Bivariate Genetic Modeling of Cardiovascular Stress Reactivity: Does Stress Uncover Genetic Variance?

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Objective: To test the existence of gene-by-stress interaction by assessing cardiovascular stress reactivity in monozygotic and dizygotic twins. **Methods:** We studied 160 adolescent (mean age 16.7 ± 2.0 years; range 13–22 years) and 212 middle-aged twin pairs (mean age 44.2 ± 6.7 years; range 34–63 years). Systolic (SBP) and diastolic (DBP) blood pressure, heart rate (HR), pre-ejection period (PEP), and respiratory sinus arrhythmia (RSA) were measured at rest and during a choice reaction time and a mental arithmetic task. We used a bivariate analysis of the resting and mean stress levels to test for gene-by-stress interaction, which can be caused by the emergence of new genetic variance specific to stress or by stress-induced amplification of the existing genetic variance at rest. **Results:** Genetic factors significantly contributed to individual differences in resting SBP, DBP, HR, PEP, and RSA levels in the adolescent (heritability range 0.31–0.70) and middle-aged (heritability range 0.32–0.64) cohorts. The effect of these genetic factors was amplified by stress for all variables in the adolescent cohort, and for SBP in the middle-aged cohort. In addition, stress-specific genetic variation emerged for HR in both cohorts and for PEP and SBP in the adolescent cohort. Heritability of stress levels of SBP, DBP, HR, PEP, and RSA ranged from 0.54 to 0.74 in the adolescents and from 0.44 to 0.64 in the middle-aged cohort. **Conclusions:** Stress uncovers genetic variance in BP, HR, and cardiac sympathovagal balance through the emergence of new stress-specific genetic effects and the amplification of existing genetic effects that also affect the resting values.
Key words: twin study, heritability, heart rate, blood pressure, pre-ejection period, respiratory sinus arrhythmia.

SBP = systolic blood pressure; **DBP** = diastolic blood pressure; **HR** = heart rate; **PEP** = pre-ejection period; **RSA** = respiratory sinus arrhythmia; **MZ** = monozygotic; **DZ** = dizygotic; **MZM** = monozygotic males; **MZF** = monozygotic females; **DZM** = dizygotic males; **DZF** = dizygotic females; **DOS** = dizygotic twin pairs of opposite sex; $\mathbf{h^2}$ = heritability; **LDL-C** = low-density lipoprotein cholesterol; **HDL-C** = high-density lipoprotein cholesterol; **BMI** = body mass index; **ECG** = electrocardiogram; **ICG** = impedance cardiogram; **PCG** = phonocardiogram.

INTRODUCTION

Twin studies have demonstrated a significant genetic contribution to cardiovascular morbidity (1) and mortality (2,3). These genetic influences are likely to arise through genetic effects on behavioral and physiological risk factors, i.e., smoking (4), exercise behavior (5), body mass index (BMI) (6), diastolic (DBP) and systolic blood pressure (SBP) (7,8), plasma low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels (9). Heritability (h^2) estimates for these established risk factors are ≥50% in most adult twin samples and these estimates remain remarkably similar across the adult life span (10,11). Population variance in a number of other suspected risk factors, including insulin sensitivity (12), coagulation/fibrinolysis balance (13,14), inflammation (15), and heart rate (HR) variability (16) have also shown to be subject to substantial genetic variation.

In addition to the above risk factors, physiological reactivity to mental and emotional stressors has long been regarded as a potential contributor to individual differences in cardiovascular disease risk (17). Psychometric studies have established satisfactory temporal stability of the commonly used cardiovascular reactivity measures, particularly when aggregated over multiple stressors (18,19). Prospective studies have shown that these individual differences in cardiovascular reactivity predict future hypertension (20–22) and atherosclerosis (23,24). An obvious next question is whether the genetic risk for cardiovascular disease is mediated in part through genetic factors that influence individual differences in response to stress.

Turner and Hewitt (25,26) reviewed several studies that explored the genetic and environmental origins of individual differences in HR and BP reactivity to psychological challenge by using the classic twin study methodology. Their conclusion was that heritability of HR and BP reactivity is substantial and there is very little evidence of shared environmental influence. Further twin studies of cardiovascular reactivity have later confirmed heritability of HR and BP reactivity, but estimates for DBP, SBP, and HR reactivity to the same task can be very different across studies or, within the same study, across different tasks, and have ranged from 0.00 to 0.85 (25–33). The small sample size of some of the twin studies may partly account for this, but the large age range across studies may also contribute because genetic effects on reactivity need not be stable across age. To address these issues, we examined genetic contributions to HR and BP reactivity in a large adolescent and a large adult twin cohort. Furthermore, only one study has addressed the heritability of cardiac vagal reactivity (34) and no study to date has looked at the heritability of cardiac sympathetic reactivity. We, therefore, added reactivity of two indices of sympathovagal balance: pre-ejection period (PEP) and respiratory sinus arrhythmia (RSA) (35–37).

In genetic designs, the added value of the use of stress tasks depends on their ability to uncover genetic variance that remains hidden in an analysis of resting values alone. However, when analyzed as a change score, the heritability of reactivity will reflect an inseparable mix of newly emerging genetic or environmental influences during stress and an am-

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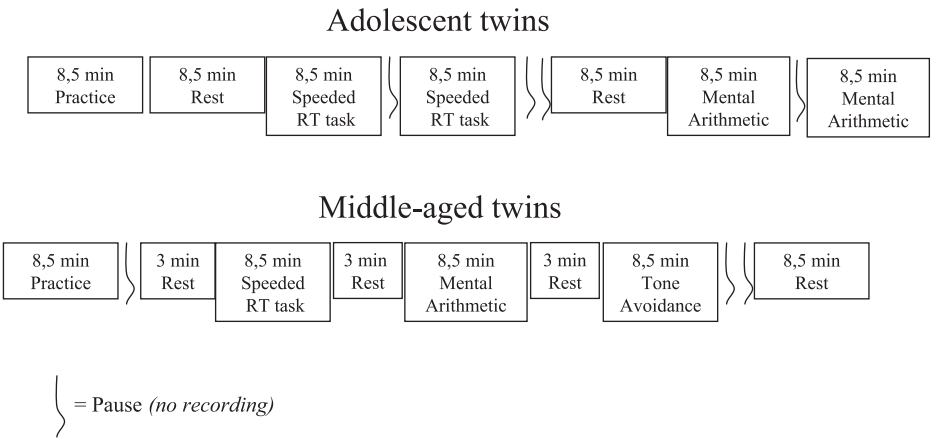

Figure 1. The timelines for the experimental protocol for reactivity testing in the middle-aged and adolescent twins. Short pauses varying in duration from 2 to 10 minutes were inserted to repeat the instructions or the refasten electrodes/respiration band to bolster signal recording. RT = reaction time.

plification or deamplification of genetic or environmental influences already present at rest. Emerging genes are ones that are expressed only during stress. They contribute to the heritability of a cardiovascular trait only when it is measured under stressful conditions. Amplified genes are ones that have an effect on individual differences in a cardiovascular trait at rest, but these effects become stronger under stress. To explicitly test for emergence, Ditto (27) and Busjahn et al. (30) used a bivariate approach on resting levels and reactivity scores to test if new genetic factors emerged during stress. We expand on their approach by using a bivariate analysis of resting and stress levels, which allows a more transparent test of emergence as well as amplification.

METHODS

Subjects

A sample of 160 adolescent twin pairs (age range 13–22 years) was measured between 1985 and 1988 and a sample of 212 middle-aged twin pairs (age range 34–63 years) was measured between 1992 and 1994. Informed written consent was obtained from all subjects, and approval for the protocols of both studies was obtained from the Medical Ethics Committee of the Vrije Universiteit. In all same-sex twin pairs, zygosity was determined from DNA polymorphisms. Grouped according to their zygosity and sex, the sample consisted of 35 pairs of adolescent and 46 pairs of middle-aged monozygotic males (MZM), 31 pairs of adolescent and 37 pairs of middle-aged dizygotic males (DZM), 35 pairs of adolescent and 49 pairs of middle-aged monozygotic females (MZF), 30 pairs of adolescent and 40 pairs of middle-aged dizygotic females (DZF), and 29 pairs of adolescent and 40 middle-aged dizygotic twin pairs of opposite sex (DOS). For middle-aged twins, valid BP measurements were not available in one member of three different twin pairs (1 MZM, 1 DZF, 1 DOS). No valid RSA data were available for eight twins (from 2 DZM, 4 MZF, and 2 DOS) and no valid PEP data were available for one twin (from an MZF pair). Data from the co-twin were nonetheless retained in the analysis because they improve estimation of means and variance.

Experimental Procedures

The middle-aged twins were always tested in the morning (10:00 AM); the younger twins were tested in the morning (10:00 AM) or the afternoon (2:00 PM). All subjects were asked to refrain from smoking, drinking alcohol, coffee, or tea after 11:00 PM the night before. The experimental protocol for both age cohorts was largely similar. Subjects underwent mental stress testing interspersed by periods of quiet rest. For stress testing, two identical mental stress tasks were used in both age cohorts consisting of a (choice) reaction time task and a speeded mental arithmetic task. These tasks have been described previously (28,34,38). In the middle-aged twins, an additional tone-avoidance task was added to the design, but has been left out here to keep the analyses in the two cohorts as comparable as possible. The exact experimental timelines for the adolescent and middle-aged twin groups are depicted in Figure 1.

In the Speeded Reaction Time (RT) task, each trial started with the simultaneous onset of an auditory stimulus and the appearance of a vertical bar on the computer screen. The bar lowered gradually, until after 5 seconds it had disappeared completely and an auditory imperative stimulus was given. Subjects had to react to high tones by pressing a button on a panel labeled "Yes" and to low tones by pressing a button labeled "No." Two seconds later, subjects received feedback on the computer screen, indicating whether they had pushed the correct button and, in case the response was correct, also their reaction time. Subjects were instructed to perform the task both as accurately and as fast as possible.

In the Mental Arithmetic (MA) task, subjects had to add three numbers that were presented in succession on the screen. Five seconds after the first number, the answer to the addition problem appeared on the screen. Half of the answers presented were correct; half were incorrect. Subjects were required to press the "Yes" key if the answer was correct, and the "No" key if it was incorrect. They received the same feedback as in the RT task and after 2 more seconds, the next trial was started. The MA problems contained 10 levels of difficulty: ranging from three 1-digit numbers (e.g., 9 + 4 + 5) to three 2-digit numbers (e.g., 85 + 79 + 47). The level reached by the subject after 36 practice trials determined the level at which he or she started in the MA task. This procedure was developed so that the MA task would be equally demanding for all subjects.

Physiological Recording

The electrocardiogram (ECG) signal was recorded from three disposable pregelled Ag-AgCl ECG electrodes (AMI type 1650-005 Medtronic, Minneapolis, Minnesota) using a bioelectric amplifier (AB 601G, Nihon Kohden, Tokyo, Japan). The ECG was converted to a heart period time series using automated software detection of the R waves in the ECG. Correction of excessively short/long beats was attempted by the program by rescanning the original ECG signal using a higher/lower trigger level. If this failed, the error was brought to the attention of the user, who could either manually correct the time series if the source of the error was obvious, or delete the fragment from further analysis.

The thorax impedance (Z), impedance change (dZ), and its first derivative (dZ/dt) were recorded with the Nihon Kohden Impedance Plethysmograph (AI-601G) and Nihon Kohden Differentiator (ED-601G), using a tetrapolar spot electrode system (39). The phonocardiogram (PCG) was recorded using an AB microphone (Siemens-Elema, Solna, Sweden) placed over the heart

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between the third and fourth ribs. The ECG, impedance cardiogram (ICG), and PCG tracings (all recorded at 250 Hz) were averaged over 60 seconds time-locked on the Q onset in the ECG. The B point and incisura in the ICG were interactively determined for each ensemble averaged segment according to the methods described previously (39,40). The minute-mean values for HR and PEP were averaged per condition to obtain mean rest and task values.

The respiration signal was recorded as the phase shift in an acoustic tone transmitted in a strain-gauge of hollow Silastic tube strapped around the waist at a level 7 cm above the umbilicus (41). The combined ECG and respiration signals were computer scored to obtain RSA (in ms) on a breath-to-breath basis by the peak-to-valley method (34,41). Mean RSA was computed for rest and task conditions by averaging the RSA values of all breaths falling within those conditions (including breaths with zero RSA). Automatic scoring of respiratory variables was checked by visual inspection of all respiratory signals in all conditions. Breathing cycles that showed irregularities like gasps, breath holding, and coughing, were not considered valid and were rejected and removed from further processing.

SBP and DBP were measured every 2 minutes during each of the conditions with a Dinamap Vital Signs Monitor (845 XT, Critikon, Louisville, Kentucky). These values were averaged to yield a mean value for rest and task conditions. The BP cuff was always attached to the nondominant arm.

Correction for Medication Use

In the adult twin sample, 35 subjects used antihypertensive medication. These agents reduce the absolute levels of DBP and SBP. Because a part of the genetic variance in BP is reportedly lost when medicated subjects are excluded (8), we did not exclude these subjects but instead we added drug-class specific average treatment effects to the observed values at rest and during stress. These drug-class specific treatment effects of antihypertensive medication on absolute DBP (average reduction of 10.5 mm Hg) and SBP (average reduction of 14.5 mm Hg) were obtained from various systematic reviews of the effect of antihypertensive treatment on BP levels (42,43). Adding these effects to both resting and stress levels does not affect reactivity. Some antihypertensive medication (β blockers) may also affect PEP and HR levels. Unlike BP, there is no systematic review from which we could estimate the average effects of β blockade on PEP and HR. Therefore, analyses on these measures were performed twice, once with and once without the exclusion of 18 subjects taking β blockers.

Analytical Approach

Previous studies have shown increased reliability of interindividual differences in the response to stress when multiple stressors are aggregated to a single stress level (18). Based on these findings, we summed the mean levels of BP, HR, PEP, and RSA across all observations in both 8.5-minute sessions of the speeded RT and MA tasks for the adolescent twins and over all observations in the 8.5-minute session of these same tasks for the middle-aged twins. This result yielded a single score for the mean stress level for each parameter in both twin cohorts.

To obtain a comparable resting baseline for both twin cohorts, we used the mean values obtained during the first and second 8.5-minute resting conditions for the adolescent twins and during the first (3-minute) and the last (8.5-minute) resting condition for the middle-aged twins. During all resting periods, the twin was asked to sit back and relax as much as possible.

Genetic Modeling

We used a bivariate analysis of rest and stress levels corresponding to the path diagram shown in Figure 2. This path diagram depicts the typical structural equation modeling approach to twin resemblances, which has been described previously (44–46). In this approach, the variance in the observed traits (e.g., SBP at rest and SBP during stress) is decomposed into latent additive genetic, shared environmental, and unique environmental components. The model is identified because correlations between latent genetic and environmental factors are known for MZ and DZ twins from biometrical genetic theory. The model implied by the path diagram specifies an expected covariance matrix (47). Estimates for the path coefficients, i.e., the model parameters (e.g., a_{11}, c_{11}, e_{11}), are obtained by using a fitting function that minimizes the difference between the observed covariance matrix and the expected covariance matrix implied by the model.

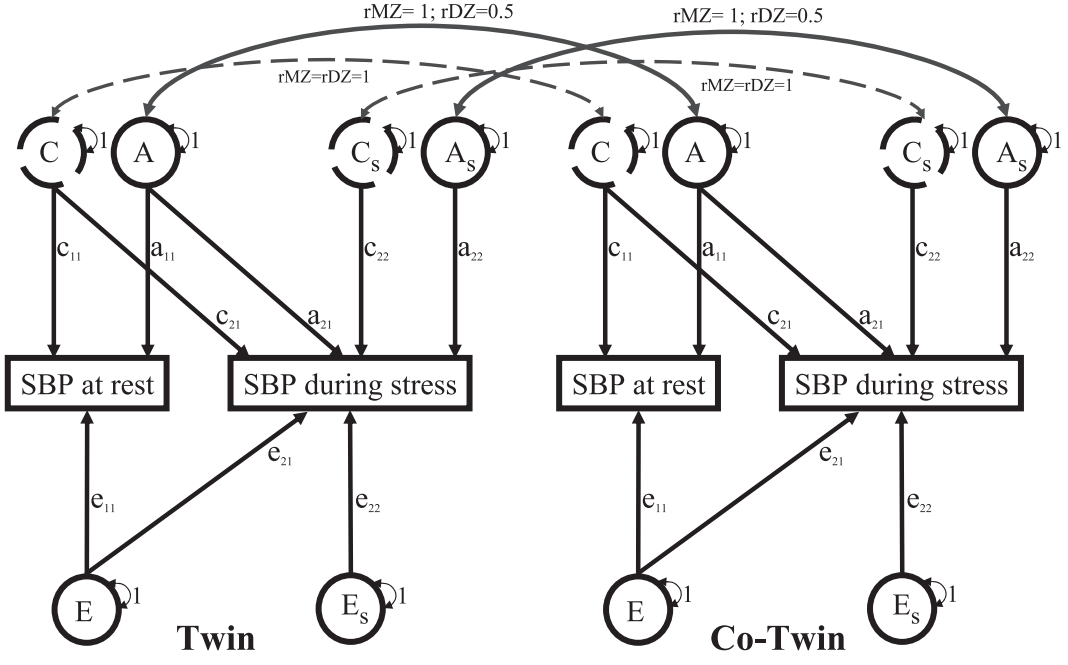

Figure 2. Bivariate twin model for genetic and environmental influences on systolic blood pressure (SBP). Biometrical genetic theory specifies that the additive genetic factors (denoted by A and A*s*) of (monozygotic) MZ twins are perfectly correlated (1.0), whereas those of dizygotic (DZ) twins are correlated 0.5. Common environmental factors shared by twins from the same family (denoted by C and C*s*) are correlated unity for both types of twins, whereas the unique environmental influences (E and E*s*) are always uncorrelated. Path coefficient a_{11} quantifies the effect of genetic influence A on SBP at rest, a_{21} quantifies the effect of A on SBP during stress, and a_{22} quantifies the effect of emergent genes in A*s* on SBP during stress. In a similar way, path coefficients e_{11}, c_{11}, e_{21}, and c_{21} quantify the effects of common and unique environmental influences E and C on SBP at rest and during stress. e_{22} and c_{22} quantify the effect of emergent environmental influences in E*s* and C*s* on SBP during stress.

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The relative contribution of genetic variance to the total variance in the SBP at rest, also known as its heritability, is the effect of the genetic factor A, and obtains as the ratio of $a_{11}^2/(a_{11}^2 + c_{11}^2 + e_{11}^2)$. The heritability of SBP during stress is the summed effect of the genetic factors A and A*s*, and obtains as the ratio of genetic to total variance, or $a_{21}^2 + a_{22}^2/(a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$. When going from rest to stress, the effects of the genetic differences between subjects may be amplified ($a_{21} > a_{11}$) or deamplified ($a_{21} < a_{11}$) by the stressors. In addition, entirely new genetic variation between subjects may emerge only during stress, depicted by factor A*s*. In this case, the path-coefficient a_{22} will differ significantly from zero ($a_{22} > 0$). This part of the total heritability of the stress level represents the influence of novel genetic effects only expressed during and is equal to $a_{22}^2/(a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$. Both amplification and emergence effectively constitute forms of gene-by-stress interaction.

For comparison with previous studies, we also computed heritability of reactivity as a change score. This was done within the bivariate model by adding the difference score as a latent factor with fixed loadings of +1 and −1 on rest and stress, respectively. Based on well-known statistical theory, the total variance of reactivity scores can be calculated as Var(stress − rest) = Var(rest) + Var(stress) − 2Cov(stress, rest). Written in terms of our bivariate model (Figure 2), the genetic part of the variance equals $a_{11}^2 + a_{21}^2 + a_{22}^2 - 2\,a_{11} \times a_{21}$, which can be simplified to $(a_{21} - a_{11})^2 + a_{22}^2$. Here, the first term reflects (de)amplification and the second term the emergence of novel genetic effects. The expectancy for the heritability of reactivity can be derived $((a_{21} - a_{11})^2 + a_{22}^2)/((a_{21} - a_{11})^2 + a_{22}^2 + (c_{21} - c_{11})^2 + c_{22}^2 + (e_{21} - e_{11})^2 + e_{22}^2)$. In contrast to the bivariate approach, the single estimate for heritability of reactivity does not allow one to assess separate contributions of amplification ($(a_{21} - a_{11})^2 > 0$), deamplification ($(a_{21} - a_{11})^2 < 0$), and emergence ($a_{22}^2 > 0$).

Model Fitting Procedures

All quantitative genetic modeling was carried out separately for the adolescent and adult age cohorts, using the Mx software package (48). Before genetic analysis, RSA was log-transformed to obtain better approximations of normal distributions. Effects of age cohort, sex and experimental condition (rest, stress) on the mean values were tested by mixed model analysis of variance, which takes nonindependency of twin data into account and yields unbiased *p* values. Sex and within-cohort age effects on the mean were regressed out simultaneously with variance decomposition.

For genetic modeling, a series of submodels nested within the full parameter ACE triangular (Cholesky) model were fitted to the multivariate variance-covariance matrices (an ADE model was not considered based on inspection of the twin correlations). The significance of variance components A, C, E was assessed by testing the deterioration in model fit after each component was dropped from the full ACE model, leading to the most parsimonious (or "best fitting") model in which the pattern of variances and covariances is explained by as few parameters as possible. Sex differences in (co)variance were examined by comparing the full model, in which parameter estimates are allowed to differ in magnitude between males and females, with a reduced model in which parameter estimates are constrained to be equal across the sexes. Emergence of new genetic or shared environmental factors was tested by a submodel that constrains the a_{22} and c_{22} parameters to zero. Amplification (or deamplification) of genetic or shared environmental factors was tested by a submodel that constrains a_{21} and a_{11}, or c_{21} and c_{11} to be equal.

Hierarchic χ^2 tests were used to compare submodels with the full model at a significance level of $p < .05$. The difference in χ^2 values between submodel and full model is itself approximately distributed as χ^2, with degrees of freedom (*df*) equal to the difference in *df* of submodel and full model. Model selection was also guided by Akaike's Information Criterion (AIC = $\chi^2 - 2df$). The model with the lowest AIC reflects the best balance between goodness-of-fit and parsimony.

RESULTS

Table 1 presents the general characteristics of the adolescent and middle-aged twins. Males were taller and heavier but no significant sex difference in BMI was found in either age cohort.

Table 2 presents the cardiovascular data of participants at rest and averaged across the two stressors. Sex × Cohort × Condition ANOVA showed significant main effects for Sex on SBP ($F(1,740) = 44.3$, $p < .0001$), HR ($F(1,740) = 26.4$, $p < .0001$), and PEP ($F(1,740) = 14.5$, $p < .0001$). Independent of experimental condition, males had higher SBP than females in both age cohorts. Females had significantly higher resting and stress HR than males in both cohorts. This may be caused by differences in intrinsic HR because resting PEP was significantly longer in females than in males, whereas RSA levels were similar. Sex significantly interacted with Cohort for DBP ($F(1,740) = 28.6$), $p < .0001$). DBP was higher in females in the adolescent cohort but higher in males in the middle-aged cohort.

A significant main effect of Cohort was found on DBP ($F(1,740) = 228.4$, $p < .0001$), SBP ($F(1,740) = 90.3$), $p < .0001$), HR ($F(1,740) = 9.7$), $p = .002$), and RSA ($F(1,740) = 266.9$), $p < .0001$). DBP and SBP were higher in the middle-aged subjects compared with the adolescents whereas HR and RSA were lower.

Significant main effects of Condition (rest versus stress) were found for DBP ($F(1,740) = 948.0$, $p < .0001$), SBP ($F(1,740) = 1017.2$, $p < .0001$), and RSA ($F(1,740) = 342.1$,

TABLE 1. General Characteristics of Adolescent and Middle-Aged Group

	Adolescent Group		Middle-Aged Group	
	Males	Females	Males	Females
n	161	159	206	218
Age, years	16.8 ± 1.8	16.7 ± 2.2	43.7 ± 6.5	44.7 ± 6.8
Height, m	1.76 ± 0.09	1.67 ± 0.07	1.80 ± 0.06	1.65 ± 0.06
Weight, kg	61.5 ± 10.3	56.6 ± 8.2	80.7 ± 10.1	66.0 ± 11.3
BMI, kg/m^2	19.7 ± 1.8	20.4 ± 2.3	25.0 ± 2.8	24.1 ± 3.8
Antihypertensive users, *n* (%)			19 (9)	16 (7)
β blocker users, *n* (%)			11 (5)	7 (3)
Oral contraceptive users, *n* (%)		24 (15)		27 (12)

Data are depicted as mean ± standard deviation, unless otherwise indicated.
BMI = body mass index.

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TABLE 2. Cardiovascular Data at Rest and During Stress in the Adolescent and Middle-Aged Twins

	Males		Females	
	Rest	Stress	Rest	Stress
Adolescent group				
n	161	161	159	159
SBP, mm Hg	119.8 ± 8.8	128.8 ± 10.3	115.2 ± 6.6	124.1 ± 9.1
DBP, mm Hg	65.6 ± 6.9	72.0 ± 7.2	67.8 ± 5.2	73.7 ± 5.7
HR, beats/min	65.8 ± 10.8	72.6 ± 12.7	69.3 ± 9.5	77.0 ± 12.0
PEP, ms	113.9 ± 17.1	109.1 ± 20.2	118.2 ± 13.3	112.6 ± 16.3
RSA, ms	115.8 ± 57.9	84.7 ± 45.1	109.1 ± 57.9	89.5 ± 46.9
Middle-aged group				
n	206	206	218	218
SBP, mm Hg	128.6 ± 11.9	138.1 ± 13.7	122.7 ± 14.3	130.8 ± 16.2
DBP, mm Hg	79.5 ± 9.3	84.7 ± 9.7	74.7 ± 10.9	79.4 ± 11.5
HR, bpm (no exclusion)	62.5 ± 9.8	71.1 ± 10.7	66.1 ± 9.7	75.4 ± 11.1
HR, bpm (β blockers excluded)	62.6 ± 9.9	71.3 ± 10.7	66.4 ± 9.7	75.8 ± 11.1
PEP, ms (no exclusion)	108.0 ± 23.0	100.5 ± 22.9	116.2 ± 21.1	106.6 ± 20.7
PEP, ms (β blockers excluded)	107.9 ± 23.1	100.3 ± 22.9	115.5 ± 21.0	105.8 ± 20.2
RSA, ms	57.7 ± 30.6	47.1 ± 24.2	62.6 ± 35.6	51.1 ± 27.2

Data are depicted as mean ± standard deviation, unless otherwise indicated.
n = number; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; bpm = beats/minute; PEP = pre-ejection period; ms = millisecond; RSA = respiratory sinus arrhythmia.

$p < .0001$), showing significant reactivity of these parameters to the stressors. For HR and PEP, a Condition × Cohort effect (HR ($F(1,740) = 21.9$, $p < .0001$); PEP ($F(1,740) = 27.2$, $p < .0001$)) was found. Under stress, PEP was shortened more in the middle-aged cohort than in the adolescent cohort, and this was paired to a lager increase in HR.

In the middle-aged subjects, chronic β blockade users did not have significantly different resting levels of HR and PEP or the reactivity of these measures compared with nonusers.

Table 3 presents the twin correlations for the five zygosity groups in the adolescent and middle-aged cohorts. In the adolescents, a number of MZ and DZ correlations were of

TABLE 3. Twin Correlations by Zygosity in the Adolescent and Middle-Aged Twins

	Adolescent Group					Middle-Aged Group				
	MZM	DZM	MZF	DZF	DOS	MZM	DZM	MZF	DZF	DOS
Total pairs[a]	35	31	35	30	29	45	37	49	39	39
SBP										
Rest	0.50	0.63	0.45	0.23	0.22	0.47	0.16	0.51	0.28	0.14
Stress	0.68	0.41	0.64	0.38	−0.01	0.55	0.29	0.52	0.11	0.36
SBP reactivity	0.56	0.24	0.24	0.41	0.04	0.38	−0.19	0.25	−0.16	0.39
DBP										
Rest	0.60	0.58	0.30	0.44	0.11	0.46	0.26	0.60	0.23	0.09
Stress	0.65	0.45	0.46	0.30	−0.06	0.62	0.36	0.60	0.03	0.29
DBP reactivity	0.12	0.11	0.15	0.27	−0.06	0.14	0.11	0.27	−0.06	0.07
HR										
Rest	0.69	0.78	0.61	0.41	0.25	0.61	0.14	0.60	0.33	0.50
Stress	0.67	0.70	0.59	0.59	0.21	0.62	0.18	0.62	0.28	0.39
HR reactivity	0.37	0.01	0.50	0.26	0.27	0.45	0.45	0.44	0.11	0.15
PEP										
Rest	0.73	0.40	0.73	0.21	0.28	0.64	0.39	0.48	0.35	0.01
Stress	0.77	0.33	0.72	0.43	0.30	0.61	0.37	0.48	0.32	−0.07
PEP reactivity	0.60	0.25	0.38	0.36	0.06	0.54	0.31	0.05	−0.02	0.12
logRSA										
Rest	0.31	0.13	0.31	0.05	0.20	0.61	0.29	0.50	0.35	−0.04
Stress	0.42	0.32	0.52	0.20	0.28	0.59	0.31	0.50	0.33	−0.18
RSA reactivity	0.06	0.04	−0.04	0.03	0.00	0.54	0.24	0.06	−0.01	0.08

MZM = monozygotic males; DZM = dizygotic males; MZF = monozygotic females; DZF = dizygotic females; DOS = dizygotic twin pairs of opposite sex; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; PEP = pre-ejection period; logRSA = log respiratory sinus arrhythmia.
[a] The total number of pairs slightly varies across variables.

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TABLE 4. Bivariate Heritability Estimates for Adolescent and Middle-Aged Twins

	Rest Level h^2 (CI)	Stress Level h^2 (CI)	Amplification of Genes Acting on Resting Level	Specific h^2 due to Genes Emerging During Stress (CI)	Reactivity h^2 (CI)
Adolescent group					
SBP	0.59 (0.44–0.71)	0.72 (0.60–0.81)	Yes,a_{21}/a_{11}=1.23	0.16 (0.01–0.24)	0.53 (0.33–0.67)
DBP	0.59 (0.43–0.71)	0.67 (0.50–0.78)	Yes,a_{21}/a_{11}=1.12	ns	ns
HR	0.68 (0.55–0.77)	0.69 (0.56–0.78)	Yes,a_{21}/a_{11}=1.16	0.07 (0.01–0.11)	0.46 (0.28–0.61)
PEP	0.70 (0.58–0.79)	0.74 (0.63–0.81)	Yes,a_{21}/a_{11}=1.12	0.14 (0.08–0.20)	0.54 (0.37–0.67)
logRSA	0.31 (0.14–0.48)	0.54 (0.36–0.68)	Yes,a_{21}/a_{11}=1.30	ns	0.09 (0.01–0.23)
Middle-aged group					
SBP	0.49 (0.33–0.62)	0.54 (0.38–0.66)	Yes,a_{21}/a_{11}=1.18	ns	ns
DBP	0.51 (0.36–0.63)	0.57 (0.42–0.68)	No,a_{21}/a_{11}=1.00	ns	ns
HR	0.63 (0.49–0.72)	0.64 (0.49–0.74)	No,a_{21}/a_{11}=0.99	0.13 (0.08–0.18)	0.52 (0.35–0.65)
PEP	0.64 (0.51–0.73)	0.56 (0.40–0.67)	No,a_{21}/a_{11}=1.09	ns	ns
logRSA	0.32 (0.15–0.47)	0.44 (0.24–0.60)	No,a_{21}/a_{11}=0.09	ns	ns

h^2 = heritability; CI = confidence interval; SBP = systolic blood pressure; DBP = diastolic blood pressure; ns = nonsignificant; HR = heart rate; PEP = pre-ejection period; logRSA = log respiratory sinus arrhythmia.

comparable magnitude, suggesting shared environmental effects, but mostly the MZ correlations exceeded DZ correlations by almost half, suggesting genetic factors as the main source of familial resemblance in these traits. In the middle-aged twins, MZ correlations for all five traits at rest and during stress were substantially higher than DZ correlations suggesting only genetic contribution to familial resemblance in these cardiovascular traits. For reactivity, two different patterns of twin correlations were found across both cohorts. For DBP and RSA reactivity, low correlations were generally found in both MZ and DZ twins. For SBP, HR, and PEP reactivity, MZ twin correlations were generally larger than DZ twin correlations.

Exclusion of the subjects using β blockers had virtually no impact on the pattern of twin correlations for HR and PEP. We proceed below using the data from all subjects, i.e., including those taking β blockers.

Results from bivariate testing, using the model depicted in Figure 2, are shown in Table 4. Models including only an additive genetic and unique environmental component (AE model) gave the overall best fit to the data for all five traits. Because sex differences in parameter estimates were small and limited to HR and PEP in the middle-aged cohort, we estimated all parameters by combining data from males and females.

Significant heritability was found for resting and stress levels for all variables in both age cohorts. The heritability estimates were highly comparable across adolescent and middle-aged subjects. As can be judged from the 95% confidence intervals, cohort effects on heritability were limited to SBP and PEP during stress, where heritability was higher in the young adolescents than in the middle-aged subjects. A single genetic factor was found to influence both resting and stress levels for all variables. This factor represents genes that act on both resting and stress levels and corresponds to factor A in Figure 2. The effect of this common genetic factor was amplified for all variables in the adolescent cohort and for SBP in the middle-aged cohort. Furthermore, new genetic factors corresponding to factor A*s* in Figure 2 emerged for HR in both cohorts and for SBP and PEP in the adolescent cohort. These factors accounted for 7% to 14% of the total heritability of these variables during stress. Comparing this to the total heritability of the stress levels, which varied from 58% to 74%, shows that this emergent genetic factor accounts for a smaller part of the total variance during stress than the effect of the common genetic factor also acting on the resting level.

Heritability of reactivity is shown in the last column of Table 4. It is immediately clear that this parameter does pick up the effect of emerging genes, but it appears less sensitive to detect amplification. Only for RSA in adolescents did amplification result in a significant heritability of reactivity.

Inspection of the standard deviations in Table 2 already showed that the total variance in SBP, DBP, HR, and PEP generally increased from rest to stress in both cohorts, with the exception of PEP in the middle-age subjects. Table 5 decomposes total variance into its additive genetic and unique environmental parts. The increase in total variance was mostly due to an increase in the genetic variance whereas environmental variance stayed about the same when going from rest to stress (Table 5). The exception was HR, where increases in genetic and environmental variance were very similar.

RSA showed a different pattern. Instead of an increase, a decrease was seen in the total variance during stress. This was due to a decrease in environmental variance paired to an increase in genetic variance, yielding a net increase in the heritability of RSA during stress. The increase in heritability reached significance in the adolescent cohort, where it went from 31% at rest to 54% during stress.

DISCUSSION

In a classical treatise on genetics, Falconer (49) described gene-by-environment interaction as a nonunity genetic correlation between repeated measurements of a trait in different environments. A genetic correlation that is lower than unity arises when different genes contribute to the genetic variance

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TABLE 5. Genetic and Environmental Variance at Rest and During Stress

	Genetic Variance			Environmental Variance		
	Rest	Stress	Ratio Stress/Rest	Rest	Stress	Ratio Stress/Rest
Adolescent group						
SBP	35.1	67.8	1.9[a]	24.4	26.4	1.1
DBP	21.2	27.9	1.3	14.8	13.7	0.9
HR	67.7	99.7	1.5[a]	31.8	46.9	1.5[a]
PEP	153.2	240.3	1.6[a]	65.7	84.4	1.3
logRSA	1.5	2.6	1.7[a]	3.3	2.2	0.7
Middle-aged Group						
SBP	81.8	116.8	1.4[a]	85.2	99.5	1.1
DBP	50.6	62.7	1.2	48.6	47.3	1.0
HR	60.5	78.2	1.3[a]	35.5	43.8	1.2
PEP	305.90	258.94	0.8	174.2	206.9	1.2
logRSA	1.4	1.8	1.3	3.1	2.3	0.7

SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; PEP = pre-ejection period; logRSA = log respiratory sinus arrhythmia.
[a] Evidence for significant emergence and/or (de)amplification of genetic or unique environmental factors ($p < .05$ level)

in the two environments. Here we tested for such imperfect genetic correlation in cardiovascular parameters, using rest and stress as the two different environments. Evidence for the emergence of stress-specific genetic variation was found for HR in both adolescent and middle-aged cohorts and for PEP and SBP in the adolescent cohort only. This means that some of the genetic variation influencing these variables is expressed specifically in one environment only (i.e., during stress). In addition to the emergence of new genetic variation, we argue that the amplification of existing genetic influences when moving from one environment to another can also be considered gene-by-environment interaction. We found significant evidence for such amplification for all cardiovascular variables in the adolescents; in the middle-aged subjects, stress only amplified genetic influences on the SBP.

As an alternative to the bivariate modeling used here, previous twin studies have tested for gene-by-stress interaction on BP and HR using univariate analyses on the reactivity change score, i.e., the difference between rest and stress levels (25–33). To compare our BP and HR results with these earlier studies, we also estimated heritability of reactivity, calculated under our bivariate model as the average level during both stressors minus the resting level. Reactivity showed significant heritability for HR in the adolescent ($h^2 = 0.46$) and adult cohorts ($h^2 = 0.52$) that was comparable to those reported in the previous studies (h^2 range 0.30–0.61). SBP reactivity was heritable only in the adolescents ($h^2 = 0.53$) but DBP reactivity was not heritable in both cohorts. It is of note that we found largest heritability for reactivity when a new genetic factor emerged during stress. This suggests that univariate heritability of change scores does not detect amplification as well as the bivariate approach that uses all available information in the bivariate variance/covariance matrices. It is also of note that the gene-stress interaction effects were larger in the adolescents than in the middle-aged subjects. This suggests that genetic contribution to stress-reactivity varies across age. Heritability of SBP and PEP during stress was higher in the young adolescents than in the middle-aged subjects, whereas heritability of resting values was not significantly different.

Our findings on HR and BP are in line with the findings of Ditto (27) and Busjahn et al. (30) that used a slightly different bivariate strategy on their twin data. They tested whether the same genetic variation influenced resting values of HR and BP as well as their reactivity (computed as a change score). In keeping with our results, genetic factors that influenced HR and BP reactivity were found to be largely independent of the genetic factors influencing the resting level of these variables. Our results are also in keeping with previous multivariate analyses of SBP, DBP, HR, and RSA in these same cohorts (34,38,50) that showed an increase in genetic variance during stress, although these analyses did not make the distinction between amplification and emergence as sources of the increase in genetic variance. The current approach is richer because in designing gene finding studies (choosing candidate genes, determining which phenotypes to measure), it may be highly relevant to know upfront from twin studies what genes to look for—amplificating ones or emerging ones. For instance, if there had only been amplification, and the effects on total genetic variance had been small, adding stress measurements to the experimental protocol would not be worthwhile. Our data show this not to be the case for any of the measured variables. They argue in favor of stress testing when examining the genetics of these cardiovascular variables.

We further add to the previous studies by providing a genetic dissection of the main index of sympathetic reactivity used in the field, the shortening of the PEP. Using ambulatory recording, we have previously shown substantial heritability ($h^2 = 0.62$) for the absolute level of the PEP during the daytime in middle-aged adults (51). This is in good accordance with the estimate for PEP heritability during rest ($h^2 = 0.64$) and under stress ($h^2 = 0.56$) in the middle-aged subjects found here. Even higher heritability of resting PEP ($h^2 = 0.70$) and PEP under stress ($h^2 = 0.74$) was found in the adolescent twins. To the extent that the absolute PEP level reflects

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individual differences in sympathetic cardiac drive (35), these results suggest that sympathetic cardiac drive is at least as heritable as HR and BP. This converges rather well with genetic analyses of other measures of sympathetic nervous system activity. Findings in family and twin studies on plasma (29,52,53) and urine (29,53) catecholamine levels reported a heritability of 0.42 to 0.57 for plasma and 0.32 to 0.76 for urinary norepinephrine levels, and 0.61 to 0.69 for plasma and 0.47 to 0.65 for urinary epinephrine.

Taken together, our findings predict that there will be some genes that show an effect on sympathovagal cardiac control, HR, and BP at rest as well as during stress, whereas others are expressed only when these traits are measured in stressful conditions. For BP, already a number of candidate genes have been identified that support this prediction. Wang et al. (54), for instance, showed that a variant in the endothelin receptor Type A gene led to higher SBP levels at rest and during acute laboratory stress. This gene, therefore, would be part of factor A in Figure 2. Two variants in the endothelin-1 gene, on the other hand, did not influence resting SBP but led to greater SBP increases to stress. This variation in the gene seems to be expressed only during stress and would be part of factor A*s* in Figure 2. Another example of a gene effect on BP that emerges only under stress is provided by the M/Z/S polymorphism in the α_1-antitrypsin gene. At rest, subjects with an S or Z allele combination showed comparable BP to MM subjects but during stress, BP levels were much lower in the MZ/MS subjects, possibly because the advantage of less stiff vessels is evident only during stress (55).

Two findings by Li et al. (31) and McCaffery et al. (32) suggested that genetic variation at the level of the translation of sympathetic nervous system activity into organ responsiveness could constitute a source of amplification. Li et al. (31) genotyped a functional polymorphism (A + 46G) in the β_2-adrenergic receptor gene coding for an arginine to glycine substitution that is known to influence receptor sensitivity, possibly by influencing receptor density (56). Higher SBP and DBP at rest and during stress were found in Arg16 homozygotes. In addition, these subjects also showed larger DBP reactivity suggesting that stress amplifies the effect of this gene on DBP. The importance of this same polymorphism in the β_2-adrenergic receptor gene was confirmed by McCaffery et al. (32) in a genetically independent draw of their Pittsburg Twin Study sample. In that same sample, the effects of variation in the β_1-adrenergic receptor gene also seemed to be amplified under stress. Subjects who carried a glycine allele at amino acid 389 in the β_1-adrenergic receptor had higher resting SBP and DBP as well as a larger DBP response to mental challenge.

An overall summary of our findings is that exposure to stress uncovers new genetic variance and amplifies the effect of genes that already influence the resting level. This has clear implications for the attempts to find the genes influencing these cardiovascular risk factors through linkage or association approaches (57). The genetic variation that emerges exclusively during stress can *only* be found in gene finding studies that have attempted to measure the stress levels of the cardiovascular risk factor. Genetic variation that is amplified during stress can be detected using resting levels, but the genetic variance, and hence the power of the study, will be larger if stress levels are measured instead. Gene hunters for these traits, therefore, would do well to measure them during stress. Stress levels capture the main genetic influences on resting levels as well as either form of gene-by-stress interaction.

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