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Heritability of blood pressure increases during mental stress

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We studied the influence of mental stress on the contributions of genes and environment to individual variation in systolic (SBP) and diastolic (DBP) blood pressure by structural equation modelling in 320 adolescent male and female twins. Blood pressure data were collected during rest and during a reaction time and a mental arithmetic task. Univariate analyses of SBP and DBP showed familial aggregation for blood pressure. A genetic explanation for this resemblance was most likely, although during rest conditions a model that attributed familial resemblance to shared environmental factors, also fitted the data. There was no evidence for sex differences in heritabilities. Multivariate analyses showed significant heterogeneity between sexes for the intercorrelations of the blood pressure data measured under different rest and task conditions. Multivariate genetic analyses were therefore carried out separately in males and females. For SBP and DBP in females and for SBP in males an increase in heritabilities was seen for blood pressure measured during stress, as compared to rest measurements. The influence of shared environmental factors decreased during stress. For DBP in males no significant contributions of shared environment were found. The multivariate analyses indicated that the same genetic and environmental influences are expressed during rest and stress conditions.

Keywords: genetics, multivariate, model fitting, hypertension

Introduction

Research during recent decades has demonstrated the heritability of systolic (SBP) and diastolic blood pressure (DBP).[1–7] Heritability estimates for systolic blood pressure range from 13% to 82% and for diastolic blood pressure from < 1% to 64% with average levels for both at about 50%.[8]

Two approaches that frequently have been used to study the contributions of genes and environment to variation in blood pressure levels are family and twin studies. The first approach studies the resemblance in blood pressure between parents and offspring or between siblings. The second approach examines the similarity in blood pressure of monozygotic (MZ) and dizygotic (DZ) twin pairs. Resemblance between family members (including twins) can arise from a common environment shared by family members or from a (partially) shared genotype. Twins offer a unique opportunity to distinguish between the influences of environment and heredity on resemblance between family members. In a twin design the separation of genetic and environmental variance is possible because MZ twins share 100% of their genetic make-up and DZ twins share on average 50% of their additive genetic variance. If a trait is influenced by genetic factors, MZ twins should resemble each other to a greater extent than DZ twins. Heritability (h^2) can be estimated as twice the difference between MZ and DZ correlations[9] and is a measure of the amount of total phenotypic variance explained by genetic factors. When twice the DZ correlation is larger than the MZ correlation, this may indicate that part of the resemblance between twins is caused by shared environmental factors.[10]

Heritability estimates may depend on sex, age and situational factors. Most twin studies have estimated heritability from samples of male twins, have pooled data from different age groups, and usually have assessed blood pressure during resting conditions only. A few studies have taken an explicit interest in the effect of age[11–13] or sex[14,15] on heritability. Sims et al[12] found a decrease in heritability from 68% to 38% from young adulthood to middle age for DBP. This reduction was caused by a threefold increase in the contribution of individual environmental factors as people grow older. The same trend was seen for SBP.[13] A decrease in heritability estimates for blood pressure with age is consistent with results from family studies. Heritability estimates from family studies, which usually measure pairs of subjects at different ages, such as parents and offspring, generally are lower than estimates obtained from twin studies, which measure pairs of subjects at the same age. Heritability estimates from family studies range from 19 to 45% for SBP and from 15 to 52% for

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DBP,[3,6] while estimates from twin studies range from 41 to 82% for SBP and from 51 to 66% for DBP.[1,5,14–16]

Studies with respect to sex differences in the genetic architecture of resting blood pressure levels show mixed results. McIlhany, Shaffer and Hines[14] observed higher heritabilities in females than in males for both SBP (78 and 41%) and DBP (61 and 56%) in a study of 200 twin pairs aged 14 years on average. Schieken et al[15] did not find a difference in heritability for SBP (66%) between males and females in a group of 251 twin pairs aged 11 years on average. For DBP a somewhat higher estimate for males (64%) than for females (51%) was observed. Tambs et al[7] did not find sex-specific genetic effects on either SBP or DBP in a very large Norwegian sample consisting of nearly 75 000 family members.

Data regarding the heritability of blood pressure measures during stress are limited.[17] These data are of interest because an enhanced cardiovascular response to stress may be an early predictor for the development of essential hypertension.[18,19] McIlhany, Shaffer and Hines[14] conducted one of the first twin studies in which blood pressure data were collected during rest and during a physical stressor. Two hundred twin pairs of both sexes (mean age 14 years) were tested using the cold pressor test as a stressor. Heritability estimates for blood pressure levels during the test were larger for females than for males for both SBP (72 and 48%) and DBP (62 and 58%), but were not different from estimates during rest. Theorell et al[20] measured blood pressure during rest and during a stressful interview in 17 MZ and 13 DZ male twin pairs aged 51–74 years and observed significant genetic influences during the interview but not during rest. Sokolov et al[21] reported heritabilities of 47% for SBP during rest and 81% during a stress task in 24 MZ and 15 DZ twin pairs. For DBP heritabilities were 73 and 77%, respectively. The stress task is described as mental effort under time pressure. Sokolov et al[21] concluded that heritabilities increase under stress conditions. However, the small sample sizes of the last two studies preclude any firm conclusions. Rose, Grim and Miller[22] present blood pressure data measured in 111 MZ and 66 same-sex DZ twin pairs aged 16–24 years during the Stroop test. They found a higher correlation for SBP mean level in MZ (0.61) than in DZ twins (0.44), suggesting genetic influences. These correlations did not differ very much from the values obtained for resting SBP (0.70 for MZ and 0.50 for DZ twins). However, when the SBP data were analysed separately by sex, correlations for MZ and DZ females were 0.68 and 0.39 and for MZ and DZ males 0.42 and 0.40, suggesting little genetic influence on SBP in males. Hunt et al[16] studied 73 MZ and 81 DZ male twin pairs (mean age 34.5 years) and obtained heritabilities of 54 and 60% for sitting SBP and DBP and 44% for both SBP and DBP during serial subtraction.

These studies clearly demonstrate genetic influences on blood pressure, both during rest and physical and mental stress conditions. It is unclear, however, whether heritabilities differ as a function of stress and if these differences depend on the sex of the subject. None of the studies reviewed above gives formal tests of differences in parameter estimates or carries out a multivariate analysis. An additional problem with these studies is their limited comparability which arises from differences in estimation techniques for heritabilities. This led Hunt et al[16] to state that 'the use of multiple applicable models may give a clearer picture of how heritable a trait is'. Structural modelling[22,23] offers a solution to this problem.

In the present study structural modelling techniques were used to examine the influence of mental stress tasks on the relative contributions of genes and environment to individual variation in SBP and DBP. The mental stressors consisted of tasks that are often employed in psychophysiological research, ie a reaction time and a speeded mental arithmetic task. Subjects were 160 Dutch adolescent male and female twin pairs. We first present a series of univariate genetic analyses in which several models for sex differences in genetic architecture of SBP and DBP are considered. Next, data from rest and task conditions are analysed simultaneously in a multivariate model. A multivariate genetic analysis offers the possibility of testing whether the magnitude of the genetic and environmental influences differ during rest and stress conditions, and additionally to what extent the genetic and environmental influences that determine blood pressure levels during rest are correlated with the genetic and environmental influences that determine blood pressure levels during stress.[24–26]

Subjects

This study is part of a larger project in which cardiovascular risk factors were studied in 160 adolescent twin pairs and their parents.[27–29] Addresses of twins (of 14–21 years of age) living in Amsterdam and neighbouring cities were obtained from City Council population registries. Twins still living with their biological parents were contacted by letter and included in the study, if the twins and both parents were willing to participate. Between 30 and 40% of families complied. In addition, a small number of families who heard of the study from other twins also volunteered to participate. Zygosity

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was initially determinated by typing the following polymorphisms: ABO, MNS, P, Rhesus, Lutheran, Kell, Duffy, Kidd, Gm, Am and Km. In a later stage of the project most same-sex twin pairs were also typed by DNA fingerprinting.[30] Three series of triplets were included by discarding the data from the middle child. Data from two twin pairs were not used because of apparatus failure during the experiment, from one pair because one of the twins was deaf, and from one pair because one of the twins had extremely low diastolic blood pressure (< 50 mmHg). This left for analysis 33 MZ female (MZF, average age 16.1 years, sd = 2.3), 35 MZ male (MZM, average age 16.6 years, sd = 1.8) 29 DZ female (DZF, average age 17.7 years, sd = 17.7, sd = 2.0), 31 DZ male (DZM, average age 17.2 years, sd = 1.7), and 28 DZ opposite-sex pairs (DOS, average age 16.4 years, sd = 1.9). All subjects were paid Dfl. 25 for their participation.

Procedure

Blood pressure was measured during rest and during two task conditions. Testing took place in a sound attenuated, electrically shielded cabin. The two experimental tasks consisted of a choice reaction time (RT) task and a speeded mental arithmetic (MA) task. Each condition was repeated once and lasted 8.5 minutes. During the resting periods subjects were asked to relax as much as possible. Subjects changed places in the cabin several times. When one subject was tested, the other subject filled out questionnaires. Sequence of events was: Practice sessions, pause, Rest1 followed by RT1 and RT2, pause, Rest2 followed by MA1 and MA2. During each condition blood pressure was measured three times (beginning, middle, end). Data were averaged over these three measures.

Tasks

In the RT task each trial was started with the simultaneous onset of an auditory warning stimulus and the appearance of a vertical bar on a television screen. After 5 seconds a reaction stimulus was heard. Subjects had to react to high tones by pressing a key labelled 'Yes' and to low tones by pressing a key labeled 'No'. Two seconds later subjects received feedback on the screen, indicating whether they had pushed the correct key and, if the response was correct, also their reaction time.

In the MA task subjects had to add up 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 presented answers were correct, half incorrect. Subjects were required to press the 'Yes' key if the presented answer was correct, and the 'No' key if it was incorrect. They received the same feedback as in the RT task and after two more seconds the next trial was started. The MA problems contained 10 levels of difficulty: ranging from three 1-digit numbers (eg 9 + 4 + 5) to three 2-digit numbers (eg 85 + 79 + 47). The level reached by the subject after 36 practise 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 stressful for all subjects.

Apparatus

Subjects were seated in a comfortable chair in front of a Barco colour television screen, that was used for presentation of visual stimuli. Auditory stimuli were binaurally presented through padded earphones. Two reaction time keys were mounted on both the left and right arm of the chair. Subjects pushed the keys with their preferred hand (278 right handed, 42 left handed). Blood pressure was measured by the Dinamap 845XT using the oscillometric technique.

Statistical analyses

To study the contributions of genetic and environmental factors to blood pressure variability a structural modelling approach was used. First, a series of univariate models was fitted to the blood pressure data from each task condition. Model fitting was carried out on the 2 $\times$ 2 variance–covariance matrices (BP-Twin 1, BP-Twin 2) of the five different sex-by-zygosity groups (MZ male and female pairs, DZ male, female and opposite-sex pairs). Genetic models specified variation in phenotype to be due to genotype and environment. Sources of variation considered were G, additive genetic influences; C, common environment shared by siblings growing up in the same family, and E, a random environmental deviation that is not shared between siblings. Their influence on the phenotype is given by parameters h, c, and e that are equivalent to the standardised regression coefficients of the phenotype on G, C and E, respectively. The square of these parameters gives the proportion of variance, Vg, Vc and Ve, due to each source. Correlations between the genetic factors of the first and second twin are unity for MZ twins and 0.5 for DZ twins. Correlations between shared environmental factors are one. A series of alternative explanations for the pattern of variation in each

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condition was compared and the fit of these theoretical models to the observed data was tested by χ^2 difference tests. To study sex differences in genetic inheritance three different models were examined:

1 Full model in which estimates for Vg, Vc, and Ve are allowed to differ in magnitude between males and females, and thus total variances as well as heritabilities may be different in the two sexes;

2 scalar model in which heritabilities are constrained to be equal across sexes, but in which total variances may be different. In the scalar model, the variance components for males are constrained to be equal to a scalar multiple, β, of the female variance components, such that $Vg_m = \beta Vg_f$, $Vc_m = \beta Vc_f$, and $Ve_m = \beta Ve_f$. As a result, the standardised variance components such as heritabilities are equal across sexes, even though the non-standardised components differ;[24]

3 constrained model in which parameter estimates for Vg, Vc and Ve are constrained to be equal in magnitude across sexes and total variances are thus also the same in males and females.

Parameters were estimated by maximum likelihood, using the computer program LISREL7.[35] Fit was assessed by likelihood ratio χ^2 tests. The overall χ^2 tests the agreement between the observed and the predicted variances and covariances in the five sex-by-zygosity groupings. A large χ^2 indicates a poor fit, while a small χ^2 indicates that the data are consistent with the model. Submodels were compared by hierarchic χ^2 tests. The scalar model B is a submodel of the full model A and the constrained model C is nested under B. The χ^2 statistic is computed by subtracting the χ^2 for the full model from that for a reduced model. The degrees of freedom (df) for this test are equal to the difference between the df for the full and the reduced model.

For the multivariate analyses 6 $\times$ 6 (two rest, two RT task and two MA task conditions for each subject) matrices of mean squares and cross-products between and within twin pairs were constructed. To these matrices we first fitted a factor model with one genetic and one environmental common factor[25,31] and a simplex model with a first-order autoregressive genetic and a first-order autoregressive environmental series.[32] In the factor model correlations between observations are explained by their loadings on the same genetic and environmental factors. In addition, unique genetic and environmental factors can be specified for that part of the variance that is not shared between measures. In the simplex model correlations are explained by the autocorrelation among genes and among environmental factors that influence the phenotype at each different time point. In this model the variance unique to each observation is accounted for by an innovation term that can come into play at each time point and by measurement errors that are uncorrelated across time.

Results

Means for SBP and DBP during rest and during the RT and MA tasks are presented in Figure 1 for males and females. Body weight in males correlated with SBP (correlations between 0.27 and 0.42) and DBP (correlations between 0.20 and 0.30) in all conditions. For females the correlations between body weight and SBP (correlations between 0.05 and 0.24) and body weight and DBP (correlations between 0.07 and 0.15) were somewhat lower than for males and highest for blood pressure measured during rest. Data were therefore corrected for body weight, separately for males and females. In the corrected data there was no correlation between blood pressure levels and age.

Analyses of variance for repeated measures were performed on SBP and DBP levels measured during rest and stress with sex and zygosity as grouping factors and condition (ie Rest, RT and MA Task) and repeated presentation of each condition as within-

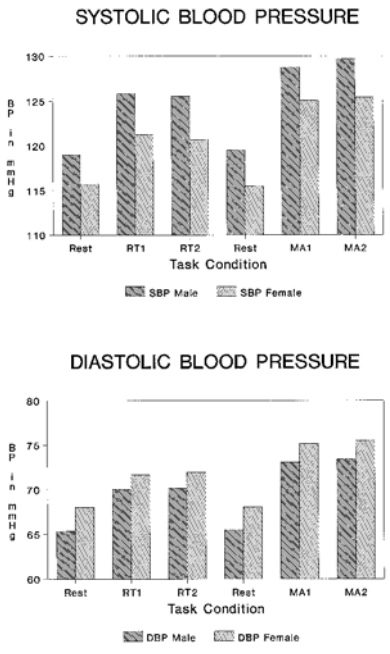

Figure 1 Mean values for blood pressure during rest and stress (reaction time (RT) and mental arithmetic (MA) task) in 160 adolescent male and 152 adolescent female twins.

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subject factors and with body weight as a covariant (probability levels for within-subject effects Greenhouse–Geisser[33] adjusted). Because twins do not represent independent observations, the residual degrees of freedom for the F-tests have been taken as half those available. This adjustment is conservative, because dizygotic twins share on average only 50% of their genetic material. Results showed a significant effect of sex and task on both SBP and DBP (for SBP $F(1,153) = 16.82$, $P = 0.00$ for sex and $F(2,313) = 365$, $P = 0.00$ for task; for DBP $F(1,153) = 8.65$, $P = 0.01$ for sex and $F(2,313) = 525$, $P = 0.00$ for task). There were no main effects of zygosity and no interactions between sex, zygosity or task. In all conditions males had higher SBP and lower DBP levels than females. The effect of the tasks was in the expected direction: blood pressure levels were lowest during rest, intermediate during the reaction time task and highest during mental arithmetic.

Table 1 lists the standard deviations for SBP and DBP in males and females in each condition. Both during rest and during task conditions variances for SBP and DBP were higher in males than in females. In both sexes, the variances in SBP and DBP increased during task compared with rest conditions.

Table 2 gives twin correlations for SBP and DBP for each sex by zygosity group in each condition. Overall, correlations were higher in MZ than in DZ twins suggesting that genetic factors play a role in individual differences in blood pressure levels. However, several of the MZ and DZ correlations were of the same magnitude, especially during rest. The correlations of DZ opposite-sex twins were not systematically lower than DZ same-sex correlations, indicating that a model in which the same genes and the same environmental factors influence blood pressure levels in males and females is appropriate.[34]

Table 1 Standard deviations for systolic (SBP) and diastolic (DBP) blood pressure for males and females in different task conditions

		Rest1	Rest2	RT1	RT2	MA1	MA2
SBP	Males	8.70	8.73	10.08	10.15	11.04	11.66
	Females	6.82	7.28	8.97	8.54	10.12	11.34
DBP	Males	6.84	7.21	7.44	7.48	8.18	8.33
	Females	5.56	5.26	6.07	5.94	6.23	6.23

Table 2 Twin correlations for systolic (SBP) and diastolic (DBP) blood pressure measured during rest and reaction time (RT) and mental arithmetic (MA) tasks

SBP	Rest1	Rest2	RT1	RT2	MA1	MA2
MZM	0.48	0.47	0.58	0.63	0.56	0.58
DZM	0.43	0.52	0.28	0.23	0.32	0.27
MZF	0.47	0.40	0.61	0.63	0.37	0.66
DZF	0.23	0.35	0.48	0.41	0.28	0.48
DOS	0.31	0.32	0.18	0.33	0.24	0.17
DBP	Rest1	Rest2	RT1	RT2	MA1	MA2
MZM	0.64	0.48	0.46	0.53	0.57	0.47
DZM	0.57	0.48	0.33	0.34	0.36	0.37
MZF	0.30	0.47	0.49	0.55	0.51	0.43
DZF	0.37	0.38	0.53	0.32	0.18	0.21
DOS	0.46	0.14	0.37	0.17	0.16	0.16

Table 3 summarises the results of univariate model fitting to SBP and DBP data by presenting χ^2 statistics and probability levels for the different univariate models of sex differences in the genetic architecture of blood pressure. For SBP measured during rest, a scalar model that specified equal heritabilities in males and females gave the most parsimonious account of the data, while a model without sex differences showed a significant increase in χ^2. Although the χ^2 for the scalar CE model for SBP during rest is somewhat larger than the scalar GE model, we recognise that we cannot really distinguish between the two. In contrast, for SBP measured during task conditions a simple GE model without sex differences showed a good fit to the observed data, as indicated by the non-significant χ^2s. For SBP measured during mental stress, it is clear that a common environmental model does not fit the data. For DBP, a scalar GE model gave the best fit and most parsimonious account of the data under all conditions, except the first rest condition where a scalar CE model showed a better fit.

Table 4 gives the estimates of genetic and environmental variances based on the univariate GE models and heritabilities for each task condition. Genetic factors explained around 50% of the variance in SBP and DBP during rest and task conditions. These heritabilities were the same for males and females, and for both SBP and DBP tended to increase somewhat under mental stress conditions as compared to rest. As can be seen, the amount of genetic variance as well as the amount of specific environmental variance increased in stress as compared to rest conditions.

In Table 5 the intercorrelations for blood pressure measured in different conditions are given for SBP and DBP. The phenotypic correlation structure was significantly different for males and females. For females, intercorrelations between the measures from different conditions were lower than for males. For the multivariate model fitting, the factor and simplex models were therefore fitted separately to data from males and females.

Table 6 shows the χ^2s and probabilities that were obtained after we fitted the simplex and factors models described above to data from male and female twins. For the simplex models we tested if a

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Table 3 Univariate genetic model fitting for systolic (SBP) and diastolic (DBP) blood pressure: χ^2 and probability

	Rest1		Rest2		RT1		RT2		MA1		MA2	
SBP full model: Sex differences in parameter estimates												
GCE	3.25	(0.95)	6.62	(0.68)	1.94	(0.99)	1.16	(0.99)	23.14	(0.01)	8.30	(0.50)
GE	4.02	(0.97)	9.09	(0.61)	3.62	(0.98)	1.36	(1.0)	23.33	(0.02)	8.99	(0.62)
CE	5.71	(0.89)	8.95	(0.63)	9.58	(0.57)	9.08)	(0.61)	31.21	(0.00)	20.13	(0.04)
SBP scalar model: Equal heritabilities												
GCE	3.75	(0.98)	7.97	(0.72)	3.83	(0.98)	1.42	(1.0)	23.33	(0.02)	10.52	(0.49)
GE	4.05	(0.98)	9.09	(0.70)	3.83	(0.98)	1.42	(1.0)	23.33	(0.03)	10.52	(0.57)
CE	6.18	(0.91)	9.50	(0.66)	10.32	(0.59)	9.68	(0.64)	32.38	(0.00)	29.65	(0.00)
SBP constrained model: No sex differences												
GCE	11.03	(0.53)	11.37	(0.50)	5.85	(0.92)	5.22	(0.95)	23.73	(0.02)	10.72	(0.55)
GE	11.56	(0.56)	12.79	(0.46)	5.85	(0.95)	5.22	(0.97)	23.73	(0.03)	10.72	(0.63)
CE	13.07	(0.44)	12.66	(0.47)	12.48	(0.49)	13.78	(0.39)	32.79	(0.00)	21.11	(0.07)
DBP full model: Sex differences in parameter estimates												
GCE	5.02	(0.83)	4.41	(0.88)	6.75	(0.66)	5.46	(0.79)	17.46	(0.04)	8.76	(0.46)
GE	9.73	(0.55)	7.00	(0.80)	8.41	(0.68)	5.98	(0.88)	18.14	(0.08)	9.25	(0.60)
CE	5.71	(0.89)	7.38	(0.77)	9.32	(0.59)	12.62	(0.32)	28.72	(0.00)	14.18	(0.22)
DBP scalar model: Equal heritabilities												
GCE	9.18	(0.61)	6.27	(0.86)	7.51	(0.76)	5.98	(0.88)	18.46	(0.07)	9.53	(0.57)
GE	13.65	(0.32)	7.09	(0.85)	8.52	(0.74)	5.98	(0.92)	18.46	(0.10)	9.53	(0.66)
CE	9.42	(0.67)	7.65	(0.81)	9.91	(0.62)	12.67	(0.39)	29.84	(0.00)	14.87	(0.25)
DBP constrained model: No sex differences												
GCE	11.25	(0.51)	18.52	(0.10)	13.54	(0.33)	11.86	(0.46)	27.05	(0.01)	18.68	(0.10)
GE	15.74	(0.26)	19.45	(0.11)	14.23	(0.36)	11.86	(0.54)	27.05	(0.01)	18.68	(0.13)
CE	11.61	(0.56)	19.83	(0.10)	16.24	(0.24)	18.43	(0.14)	38.70	(0.00)	24.04	(0.03)

Degrees of freedom (df) for models with sex differences in parameter estimates: GCE df = 9; GE and CE df = 11; for scalar models: GCE df = 11, GE and CE df = 12; for models without sex differences: GCE df = 12, GE and CE df = 13.

model without any genetic or environmental innovations or without any innovations (ie no new genetic or environmental influences at any time point) would lead to a significant increase in χ^2. None of these models fitted the data. Second-order autoregressive models were also fitted to the data with additional paths from Rest1 to Rest2 and from RT2 to MA1. These separate paths test whether there is a significant independent influence from the first to the second resting period, that is specific to rest and not to task, and if there is an independent influence from the RT to the MA task that is not mediated by the rest period in between the two. These models did not converge after a large number of iterations, and the intermediate solutions showed unreasonable parameter estimates. Since the simplex models as well as the one-factor model did not provide a good explanation of the multivariate data structures for either males or females, a full Cholesky decomposition of the phenotypic matrices were carried out. This decomposition is a fully saturated, unconstrained model for all unique observed variances and covariances and provides the most general approach to estimating the genetic, shared environmental and

Table 4 Estimates of genetic and environmental variances and heritabilities (percentages of total variance) based on univariate genetic analyses; GE models for DBP and SBP measured during rest include scalar parameter to account for sex differences in total variance

SBP	Rest1	Rest2	RT1	RT2	MA1	MA2
Vg	25.24	30.03	55.26	56.16	72.42	91.24
Ve	22.53	24.16	36.10	32.14	43.85	46.42
h^2	52%	55%	61%	64%	62%	66%
DBP	Rest1	Rest2	RT1	RT2	MA1	MA2
Vg	18.70	13.80	21.91	20.98	25.70	21.04
Ve	14.27	13.13	15.04	15.17	14.46	18.66
h^2	57%	51%	59%	58%	64%	53%

Table 5 Phenotypic intercorrelations for systolic and diastolic blood pressure measured during rest and mental stress; males lower diagonal, females upper diagonal

SBP	Rest1	RT1	RT2	Rest2	MA1	MA2
Rest1	–	0.754	0.698	0.658	0.556	0.550
RT1	0.823	–	0.875	0.649	0.743	0.755
RT2	0.758	0.883	–	0.588	0.755	0.773
Rest2	0.758	0.733	0.697	–	0.624	0.618
MA1	0.661	0.791	0.785	0.756	–	0.885
MA2	0.674	0.793	0.798	0.719	0.896	–
DBP	Rest1	RT1	RT2	Rest2	MA1	MA2
Rest1	–	0.767	0.751	0.770	0.603	0.599
RT1	0.812	–	0.843	0.705	0.743	0.722
RT2	0.811	0.892	–	0.727	0.721	0.745
Rest2	0.825	0.784	0.770	–	0.646	0.659
MA1	0.702	0.816	0.797	0.793	–	0.844
MA2	0.708	0.817	0.780	0.779	0.924	–

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unique environmental components of variance and covariance.[24] The second part of Table 6 shows the χ^2s and probabilities for the full Cholesky decomposition and for several more constrained submodels. Both the genetic and the shared environmental components show a one factor solution, indicating that the same genes and the same shared environmental factors influence blood pressure during rest and stress conditions. However, in contrast to the results from the univariate analyses, the shared environmental component is significant for SBP in males and females and for DBP in females. Only for DBP in males the contribution of shared environment to variation and covariation could be omitted without significant loss of fit. The increase in χ^2 is significant, however, if the genetic contributions are omitted from the model. Probably the most interesting model test is listed on the last line of Table 6 It appeared that for the unique environmental covariance structure no simple factor or time series model could be specified. At every time point the specific environmental factors influencing each blood pressure measure were associated with all their earlier values in a non-reducible way.

Table 7 lists the components of variance as obtained from a Cholesky decomposition with one genetic and one common environmental factor shared by siblings and with a full structure for the unique environmental part of the model. For SBP in males and females genetic variances increase during stress as compared to rest conditions. Specific environmental variance also increases, but not to the same extent and consequently heritabilities become larger. The influence of common environmental factors decreases during rest compared with stress tasks. The same results were obtained for DBP in females, but not in males.

Discussion

In a series of univariate analyses of systolic and diastolic blood pressure measured in male and female adolescent twins during rest and mental stress, we obtained heritability estimates for SBP that were between 52% (during rest) and 66% (during mental arithmetic). For DBP, heritabilities were between 51 and 64%. We found no evidence for sex differences in genetic heritabilities. The univariate pattern of twin correlations had suggested some contribution of common environmental factors to individual differences in blood pressure levels, especially for blood pressure measured under resting conditions. The univariate likelihood-ratio tests, however, indicated no significant contribution of shared environment. For blood pressure assessed during rest, a model in which shared environment explained familial resemblance fitted the data almost as good as a genetic model, whereas for blood pressure measured during mental stress, the results clearly indicated the importance of genetic factors. These results are in accordance with the 12 studies reviewed by Snieder et al[36] which also reported little evidence for shared environment.

Multivariate analyses yielded a similar pattern of results with respect to the increase in heritabilities during stress. In addition, these analyses provided insight into the stability of genetic and environmental influences across tasks. They also had more power to detect the presence of sex differences and shared environmental influences. The correlation among blood pressure values obtained under different task conditions was different in males and females. On average, blood pressure values of males were more highly correlated across task conditions

Table 6 Multivariate model fitting to systolic (SBP) and diastolic (DBP) blood pressure data from males and females, χ^2 and probability

		Females SBP		Females DBP		Males SBP		Males DBP	
	df	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Simplex model:	59	91.08	0.005	93.58	0.003	91.59	0.004	73.87	0.092
No. G innovations:	64	113.68	0.000	104.72	0.001	110.60	0.000	77.90	0.114
No. E innovations:	64	103.20	0.001	104.19	0.001	119.04	0.000	96.55	0.005
No. innovations	69	131.25	0.000	114.02	0.001	151.57	0.000	109.67	0.001
Factor model:	60	117.61	0.000	110.78	0.000	127.33	0.000	103.00	0.000
Cholesky decomposition									
Full	21	54.37	0.000	47.56	0.001	63.85	0.000	46.81	0.001
C one factor	36	56.31	0.017	47.56	0.094	64.41	0.003	48.13	0.085
G one factor	51	67.41	0.062	58.63	0.216	67.94	0.056	54.84	0.331
No. C	57	91.39	0.003	77.01	0.040	88.83	0.004	61.50	0.318
No. G	57	97.36	0.001	83.32	0.013	85.67	0.008	62.52	0.287
G, C and E one factor and specifies	61	168.20	0.000	157.69	0.000	128.84	0.000	180.01	0.000

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Table 7 Parameter estimates from Cholesky decomposition (1 factor for G and C, full decomposition for E) carried out separately on data of male and female twins

	Rest1	Rest2	RT1	RT2	MA1	MA2
Females SBP						
Vg	6.42	3.50	21.05	30.01	45.83	93.92
Ve	29.50	34.05	30.58	26.87	52.04	42.11
Vc	11.18	11.18	28.74	17.31	5.24	5.19
h^2	14%	7%	26%	40%	44%	67%
c^2	24%	23%	36%	23%	5%	4%
Females DBP	Rest1	Rest2	RT1	RT2	MA1	MA2
Vg	1.19	2.99	7.42	1.85	19.70	16.20
Ve	18.46	14.07	15.46	18.30	14.16	19.86
Vc	10.75	10.29	15.67	13.56	3.08	3.18
h^2	4%	11%	19%	5%	53%	41%
c^2	35%	38%	41%	40%	8%	8%
Males SBP	Rest1	Rest2	RT1	RT2	MA1	MA2
Vg	19.50	7.76	52.66	57.81	60.03	76.55
Ve	40.18	39.83	40.84	33.95	40.07	49.53
Vc	20.58	26.98	9.40	14.80	15.03	13.02
h^2	24%	10%	51%	54%	52%	55%
c^2	26%	36%	9%	14%	13%	10%
Males DBP	Rest1	Rest2	RT1	RT2	MA1	MA2
Vg	32.23	26.80	27.79	32.73	27.86	27.92
Ve	13.10	21.96	22.48	21.20	30.90	34.02
h^2	71%	55%	55%	61%	47%	45%

than blood pressure values of females. Therefore, in contrast to the univariate analyses, multivariate analyses were carried out separately in males and females. We used an exploratory multivariate model, ie a fully saturated unconstrained model for the genetic, shared environmental and specific environmental variances and covariances. In these analyses, shared environment contributed significantly to blood pressure levels at rest and during stress, although the contribution during the most stressful task (mental arithmetic) became very small.

For males as well as for females genetic and shared environmental influences clearly indicated a one-factor structure. Thus, the genetic and shared environmental factors that influence blood pressure during rest do not differ from the genetic and shared environmental factors that influence blood pressure during stress conditions. A more complicated structure was seen for the specific environmental part of the model. It is possible that this specific environmental covariance structure includes variance caused by genotype $\times$ environment interaction, which in structural models such as employed in our analyses cannot be distinguished from the random environmental component. This complex structure for the specific environmental part of the model is probably the reason that the simplex and factor models we initially fitted to the multivariate data did not give an adequate account of the covariance structure.

In agreement with the univariate analyses, multivariate analyses demonstrated an increase in the heritability of blood pressure as a consequence of stress. The effect was more pronounced than in the univariate analyses and, tentatively, a dose-response effect was suggested such that the heritability increased most in the task (MA) that yielded the greatest blood pressure increases. This same effect was also observed by Snieder et al[36] when univariate genetic models were fitted to blood pressure reactivity scores of the same subjects. Diastolic blood pressure in males, however, formed an exception, possibly because heritability of blood pressure was already high at rest. Overall, the analyses of the blood pressure data clearly demonstrate the increased power of multivariate as compared to univariate analyses[37] to detect both genetic and shared environmental components of variance and covariance.

With regard to the stress-induced blood pressure increase, this study presents us with some enigmatic results. The same genetic factors were found to influence individual differences in blood pressure at rest and under stress, but the multivariate results suggested a clear increase in the impact of these genetic factors during stress. Apparently, there is a

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genetic tendency towards high resting blood pressure levels that is amplified during stress. What could be the nature of such a 'genes by stress' interaction? It is well-known that blood pressure regulation is a complex multifactorial phenomenon influenced by various nervous and hormonal control systems like the sodium retention system, the renine-angiotensin system, the baroreflex-regulation and sympathetic nervous control of cardiac output and vasoconstriction. All these blood pressure regulation systems are known to have a genetic component[38–44] and all these systems are engaged by the type of stressors used in this study.[45] Thus, it is plausible that the impact of genetic influences in one or more of these systems is amplified during stress. As a single example of such a mechanism we can point to subjects with alpha-1-antitrypsin (AAT) deficiency. These subjects have lower blood pressure levels during rest and stress, but the effect of AAT on blood pressure is much more pronounced during stress than it is during rest.[46] Possibly their aberrant regulation of elastase prevents the loss of vascular elasticity with aging. The advantage of less stiff vessels may be amplified during stress because noradrenergically induced vasoconstrion is attenuated.

Clearly, the present study cannot decide to what extent genetic variation in the various regulatory systems accounts for increased genetic control over blood pressure during stress. To address this problem, we would need to include indices of renal sodium retention and renin-angiotensin systems, cardiac and vascular baroreflex control and cardiac and vascular sympathetic nervous system activity in one study. Many such indices do exist in fact, and can be derived by simple venipuncture or even non-invasively. Examples include aldosteron, angiotensin converting enzyme, baroreflex sensitivity, respiratory sinus arrhythmia (cardiac parasympathetic tone),[47] pre-ejection period (cardiac sympathetic tone), cardiac output and peripheral vascular resistance. All these indices are now routinely assessed in (behavioural) medicine.[48–50] The added value of assessing these variables in a twin study is that it allows the computation of genetic covariance between blood pressure levels and indices of the blood pressure regulatory systems. Such a twin study would yield a clear picture of the relative contributions of these systems to the genetic variation in blood pressure. Model fitting on the complete set of blood pressure and underlying regulatory variables – both at rest and during stress – would further provide us with a multivariate genetic factor score for blood pressure that is a far more informative phenotype than resting blood pressure by itself. Such a multivariate phenotype is known to increase the statistical power of genetic linkage substantially[51,52] making it feasible to hunt down the most relevant 'blood pressure genes' in humans.

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