

Heritability and Stability of Resting Blood Pressure

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We examined the contribution of genetic and environmental influences to variation in resting systolic (SBP) and diastolic (DBP) blood pressure in participants from 4 twin studies carried out between 1986 and 2003. A total of 1577 subjects (682 males, 895 females) participated. There were 580 monozygotic twins, 664 dizygotic twins and 333 of their siblings. The 4 studies sampled subjects in different age groups (average age 17, 32, 37, 44 years), allowing for comparison of the relative contribution of genetic and environmental factors across the first part of the life span. Blood pressure was assessed under laboratory conditions in 3 studies and by ambulatory monitoring in 1 study. Univariate analyses of SBP and DBP showed significant heritability of blood pressure in all studies (SBP h^2 48% to 60%, DBP h^2 34% to 67%). Overall, there was little evidence for sex differences in blood pressure heritability, and no evidence for differences in heritability due to measurement strategy (laboratory vs. ambulatory). For 431 subjects there were data from 2 or more occasions that allowed us to assess the tracking of blood pressure over time and to estimate the genetic and environmental contributions to blood pressure tracking. Correlations over time across an average period of 7.1 years (tracking) were between .41 and .70. Multivariate genetic analyses showed that blood pressure tracking was entirely explained by the same genetic factors being expressed across time. It was concluded that whole genome scans for resting blood pressure can safely pool data from males and females, laboratory and ambulatory recordings, and different age cohorts.

Research during the last decades has demonstrated a significant genetic contribution to resting systolic (SBP) and diastolic blood pressure (DBP). Heritability estimates from twin studies range from 11% to 78% for SBP and from 22% to 74% for DBP (reviewed in Snieder, 2004) with average levels at about 50% (Evans et al., 2003). There is a nonlinear increase in the means and variances of SBP and DBP with age, but longitudinal studies also suggest a substantial degree of stability in individual differences in blood pressure values (Burton et al., 2005; Eisenmann et al.,

2004; Evans et al., 2003). This stability may be caused by genes or environmental factors whose influences are stable across time. Nonstable genetic influences could represent the emergence of new genetic effects at later ages or age-selective amplification of the effects of some of the blood pressure genes and reduction of others. Similarly, environmental factors may decrease stability in blood pressure values. These phenomena can have important implications for gene finding studies. The age-dependent emergence of gene effects has direct consequences for whole genome scans attempting to identify the genetic loci harboring blood pressure genes. If genes are switched on only after a certain age, analyzing data from younger and older subjects in a single genome scan may detract from the normal strength of pooling data and reduce, rather than increase, statistical power to find linkage.

Many population-based studies confirm a significant tracking of blood pressure across large parts of the life span, but informative studies on the genetic contribution to blood pressure tracking are limited. In a large Norwegian sample with 43,751 parent-offspring pairs, 19,140 pairs of siblings, and 169 pairs of twins, correlations between relatives decreased as age differences between them increased. For SBP, 62% of the genetic variance at age 20 and at age 60 was explained by genes that were common to both ages, but 38% was explained by age-specific genetic effects. For DBP these values were 67% and 33%, respectively (Tambs et al., 1993). With a design including twins, their parents and a separate group of middle-aged twins of the same age as the parents, no support was found for the existence of age-specific genetic effects on blood pressure as well (Snieder et al., 1995).

To date only two longitudinal twin studies have addressed the potential emergence of new genetic factors for blood pressure directly. Colletto et al. (1993) measured resting SBP and DBP in 254

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monozygotic (MZ) and 260 dizygotic (DZ) male middle-aged twin pairs (average age 48 years) and again 9 and 24 years later. Iliadou et al. (2002) followed 298 same-sex elderly twin pairs at an average age of 65 years and again 6 years later. In a longitudinal twin study, a twin's blood pressure at time one is used to predict their co-twin's blood pressure at later time points. Such a prediction can be significant only if shared familial factors affect the blood pressure of both twins. These familial factors can be further decomposed in genetic and shared environmental effects, by testing whether the cross-time cross-twin prediction is larger in MZ twins than in DZ twins. If so, this constitutes evidence for a genetic source of stability in blood pressure. Using structural equation models that formalize this twin logic, a considerable genetic stability in both diastolic and systolic blood pressure was found over the 24- and 6-year follow-up periods in both longitudinal twin studies respectively (Colletto et al., 1993; Iliadou et al., 2002). However, the two studies partly disagreed on the question whether changes in individual differences in blood pressure across time were caused by genetic factors. In the first study on middle-aged subjects, genetic variation present at time one contributed to about 60% of that present at the second time 9 years later, but the remaining 40% of genetic variation was new. At the third measurement occasion 15 years later, no new genetic variation was evident (Colletto et al., 1993). In the second study on elderly twins, however, the same set of genes explained all genetic variance in blood pressure across the 6-year follow-up and no evidence was found for new genes being switched on or off at different points in time (Iliadou et al., 2002).

In this article we readdress the issue of the relative contributions of genes and environment to variation in SBP and DBP across the life span. Data come from four different studies of twins and their siblings carried out between 1986 and 1988 (Boomsma et al., 1998), between 1992 and 1994 (Snieder et al., 1995), between 1997 and 2002 (Posthuma et al., 2001) and between 1998 and 2003 (Kupper et al., 2005). As there was considerable overlap between the subjects who took part in these studies, we used the entire dataset to study the heritability of blood pressure at different ages, and second, to study the etiology of tracking in blood pressure. Across the four studies, resting blood pressure was measured by two different strategies. In three studies blood pressure was assessed during quiet resting in a laboratory baseline setting, and in one study blood pressure was obtained from home recordings during quiet sitting in leisure time. To date, heritable and environmental influences on both conventional and ambulatory blood pressure have been studied in the same twin sample only once (Fagard et al., 1995, 2003; Vinck et al., 2001). No significant differences in heritability across measurement strategies were found, which suggests that genome research could pool laboratory and ambulatory data

(Vinck et al., 2001). Here we will reexamine the validity of such pooling.

In two of our studies we not only recruited MZ and DZ twins, but also their siblings. If siblings do not differ from twins, then it is possible, for linkage studies, to employ the larger families, realizing a considerable gain in statistical power (Dolan et al., 1999). The use of additional siblings enables alternative methods of analysis, and improved power to estimate heritability (Posthuma & Boomsma, 2000). We examined whether there are differences in heritability estimates and results of structural equation modeling when data from larger sibships are analyzed with a family-based approach, or an all-possible-pairs-based approach in which all possible pairs within a family are formed. This latter approach is applied in genome scans (e.g., Fallin et al., 2004; Holmans et al., 2004).

Materials and Methods

Participants

Blood pressure data of Dutch twins and siblings participating in four different studies of the Netherlands Twin Register (NTR) were collected over the last decade (Boomsma et al., 1998; Kupper et al., 2005; Posthuma et al., 2001; Snieder et al., 1995). These studies were approved by the medical ethical committee of the Free University of Amsterdam. Four triplets were included as pairs by discarding the data from the middle child. Study 1 was part of a project in which cardiovascular risk factors were studied in 160 twin families (Boomsma et al., 1998). Addresses of twins living in and around Amsterdam were obtained from city council population registries. Twins and both their biological parents were invited to participate. Twins were between 14 and 21 years of age at the time of blood pressure measurement. Study 2 was also part of a cardiovascular risk factor project. Here, middle-aged Dutch twins were recruited by a variety of means including advertisement in twin newsletters and through city councils (Snieder et al., 1995). A total of 213 families participated and twins were between 34 and 63 years old at the time of measurement. In Study 3 blood pressure was measured in 261 extended Dutch twin families (twins and their siblings) who took part in a study of cognition and brain function (Posthuma et al., 2001). The age of the subjects ranged between 14 and 71 years at the time of measurement, with a somewhat bimodal distribution. Study 4 was a project studying the genetics of anxious-depression and cardiovascular risk factors (Kupper et al., 2005). Twins and siblings from the NTR were invited and 792 subjects from 339 families agreed to participate. The age of twins and siblings ranged between 17 and 81 years at the time of blood pressure measurement.

Families that were included in this study had blood pressure data on at least two subjects (twin-twin, twin-sib or sib-sib pairs). Hypertension or the use of antihypertensive medication was not an

exclusion criterion in any of the studies, but blood pressure values of subjects using medication were adjusted (see below). The maximum number of family members was restrained to six (two families had more than six siblings, here sibs were randomly selected). This yielded a final total of 1577 subjects (682 males, 895 females) from 648 twin families across the four studies. Zygosity of the same-sex twin pairs was originally determined by various means (e.g., questionnaires, blood polymorphisms) but later verified by DNA typing in all twin pairs.

Procedures

In total 2061 SBP and DBP blood pressure measurements were obtained in the four studies for the 1577 subjects. The number of subjects with one, two and three blood pressure measurements were 1146, 378 and 53, respectively. Subjects with more than one measurement showed an average age difference of 7.1 years and a maximum 15.1 years between the earliest and latest measurement. Study 1 and Study 2: Brachial SBP and DBP of seated subjects was measured in a sound attenuated, electrically shielded cabin using an oscillometric technique (Dinamap 845XT) during rest and two or three mental stress tasks (Boomsma et al., 1998; Snieder et al., 1995). For the current study, we used resting blood pressure only, which was the average of six readings during two 8-minute rest conditions (beginning, middle, end) in Study 1, and the average of three values in Study 2 in which one 8-minute rest condition was included. In study 3, subjects came to the laboratory to undergo extensive testing on cognitive functioning, including reaction time tests and electroencephalography (de Geus et al., 2001; Posthuma et al., 2001). Throughout these measurements participants were sitting in a dimly lit cabin. After acclimatization and instructions, resting blood pressure level was determined as the average of three readings with a 2- to 5-minute interval, using the same procedure as in Study 1 and Study 2 (Brachial cuff, Dinamap 845XT). In study 4, unlike the other studies, ambulatory blood pressure was measured over a whole working day using a Spacelabs 90207 monitor (Kupper et al., 2005). Blood pressure measurements were taken every 30 minutes, and in case of failure repeated after 2 minutes. The subjects were warned by a tone before the measurement to keep their arms as relaxed as possible. The subjects also needed to write down their activities around the time of measurement. From the total set of blood pressure measurements only the evening measurements were selected where subjects were seated calmly. The average of these measurements was taken as their ambulatory rest value. The number of measurements per subject was on average 3.8, with a minimum of 1 and a maximum of 10.

If subjects took antihypertensive medication at the time of measurement (mainly older subjects), a correction of +14 mmHg for SBP and +10 mmHg for DBP was made in Study 2 and Study 3 (Cui et al., 2003; Palmer et al., 2003). For Study 4 medication correction

was drug class-specific (Kupper et al., 2005), but the average treatment effects were also close to +14 mmHg for SBP and +10 mmHg for DBP. The number of subjects using blood pressure medication was 0, 27, 39 and 21 for Study 1 to Study 4, respectively.

Statistical Analysis

For each study, the contribution of genetic and environmental factors to SBP and DBP was determined using a univariate structural equation modeling approach with the Mx program (Neale, 2004; Neale & Cardon, 1992). The effects of age at time of measurement and sex on mean blood pressure levels were modeled using linear regression. The total variance of blood pressure was partitioned into additive genetic (A) variance, common environmental variance shared by siblings growing up in the same family (C), and random environmental variance (E). The covariance between pairs was modeled by setting the correlations between the genetic factors to 1 for MZ twins, and to .5 for DZ twins, twin-sib and sib-sib pairs. Correlations between shared environmental factors were set to unity in all pairs. Sex differences between the relative contributions of the ACE components were tested by estimating separate variance components in males and females, and then by equating the contributions (Medland et al., 2004; Neale & Cardon, 1992). Furthermore, a series of alternative explanations, the models AE, CE where required, and E, were tested. Model fitting was carried out on the raw data and was tested by likelihood-ratio difference tests: chi-square distributed statistic with the difference in free parameters as degrees of freedom. Equality of variances and means for twins and siblings, and equal covariance between siblings and DZ twins were formerly tested in a saturated model. The saturated model only specified a correlation between family members: the standardized covariance between twins and/or siblings. Sex differences in variances and correlations were tested under the saturated model.

Based on the univariate results, Studies 1 to 4 were analyzed simultaneously in a multivariate approach to evaluate the temporal contribution of A and E. The blood pressure measurement of each study was assigned as a variable, and analyses were done separately for SBP and DBP. In order to analyze the data from the four studies simultaneously, the raw data of the individual studies were combined. Individuals who did not participate in a particular study were assigned a missing value for the blood pressure of that study. The total variance, means, age and sex regression coefficients were allowed to differ between studies. Models using a full Cholesky (triangular) decomposition for the variance and covariance components were fitted to the data (Neale & Cardon, 1992). Subsequently, a series of nested AE models was tested. The first model did not include temporal unique environment within subjects, meaning that the influence of environment on blood pressure within an individual was assumed to be measurement specific. The second model examined

Table 1

Mean Age and Blood Pressure Levels Sitting at Rest in Male and Female Individuals of the Four Dutch Twin Studies

| Group | Sex | Variable | Study 1 | Study 2 | Study 3 | Study 4 ^a |
|-------------------|--------|----------|-------------|--------------|--------------|----------------------|
| Monozygotic twins | Male | <i>N</i> | 70 | 92 | 117 | 57 |
| | | Age | 16.6 (1.8) | 42.9 (5.6) | 36.8 (12.3) | 34.0 (13.1) |
| | | SBP | 119.8 (8.2) | 129.1 (11.9) | 129.7 (14.4) | 129.7 (11.1) |
| | | DBP | 65.6 (6.4) | 80.5 (9.6) | 77.6 (12.6) | 77.5 (9.6) |
| | Female | <i>N</i> | 70 | 98 | 147 | 108 |
| | | Age | 16.0 (2.2) | 45.4 (7.4) | 39.0 (13.1) | 29.0 (10.5) |
| | | SBP | 115.0 (5.7) | 120.7 (12.0) | 122.5 (14.4) | 124.0 (10.8) |
| | | DBP | 67.6 (4.7) | 73.5 (10.0) | 74.8 (10.0) | 77.1 (9.2) |
| Dizygotic twins | Male | <i>N</i> | 91 | 114 | 125 | 80 |
| | | Age | 16.9 (1.8) | 44.6 (7.1) | 36.2 (13.1) | 29.3 (8.8) |
| | | SBP | 119.8 (9.3) | 127.6 (11.7) | 129.6 (12.4) | 131.1 (10.7) |
| | | DBP | 65.6 (7.4) | 78.2 (8.9) | 77.6 (11.8) | 77.6 (8.9) |
| | Female | <i>N</i> | 89 | 120 | 175 | 137 |
| | | Age | 17.2 (1.9) | 44.1 (6.3) | 37.0 (12.7) | 30.9 (11.3) |
| | | SBP | 115.3 (7.3) | 124.5 (16.2) | 124.6 (16.2) | 125.4 (12.9) |
| | | DBP | 67.9 (5.6) | 75.7 (11.8) | 76.1 (11.0) | 78.0 (10.9) |
| Siblings | Male | <i>N</i> | — | — | 88 | 74 |
| | | Age | — | — | 37.3 (14.2) | 35.2 (13.1) |
| | | SBP | — | — | 128.3 (13.0) | 130.4 (9.7) |
| | | DBP | — | — | 78.3 (11.2) | 79.2 (8.4) |
| | Female | <i>N</i> | — | — | 99 | 110 |
| | | Age | — | — | 37.3 (12.8) | 36.7 (11.9) |
| | | SBP | — | — | 124.3 (16.4) | 122.6 (12.2) |
| | | DBP | — | — | 76.4 (10.2) | 77.0 (9.7) |

Note: *N* = number of subjects; Age = age at time of blood pressure measurement; SBP = systolic blood pressure; DBP = diastolic blood pressure. Standard deviation given in brackets. Blood pressure levels were corrected for medication; see text for details.

a = ambulatory blood pressure measurement.

Table 2

Age at Measurement and Sex-Adjusted Intrapair Twin and Sibling Correlations in the 4 Dutch Twin Studies for Resting Blood Pressure

| Blood pressure | Pairtype | Study 1 | Study 2 | Study 3 | Study4 ^a |
|----------------|----------|------------------|-----------------|------------------|---------------------|
| SBP | MZM | .56 (.25; .74) | .44 (.18; .63) | .63 (.46; .74) | .38 (.07; .61) |
| | DZM | .57 (.32; .74) | .10 (−.21; .38) | −.02 (−.34; .31) | .05 (−.34; .42) |
| | MZF | .52 (.19; .72) | .60 (.35; .74) | .55 (.38; .68) | .61 (.39; .75) |
| | DZF | .19 (−.11; .46) | .34 (.09; .55) | .34 (.11; .53) | .20 (−.05; .43) |
| | DOS | .21 (−.16; .52) | .10 (−.21; .39) | .42 (.17; .62) | .10 (−.26; .43) |
| | SM | — | — | .16 (−.04; .36) | .23 (.03; .42) |
| | SF | — | — | .38 (.21; .53) | .20 (.00; .37) |
| | SOS | — | — | .24 (.00; .43) | .24 (.05; .38) |
| DBP | MZM | .65 (.40; 0.79) | .44 (.19; .63) | .65 (.48; .76) | .34 (.01; .58) |
| | DZM | .54 (.27; 0.72) | .20 (−.14; .49) | .01 (−.27; .30) | .10 (−.34; .47) |
| | MZF | .35 (−.02; 0.61) | .65 (.46; .78) | .51 (.34; .64) | .62 (.39; .75) |
| | DZF | .38 (.08; 0.61) | .32 (.04; .54) | .34 (.12; .52) | .15 (−.09; .37) |
| | DOS | .14 (−.22; 0.48) | .13 (−.19; .42) | .50 (.28; .67) | −.16 (−.49; .21) |
| | SM | — | — | .19 (−.06; .41) | .14 (−.06; .31) |
| | SF | — | — | .45 (.30; .57) | .26 (.07; .42) |
| | SOS | — | — | .05 (−.21; .32) | .15 (−.06; .35) |

Note: SBP = systolic blood pressure; DBP = diastolic blood pressure; MZM = monozygotic male twins; DZM = dizygotic male twins; MZF = monozygotic female twins; DZF = dizygotic female twins; DOS = opposite sex twins; SM = male sib–sib and sib–twin pairs; SF = female sib–sib and sib–twin pairs; SOS = opposite sex sib–sib and sib–twin pairs. 95% confidence interval around estimate given in brackets.

a = ambulatory blood pressure measurement.

whether the heritability over studies and over time-points within subjects could be equated. A third model was tested in which it was assumed that a single genetic factor influenced blood pressure at all time-points. Finally a fourth model was tested in which the single genetic factor also had an equal contribution to the blood pressure variance over time points.

In all uni- and multivariate analyses, parameters and their 95% confidence intervals were estimated by raw-data maximum likelihood with the Mx computer program. A significance level of .01 was employed. In Study 3 and Study 4, the data were analyzed using the family-based as well as an all-possible-pairs method.

Results

In Table 1 the mean SBP, DBP and age at measurement are shown for males and females of the individual

studies. Mean SBP was significantly higher in males compared to females in all studies (p values < .001). For DBP, adolescent females have higher mean values than males ($p = .007$), while for adults this changes to a higher pressure for males in Study 2 and Study 3 ($p < .001$). Mean ambulatory DBP in Study 4 did not show a significant difference between males and females ($p = .369$). SBP and DBP increase with age in all studies (p values < .002), except for SBP in adolescents (Study 1, $p = .063$). In Study 1 a possible sex difference in variances was observed (SBP, $p = .005$; DBP, $p = .011$) with males having larger variances than females.

For each study, twin correlations for the sex and age-at-measurement adjusted mean SBP and DBP levels are presented in Table 2. The correlations were generally higher in MZ than DZ twins. Differences between the male and female MZ correlations, as well

Table 3
Univariate Analysis Goodness-of-Fit Parameters and Proportion of Variance Explained by Components of Sex and Age at Time of Measurement Adjusted Systolic Blood Pressure

| Study | Analysis | Model | Sex | A | C | E | -2LL | df | $\Delta\chi^2$ | Δdf | p | |
|-----------|-----------|-----------|-----------------------|-----------------------|-----------------------|----------------|----------------|------------|----------------|-------------------------|------|--|
| 1 | Family | ACE | M | .02 (.00; .56) | .54 (.07; .70) | .44 (.27; .62) | 2165.3 | 311 | | | | |
| | | | F | .51 (.02; .72) | .02 (.00; .38) | .47 (.27; .79) | | | | | | |
| | All pairs | ACE | B | .37 (.00; .69) | .18 (.00; .50) | .45 (.31; .65) | 2179.0 | 314 | 13.6 | 3 | .003 | |
| | | | M | .54 (.34; .68) | .46 (.31; .66) | 2170.2 | 313 | 4.8 | 2 | .090^a | | |
| | | AE | M | .60 (.39; .74) | .40 (.26; .61) | 2170.3 | 313 | 5.0 | 2 | .083^a | | |
| | | | F | .52 (.20; .72) | .48 (.28; .80) | | | | | | | |
| 2 | Family | ACE | M | .36 (.00; .58) | .03 (.00; .38) | .61 (.42; .86) | 3337.7 | 415 | | | | |
| | | | F | .47 (.00; .75) | .16 (.00; .56) | .38 (.24; .61) | | | | | | |
| | All pairs | ACE | B | .51 (.20; .64) | .00 (.00; .22) | .49 (.36; .65) | 3346.9 | 418 | 9.2 | 3 | .026 | |
| | | | B | .51 (.35; .64) | .49 (.36; .65) | 3346.9 | 419 | 9.2 | 4 | .056 | | |
| | | AE | M | | | | | | | | | |
| | | | F | | | | | | | | | |
| 3 | Family | ACE | M | .56 (.31; .70) | .01 (.00; .16) | .43 (.29; .62) | 5893.5 | 742 | | | | |
| | | | F | .43 (.10; .69) | .15 (.00; .40) | .42 (.31; .57) | | | | | | |
| | | ACE | B | .56 (.30; .68) | .03 (.00; .22) | .41 (.32; .52) | 5904.3 | 745 | 1.9 | 3 | .012 | |
| | | | B | .59 (.49; .68) | .41 (.32; .51) | 5904.4 | 746 | 1.9 | 4 | .027 | | |
| | | All pairs | ACE | M | .53 (.26; .68) | .02 (.00; .15) | .46 (.32; .64) | 10,157.8 | 1277 | | | |
| | | | | F | .37 (.06; .67) | .21 (.00; .43) | .42 (.31; .56) | | | | | |
| | All pairs | ACE | B | .54 (.27; .68) | .05 (.00; .23) | .41 (.33; .53) | 10,174.7 | 1280 | 16.9 | 3 | .001 | |
| | | | B | .60 (.51; .68) | | .40 (.32; .49) | 10,175.0 | 1281 | 17.3 | 4 | .002 | |
| | | Family | ACE | M | .36 (.00; .60) | .03 (.00; .35) | .62 (.40; .86) | 4298.6 | 557 | | | |
| | | | | F | .54 (.21; .70) | .00 (.00; .19) | .46 (.30; .67) | | | | | |
| All pairs | ACE | B | .48 (.19; .61) | .00 (.00; .17) | .52 (.39; .68) | 4301.7 | 560 | 3.1 | 3 | .376 | | |
| | | B | .48 (.33; .61) | .52 (.39; .67) | 4301.7 | 561 | 3.1 | 4 | .541 | | | |
| | All pairs | ACE | M | .39 (.00; .64) | .05 (.00; .40) | .56 (.36; .81) | 7548.0 | 971 | | | | |
| | | | F | .51 (.17; .67) | .00 (.00; .19) | .49 (.33; .70) | | | | | | |
| All pairs | ACE | B | .48 (.19; .60) | .00 (.00; .16) | .52 (.40; .68) | 7548.6 | 974 | .6 | 3 | .894 | | |
| | | B | .48 (.34; .60) | | .52 (.40; .66) | 7548.6 | 975 | .6 | 4 | .962 | | |

Note: M = male; F = female; B = both sexes; A = additive genetic component (heritability); C = common familial environment component; E = unique environment component; -2LL = -2 times maximum likelihood; df = degrees of freedom. 95% confidence interval around estimate given in brackets.

a = likelihood ratio test against the ACE-MF model.

b = ambulatory blood pressure.

Most parsimonious models are given in bold. Level of significance $\alpha = .01$.

Table 4

Univariate Analysis Goodness-of-Fit Parameters and Proportion of Variance Explained by Components of Sex and Age at Time of Measurement Adjusted Diastolic Blood Pressure

| Study | Analysis | Model | Sex | A | C | E | -2LL | df | $\Delta\chi^2$ | Δdf | p | |
|----------------|-----------|----------------|----------------|-----------------------|-----------------------|-----------------------|----------------|------------|----------------|-------------------------|-------------------|--|
| 1 | Family | ACE | M | .29 (.00; .77) | .37 (.00; .70) | .33 (.20; .54) | 2001.8 | 311 | | | | |
| | | | F | .19 (.00; .62) | .23 (.00; .54) | .58 (.37; .85) | | | | | | |
| | CE | B | .38 (.00; .69) | .18 (.00; .50) | .44 (.31; .64) | 2015.8 | 314 | 14.0 | 3 | .003 | | |
| | | M | | .58 (.37; .72) | .42 (.28; .63) | 2006.6 | 313 | 4.8 | 2 | .093^a | | |
| | | F | | .24 (.02; .46) | .76 (.54; .98) | | | | | | | |
| | | AE | M | .67 (.46; .80) | | .33 (.20; .54) | 2011.5 | 313 | 9.7 | 2 | .008 ^a | |
| | F | .34 (.00; .60) | | .66 (.40; 1.00) | | | | | | | | |
| 2 | Family | ACE | M | .35 (.00; .59) | .06 (.00; .47) | .58 (.40; .82) | 3111.9 | 415 | | | | |
| | | | F | .60 (.11; .77) | .05 (.00; .45) | .34 (.22; .54) | | | | | | |
| | AE | B | .54 (.22; .66) | .00 (.00; .25) | .46 (.34; .60) | 3118.5 | 418 | 6.6 | 3 | .085 | | |
| | | B | | .54 (.40; .66) | | .46 (.34; .60) | 3118.5 | 419 | 6.6 | 4 | .157 | |
| 3 | Family | ACE | M | .56 (.31; .71) | .01 (.00; .14) | .43 (.28; .63) | 5484.4 | 742 | | | | |
| | | | F | .27 (.00; .60) | .26 (.00; .48) | .47 (.35; .62) | | | | | | |
| | | ACE | B | .56 (.29; .65) | .00 (.00; .21) | .44 (.35; .55) | 5487.8 | 745 | 3.4 | 3 | .337 | |
| | AE | B | | .56 (.46; .65) | | .44 (.35; .54) | 5487.8 | 746 | 3.4 | 4 | .497 | |
| | | All pairs | ACE | M | .51 (.26; .66) | .02 (.00; .13) | .48 (.33; .66) | 9417.1 | 1277 | | | |
| | | | | F | .26 (.00; .57) | .27 (.01; .48) | .47 (.36; .61) | | | | | |
| | ACE | B | .54 (.27; .64) | .01 (.00; .20) | .45 (.36; .56) | 9422.1 | 1280 | 5.0 | 3 | .171 | | |
| | AE | B | .56 (.46; .64) | | .44 (.36; .54) | 9422.1 | 1281 | 5.0 | 4 | .285 | | |
| 4 ^b | Family | ACE | M | .15 (.00; .48) | .11 (.00; .37) | .74 (.49; .96) | 4075.7 | 557 | | | | |
| | | | F | .55 (.15; .71) | .01 (.00; .25) | .44 (.29; .67) | | | | | | |
| | | ACE | B | .44 (.17; .58) | .00 (.00; .15) | .56 (.42; .73) | 4083.5 | 560 | 7.8 | 3 | .051 | |
| | AE | B | | .44 (.28; .58) | | .56 (.42; .72) | 4083.5 | 561 | 7.8 | 4 | .101 | |
| | | All pairs | ACE | M | .20 (.00; .53) | .13 (.00; .39) | .68 (.44; .91) | 7117.0 | 971 | | | |
| | | | | F | .54 (.17; .70) | .01 (.00; .22) | .45 (.30; .67) | | | | | |
| | ACE | B | .44 (.18; .57) | .00 (.00; .14) | .56 (.43; .71) | 7123.1 | 974 | 6.0 | 3 | .110 | | |
| | AE | B | .44 (.30; .57) | | .56 (.43; .70) | 7123.1 | 975 | 6.0 | 4 | .197 | | |

Note: M = male; F = female; B = both sexes; A = additive genetic component (heritability); C = common familial environment component; E = unique environment component; -2LL = -2 times maximum likelihood; df = degrees of freedom. 95% confidence interval around estimate given in brackets.

a = likelihood ratio test against the ACE-MF model.

b = ambulatory blood pressure.

Most parsimonious models are given in bold. Level of significance $\alpha = .01$.

as the differences between the male, female and opposite-sex DZ correlations were not significant. Study 3 and Study 4 included siblings and no significant differences were found between the DZ twin and sibling correlations for SBP and DBP, indicating similar influences on blood pressure. Overall, the point estimates of the DZ correlations vary substantially between the four studies; however, the 95% confidence intervals of the estimates are large and overlapping.

Univariate Analyses

The results of the univariate structural equation analyses of age at the time of measurement and sex-adjusted SBP are presented in Table 3. No significant sex differences were found for the contributions of A, C and E in Study 2, 3 and 4. A model without common environment did not fit significantly worse than the full ACE model in these studies. For Study 1 sex differences were

present for adolescents tested under the ACE model. The AE model, CE model and ‘AE in females/CE in males’ model all fitted the data, indicating familial influences on BP, without the statistical power to resolve whether shared genes or shared environment explained the familial aggregation. In previous analyses of these data a similar result was obtained for resting blood pressure, but when blood pressure measured during stress tasks was added, a genetic model was more likely (Boomsma et al., 1998). The heritability estimates for SBP are similar across the four studies and range from 48% to 60%. A model with unique environment only could be excluded in all studies (*p* values < .001).

For age at time of measurement and sex-adjusted DBP no significant sex differences were found for the contribution of additive genetic factors, common and unique environment in Study 2, 3 and 4 (Table 4). A model with only the influences of additive genetic factors

Table 5
Age at Time of Measurement and Sex Adjusted Correlations of Systolic and Diastolic Blood Pressure within Individuals across the Four Twin Studies

| | Study 1 | Study 2 | Study 3 | Study 4 ^a |
|--|-----------------|----------------|----------------|----------------------|
| Study 1 (<i>N</i> = 320) | | — | .48 (.33; .60) | .70 (.32; .86) |
| Study 2 (<i>N</i> = 424) | — | | .57 (.49; .64) | .51 (.35; .64) |
| Study 3 (<i>N</i> = 751) | .58 (.42; .67) | .52 (.43; .60) | | .47 (.34; .58) |
| Study 4 ^a (<i>N</i> = 566) | .55 (–.04; .79) | .41 (.26; .55) | .47 (.35; .57) | |

Note: *N* = number of subjects. Below the diagonal SBP and above DBP. 95% confidence interval around estimate given in brackets.

a = ambulatory blood pressure.

Table 6
Multivariate Analysis Goodness-of-Fit Parameters of Sex and Age at Time of Measurement Adjusted Systolic and Diastolic Blood Pressure

| Blood pressure | Model | –2 LL | <i>df</i> | $\Delta\chi^2$ | Δdf | <i>p</i> |
|----------------|--|-----------------|-------------|----------------|-------------|-------------|
| SBP | AE Cholesky | 15,559.5 | 2029 | | | |
| | A Cholesky | 15,564.3 | 2035 | 4.8 | 6 | .570 |
| | E no environmental covariance across time | | | | | |
| | A equal heritability | 15,571.0 | 2038 | 11.5 | 9 | .240 |
| | E no environmental covariance across time | | | | | |
| | A one common factor | 15,568.2 | 2041 | 8.7 | 12 | .726 |
| | E no environmental covariance across time | | | | | |
| | A one common factor with equal variance proportions | 15,574.9 | 2044 | 15.4 | 15 | .425 |
| | E no environmental covariance across time | | | | | |
| DBP | AE Cholesky | 14,515.5 | 2029 | | | |
| | A Cholesky | 14,521.2 | 2035 | 5.6 | 6 | .468 |
| | E no environmental covariance across time | | | | | |
| | A equal heritability | 14,523.9 | 2038 | 8.3 | 9 | .502 |
| | E no environmental covariance across time | | | | | |
| | A one common factor | 14,525.9 | 2041 | 10.3 | 12 | .587 |
| | E no environmental covariance across time | | | | | |
| | A one common factor with equal variance proportions | 14,527.3 | 2044 | 11.8 | 15 | .697 |
| | E no environmental covariance across time | | | | | |

Note: –2 LL = –2 times maximum likelihood of the model; *df* = degrees of freedom. Most parsimonious models are given in bold. Level of significance $\alpha = 0.01$.

and unique environment best explained the variability of DBP. Again Study 1 is an exception, as the unique environment component was substantially larger in females (58% vs. 33%). For DBP in Study 1 the effects of common environment could not be excluded ($p = .008$). Both a common plus unique environment (CE) model and an additive genetic plus unique environment (AE) model fitted the data. However, as for SBP, it was previously found that a genetic model was more likely when resting DBP was analyzed in one model with stress values (Boomsma et al., 1998). An E model could be excluded in all studies (p values < .001). Heritability estimates for DBP range from 34% to 67% and are comparable over the four studies.

Multivariate Analyses

For the subjects with multiple measurements, age at measurement and sex-adjusted correlations between studies are shown in Table 5. The phenotypic correlations range from .41 to .58 for SBP and from .47 to .70

for DBP and show a substantial temporal stability in blood pressure. Based on the univariate analyses and assuming no sex differences to be present in adolescents, multivariate models were fitted to the data to study the contribution of genetic and environmental factors to the temporal stability of blood pressure (Table 6). The results show that the influence of unique environmental factors within an individual is time and measurement specific for SBP. Furthermore, the relative contribution of genetic and environmental factors, the heritability, remains the same over studies. We tested whether the same set of genes influenced blood pressure measurements across the four studies by reducing the Cholesky structure to a single common factor. The results show that this was allowed. Finally we tested if this factor contributes to an equal percentage of the SBP variation in studies and this cannot be rejected as well. The results for DBP show a similar pattern: no evidence for shared environment, only measurement-specific environmental influences and a common factor structure with an equal

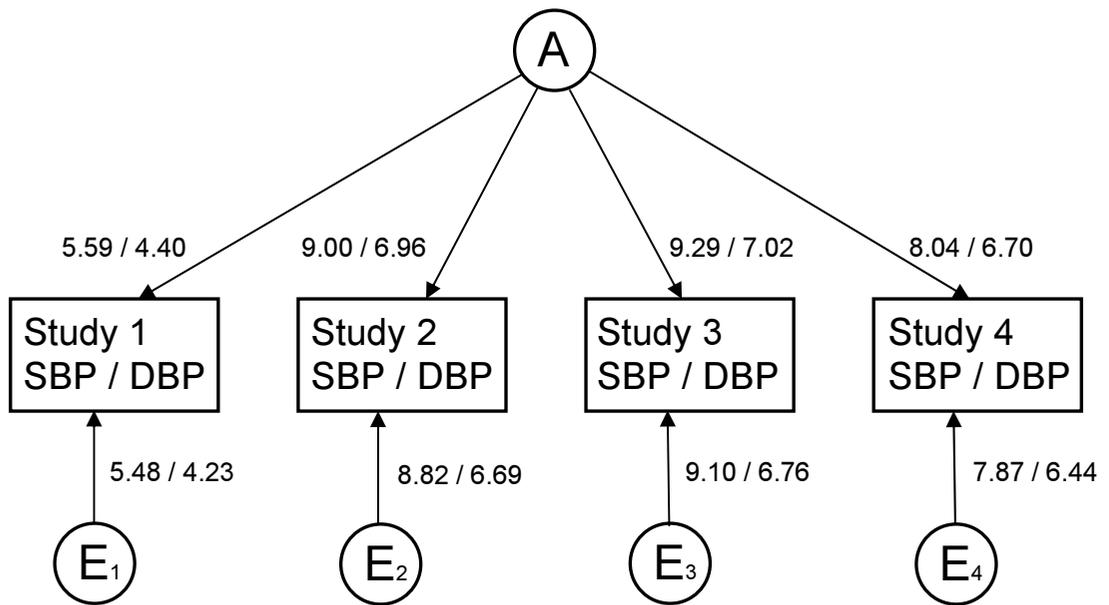


Figure 1

Pathway model showing latent genetic and environmental influences on the measured systolic and diastolic blood pressure corrected for sex and age at measurement.

Note: A represents the additive genetic factors common to the four measurements. E₁–E₄ shows the unique environmental influences at each time point/study. Path coefficients are shown. The proportions of measured variance for the latent variables are SBP: A = 51%, E = 49%; and DBP: A = 52%, E = 48%.

effect for the genetic influences across time (Table 6). As no differences were found between Study 4 and the other studies, and because the common factor model fits the data well, genetic influences on ambulatory and conventional blood pressure measures can be considered the same across measurement techniques. The most parsimonious model for SBP and DBP is given in Figure 1 with parameter estimates.

All structural equation analyses that were performed on the data structured in families were compared to the outcome from the analyses in which the data were reordered into all possible pairs where applicable. The results of these all-possible-pairs analyses show that there is very little difference in model parameter estimates (Tables 3 and 4, multivariate analyses data not shown). The significance of the all-pairs model is, however, different as compared to the family-based approach. In the case of borderline significance in the family-based model this may lead to different conclusions about the model best fitting the data, for example regarding the presence of sex differences in Study 3 for SBP.

Discussion

In a series of univariate analyses of SBP and DBP measured during rest in male and female twins and in their brothers and sisters, heritability estimates for SBP were obtained that ranged between 48% and 60%. For DBP, these estimates ranged between 34% and

67%. Additive genetic factors and unique environmental factors explained the data optimally for SBP as well as DBP. No significant difference in heritability could be detected between resting values obtained in laboratory and ambulatory studies. The estimates for resting SBP and DBP from all four studies under an AE or ACE model are in very good agreement with previous studies (Evans et al., 2003; Snieder, 2004).

Comparing the four studies in different age groups we conclude that the relative contribution of genes and environment to BP remains equal across the first part of the life span, even if absolute variance increases with age. No sex differences were found for DBP, but for SBP the presence of sex differences in the estimated variance components remains somewhat ambiguous. This is in keeping with a very large twin study over different countries where sex differences could mostly be excluded (Evans et al., 2003). Genetic architecture of blood pressure in our studies was more complex in adolescents than in adults: sex differences as well as common environment could not be excluded in the adolescent twin study. Previous multivariate analyses have shown, however, that sex differences are attenuated and an additive genetic source of familial resemblance is most likely when blood pressure measurements during different stress tasks are added to the resting values (Boomsma et al., 1998).

Substantial correlations across time were seen for both SBP and DBP. A correlation of around .50

over a time interval of at least 1 year has been suggested as a cut-off for a trait to be considered 'stable' (Bloom, 1964). Both SBP and DBP generally met this criterion. Cross-time correlations over an average span of 7 years ranged from .41 to .58 for SBP and from .47 to .70 for DBP. Multivariate analyses of the longitudinal data showed that genetic factors entirely accounted for this temporal stability. The influence of unique environment was time- and study-specific. More importantly, the results suggest that the *same* genetic factors influence blood pressure across the age range. No new genes are 'switched' on when subjects age. The tracking of blood pressure is thus based on a stable genetic component, which is very good news for large-scale linkage and association studies. Data from subjects of different ages can be pooled, although the effects of age on the *means* should be accounted for.

In linkage-based whole genome scans, an all-possible-pairs approach is used. Hence, we also examined whether different results obtain in an all-possible-pairs analysis compared to the family-based analysis. The all-possible-pairs approach is wrong in a sense that subjects are entered into the data multiple times depending on the family size. The practical advantage is a reduction of irregular pedigree structures that sometimes can make the family-based analysis difficult. Comparison of the two methods showed that the variance components estimates are very similar, as well as most conclusions about the model fitting. In the all-possible-pairs analysis, the threshold for significance is lower because the number of pairs (observations) is greatly increased. A similar finding, an increase in Type 1 error, was also found in studies testing all possible pairs compared to weighting statistics in linkage analysis (Abel et al., 1998; Abel & Muller-Myhsok, 1998).

In conclusion, whole genome scans for resting blood pressure that pool data from males and females, laboratory and ambulatory recordings, and different age cohorts can safely do so because they will largely tap into the same genetic factors.

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