 African Americans

	European Americans		*African Americans*		*Ethnicity and gender effects*		
	Males	*Females*	*Males*	*Females*	*Ethnicity* P	*Gender* P	*Ethnicity × Sex* P
N, individuals	52	49	55	36	—	—	—
Age, year	18.7 ± 3.9	18.6 ± 2.8	17.5 ± 2.6	17.0 ± 2.6	NS	NS	NS
Height, m	1.76 ± 0.08	1.62 ± 0.07	1.76 ± 0.09	1.64 ± 0.06	NS	<0.001	NS
Weight, kg	75.9 ± 17.9	62.9 ± 17.8	75.5 ± 19.8	74.9 ± 22.5	NS	0.001	0.016
BMI, kg m^{-2}	24.4 ± 4.8	23.7 ± 5.6	24.3 ± 5.3	27.7 ± 7.8	NS	NS	0.025
U_EV, μg h^{-1}	0.23 ± 0.23	0.16 ± 0.12	0.29 ± 0.31	0.25 ± 0.23	NS	NS	NS
$U_{NE}V$, μg h^{-1}	1.86 ± 1.33	1.58 ± 0.71	2.07 ± 1.54	1.96 ± 1.50	NS	NS	NS

Abbreviations: BMI, body mass index; NS, not significant; U_EV, urine epinephrine excretion rate; $U_{NE}V$, urine norepinephrine excretion rate.
Values are mean ± s.d. unless stated otherwise.
Weight, BMI, U_EV and $U_{NE}V$ were log-transformed before analysis.

Table 2 Correlations of $U_{NE}V$ and U_EV with office BP, and 24 h, daytime and night time ambulatory BP

	European American overall (male/female)		*African American overall (male/female)*	
N, *individuals*	*101(52/49)*		*91(55/36)*	
Measures	$U_{NE}V$	U_EV	$U_{NE}V$	U_EV
U_EV	**0.77(0.85/0.62)**	…	**0.84(0.86/0.80)**	…
Office SBP	−0.18(−0.22/−0.11)	−0.13(−0.18/−0.10)	−**0.23**(−**0.31**/−0.24)	−**0.21**(−0.24/−0.32)
Office DBP	0.002(−0.08/0.12)	−0.03(−0.14/0.18)	−0.04(−0.05/−0.002)	−0.08(−0.10/−0.02)
24 h SBP	−0.08(−0.11/−0.11)	−0.10(−0.10/−0.20)	−0.18(−0.10/−**0.56**)	−0.22(−0.03/−**0.73**)
24 h DBP	0.12(0.15/0.18)	−0.06(0.13/−0.32)	−0.24(0.08/−**0.51**)	−**0.33**(−0.06/−**0.61**)
Daytime SBP	−0.06(−0.11/−0.09)	−0.07(−0.08/−0.17)	−0.14(0.06/−**0.57**)	−0.20 (0.11/−**0.79**)
Daytime DBP	0.18(0.18/0.25)	0.01(0.15/−0.17)	−0.22(0.16/−**0.53**)	−0.27(0.08/−**0.63**)
Night time SBP	−0.13(−0.20/−0.10)	−0.19(−0.24/−0.18)	−0.19(−0.35/−0.38)	−0.18(−0.29/−0.34)
Night time DBP	−0.02(0.01/0.04)	−0.17(0.06/−0.36)	−0.17(−0.16/−0.28)	−0.27(−0.35/−0.21)

Abbreviations: DBP, diastolic BP; SBP, systolic BP; U_EV, urine epinephrine excretion rate; $U_{NE}V$, urine norepinephrine excretion rate.
$U_{NE}V$ and U_EV were log-transformed and adjusted for BSA;
All BP measures were adjusted for age and BMI before the analysis;
There are 29 missing values in European-American males, 26 in European-American females, 34 in African-American males and 17 in African-American females for the ambulatory BP measures.
Significant correlations ($P<0.05$) are shown in bold.

Table 3 Twin correlations for each zygosity group in European and African Americans

	European Americans		*African Americans*		*Overall*	
Measures	*MZ*	*DZ*	*MZ*	*DZ*	*MZ*	*DZ*
Pairs, *N*	23	22	24	15	47	37
U_EV, μg h^{-1}	0.69	0.54	0.67	0.42	0.68	0.48
$U_{NE}V$, μg h^{-1}	0.75	0.39	0.59	0.20	0.67	0.28

Abbreviations: DZ, dizygotic twins; MZ, monozygotic twins.
$U_{NE}V$, urine norepinephrine excretion rate; U_EV, urine epinephrine excretion rate.
$U_{NE}V$ and U_EV were log-transformed and adjusted for age, ethnicity, sex and BSA before analysis.

Univariate modeling

Parameter estimates of the best fitting models for $U_{NE}V$ and U_EV are shown in Table 4. Variance component estimates were collapsed across gender and ethnicity, because genetic and environmental parameter estimates were not significantly different between males and females or between European and African Americans (data not shown). Both traits were significantly heritable with heritabilities of 68% (50–79%) for $U_{NE}V$ and 74% (59–83%) for U_EV. The model including additive genetic and unique environmental effects without gender or ethnicity differences provided the best fit. That is, dropping common environmental (C) or dominant genetic (D) effects had virtually no effect on model fit indicating they do not contribute significantly.

Bivariate model

Subsequently, we performed bivariate model fitting to estimate to which extent phenotypic correlations can be explained by genetic or environmental factors that influence both $U_{NE}V$ and U_EV (Figure 2). As the bivariate model can also be used to estimate the variance components for each individual trait, we found very similar estimates of heritability as in the univariate model (Figure 3). The phenotypic correlation between $U_{NE}V$ and U_EV was high ($r_p = 0.81$). The genetic correlation between $U_{NE}V$ and U_EV was even higher at 0.86, while unique environmental correlations were somewhat lower but still highly significant ($r_e = 0.71$) (Figure 3). Not surprisingly, decomposition of the phenotypic correlation into its genetic and environmental parts showed it to be largely (73%) due to genetic factors.

Figure 4 presents sources of variance of $U_{NE}V$ based on the best-fitting bivariate model. Eighteen percent of the total variance of $U_{NE}V$ could be attributed to specific genetic factors that only influence $U_{NE}V$. Genetic factors that also influence U_EV contributed to the total variance for $U_{NE}V$ to a large extent (50%). Comparatively, environmental factors that also influenced U_EV contributed substantially less to the total variance of $U_{NE}V$ (16%).

DISCUSSION

The present study shows significant heritabilities for $U_{NE}V$ (0.68, 95% CI: 0.50–0.79) and U_EV (0.74, 95% CI: 0.59–0.83) using the best-fitting univariate model. The genetic correlation was 0.86 (95% CI: 0.76–0.97), indicating a large overlap in the genes influencing $U_{NE}V$ and U_EV. Fifty percent of the variance in $U_{NE}V$ was explained by genes that also influence individual differences in U_EV.

Several groups have investigated the heritability of catecholamines in blood and urine. For instance, Williams *et al.*[5] reported substantial heritability (h^2) estimates of blood NE ($h^2 = 57\%$) and E levels ($h^2 = 74\%$ for males and $h^2 = 64\%$ for females), based on data of 109 twin pairs. In addition, Jedrusik *et al.*[12] studied 39 MZ twin pairs and 37 age-matched same-gender DZ twin pairs to determine the effects of genetic factors on sympathetic activity in twins. Catecholamines in blood and urine were used as measures of sympathetic activity, and genetic contributions were 42 and 76% for NE and 69 and 65% for E, respectively. Finally, Zhang *et al.*[14] found that heritabilities for plasma and urinary E or NE ranged from 0.33 to 0.61 in a sample of Caucasian twins.[14] Similar results were reported more recently in a slightly larger sample showing that both plasma and urinary E and NE were significantly heritable and ranged from 0.49 to 0.72.[6,13]

We are not aware of other heritability studies estimating the basal sympathetic tone by means of overnight urinary excretion rates of both NE and E. In principle, catecholamine levels in blood may more accurately reflect sympathetic activity than overnight urinary excretion rates of NE and E. However, collection of overnight urine for measurement of catecholamine excretion rates has a number of advantages. Catecholamine excretion rates provide an integrated measure of 'steady-state' operating levels of the sympathetic nervous

Table 4 Parameter estimates and 95% CIs of best-fitting univariate models

	h^2 (95%CI)	c^2or d^2 (95%CI)	e^2 (95%CI)	-2LL	df	$\Delta\chi^2$	Δdf	P	AIC
U_EV, μg/h									
ACE	0.60 (0.13–0.83)	0.13 (0.00–0.54)	0.27 (0.17–0.43)	440.685	183				
AE	**0.74 (0.59–0.83)**		**0.26 (0.17–0.41)**	**440.945**	**184**	**0.259**	**1**	**0.611**	**−1.741**
CE		0.59 (0.43–0.71)	0.41 (0.29–0.57)	446.891	184	6.206	1	0.013	4.206
E	0.00 (0.00–0.00)	0.00 (0.00–0.00)	1.00 (1.00–1.00)	480.582	185	39.896	2	0.000	35.896
$U_{NE}V$, μg/h									
ADE	0.45 (0.00–0.79)	0.23 (0.00–0.79)	0.32 (0.21–0.50)	355.981	183				
AE	**0.68 (0.50–0.79)**		**0.32 (0.21–0.50)**	**356.136**	**184**	**0.155**	**1**	**0.694**	**−1.845**
E	0.00 (0.00–0.00)	0.00 (0.00–0.00)	1.00 (1.00–1.00)	386.584	185	30.603	2	0.000	26.603

Abbreviations: $U_{NE}V$, urine norepinephrine excretion rate; U_EV, urine epinephrine excretion rate; h^2, heritability; c^2, common environmental variance; d^2, dominant genetic variance; e^2, unique environmental variance; CI, confidence interval.
U_EV and $U_{NE}V$ were log-transformed and adjusted for age, ethnicity, sex and BSA prior to analysis.
Best fitting models are in bold.

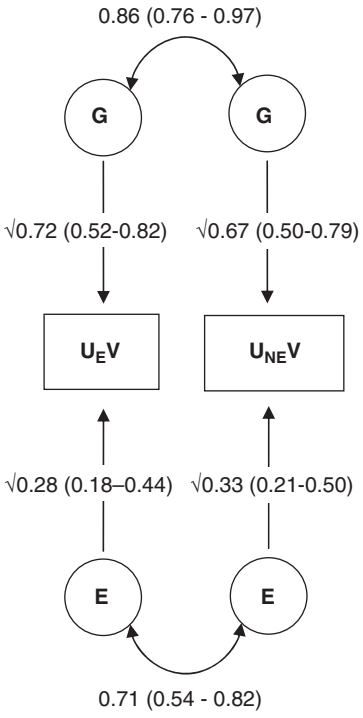

Figure 3 Best-fitting bivariate model for U_EV and $U_{NE}V$. For clarity, only one twin is depicted. Factor loadings (or path coefficients) are expressed as square roots (± their 95% confidence intervals) to make clear that squaring those factor loadings yields estimates of genetic and environmental variance components as shown in text. The genetic (r_g) and environmental (r_e) correlations between U_EV and $U_{NE}V$ are shown above and below the double-headed arrows. *A* indicates additive genetic factor; *E*, unique environmental factor.

system, capturing the more chronic effects of stress and have been widely used in studies of stress and CVD risk,[32–38] including McEwen and Seeman's allostatic load studies.[32,33] Furthermore, overnight urine can be reliably collected by adolescents, and the potential confounding effects of physical activity are minimized because subjects generally spend this time at home, mostly in bed.[39] Thus, we expected that overnight urinary excretion rates of NE and E might be less influenced by environmental factors such as mental stress and physical exercise, making it easier to delineate the genetic contribution to basal sympathetic activity. This is indeed supported by our present results, showing heritabilities on par or exceeding those previously reported for blood[5,12] and urinary [12] catecholamines measured during the daytime. Urinary catecholamine levels in the studies by Zhang *et al.*[14] and Rao *et al.*[6,40,41] were normalized for creatinine excretion in the same sample, but it is not clear whether 24 h, overnight or spot urine was used, which complicates meaningful comparisons with our study.

Several studies have reported associations between genetic polymorphisms and catecholamine secretion. One study found that the Gly364Ser polymorphism in the catecholamine storage vesicle protein chromogranin A gene *CHGA* displayed diminished inhibition of catecholamine secretion from cultured neurons. Renal NE excretion was diminished by around 26% and E excretion by around 34% in Gly/Ser heterozygotes.[40] Another study performed in a similar sample suggested that common tyrosine hydroxylase (*TH*) promoter polymorphisms with variants at C-824T and A-581G showed significant associations with urinary catecholamine excretion.[41] C-824T also exerted significant pleiotropic effects on the coupling between blood pressure response to cold stress and urinary NE. In addition, the second most frequent promoter haplotype (TGGG), based on four common promoter SNPs (C-824T, G-801C, A-581G, and G-494A) displayed copy number–dependent effects on urinary E ($P = 0.0044$, % variance explained = 5.7%) and NE excretion ($P = 0.0125$, % variance explained = 4.06%). This haplotype also showed pleiotropy, increasing both NE excretion and blood pressure during stress.[6]

Using the same Georgia Cardiovascular Twin cohort, we have performed several candidate gene studies investigating genes in the sympathetic nervous system pathway, including adrenergic receptor and signal transduction genes, for association with BP regulation and hypertension risk.[18,20] Polymorphisms of genes coding for the enzymes involved in synthesis and degradation of catecholamines could also be involved. For instance, *TH* coding for tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamines, was found to be associated with essential hypertension in a case-control study.[42] Another example is phenylethanolamine *N*-methyltransferase (*PNMT*), coding for the terminal enzyme of the catecholamine synthetic pathway, which catalyzes the synthesis of E from NE. Methods in comparative genomics have identified a genetic locus associated with BP regulation in the stroke-prone spontaneously hypertensive rat on rat chromosome 10 in a conserved syntenic group that corresponded to a gene encoding *PNMT* on chromosome 17q21-q22 in humans.[43]

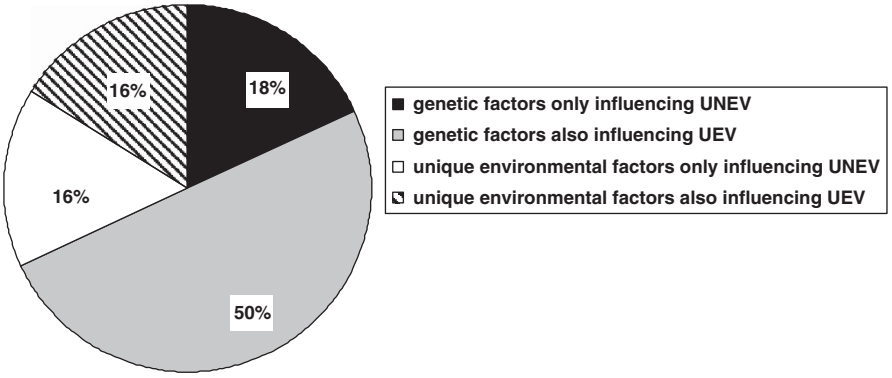

Figure 4 A decomposition of the variance of $U_{NE}V$ in its genetic and environmental components (that is, genetic and environmental sources of individual differences in $U_{NE}V$) is shown. Because we used a bivariate model in which both $U_{NE}V$ and U_EV were included, we could further discriminate between genetic and environmental factors that also influenced U_EV or were specific to $U_{NE}V$. Results are those of the best-fitting bivariate model as shown in Figure 2.

It is likely that many genes in the sympathetic system are involved in heritable NE and E excretion and these may also contribute to essential and stress-induced hypertension. Previously, we have proposed a model wherein chronic environmental stress in concert with genetic predisposition and factors, such as gender and ethnicity, might eventually lead to essential hypertension, type 2 diabetes and cardiovascular disease.[18,44] The sympathetic system is a key component of this model and the current study suggests that genetic factors are strong determinants of basal sympathetic activity. On the basis of this model, we expected positive correlations of $U_{NE}V$ and U_EV with BP measures, which is not what we observed. In spite of our wide array of BP measures, no associations were found in European Americans and, if anything, correlations in African Americans were moderately negative. One possible explanation of this latter result may be that those African Americans excreting more catecholamines are also better capable of (down) regulating their BP, for example, through more efficient sodium excretion and volume regulation.

Several limitations need to be recognized. First, as the Georgia Cardiovascular Twin Study is comprised of youth and young adults, the generalizability of these results to other adult populations remains to be determined. Second, we did not have information on the quality of the previous night's sleep. The prospect of participating in our study the next day may have caused some anticipatory stress, which could have affected sleep quality and $U_{NE}V$ and U_EV. Finally, not all subjects with excretion rates also had ambulatory BP measures available (Table 2). Further studies with larger sample sizes are warranted to more definitively determine the relation between overnight urinary excretion rates of NE and E and BP.

In summary, individual differences in both $U_{NE}V$ and U_EV and the association between them are substantially heritable, indicating that measurements of $U_{NE}V$ and U_EV provide a viable method for the study of sympathetic tone in genetic epidemiological research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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