Repeated blood pressure measurements in a sample of Swedish twins: heritabilities and associations with polymorphisms in the renin-angiotensin-aldosterone system

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Background **Twin and family studies have shown that genetic effects explain a relatively high amount of the phenotypic variation in blood pressure. However, many studies have not been able to replicate findings of association between specific polymorphisms and diastolic and systolic blood pressure.**

Methods **In a structural equation-modelling framework the authors investigated longitudinal changes in repeated measures of blood pressures in a sample of 298 like-sexed twin pairs from the population-based Swedish Twin Registry. Also examined was the association between blood pressure and polymorphisms in the angiotensin-I converting enzyme and the angiotensin II receptor type 1 with the 'Fulker' test. Both linkage and association were tested simultaneously revealing whether the polymorphism is a Quantitative Trait Locus (QTL) or in linkage disequilibrium with the QTL.**

Results **Genetic influences explained up to 46% of the phenotypic variance in diastolic and 63% of the phenotypic variance in systolic blood pressure. Genetic influences were stable over time and contributed up to 78% of the phenotypic correlation in both diastolic and systolic blood pressure. Non-shared environmental effects were characterised by time specific influences and little transmission from one time point to the next. There was no significant linkage and association between the polymorphisms and blood pressure.**

Conclusions **There is a considerable genetic stability in both diastolic and systolic blood pressure for a 6-year period of time in adult life. Non-shared environmental influences have a small long-term effect. Although associations with the polymorphisms could not be replicated, results should be interpreted with caution due to power considerations. *J Hypertens* 20:1543–1550 © 2002 Lippincott Williams & Wilkins.**

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Introduction

Blood pressure is an important risk factor for cardiovascular diseases, kidney failure and stroke. It is recognized as a multifactorial trait resulting from the effect of a combination of environmental and genetic factors. Several twin and/or family studies in recent decades have estimated the importance of the genetic influences (heritability). Heritabilities range from 12–66% for systolic blood pressure and 13–64% for diastolic blood pressure with average levels for both at about 50% [1–8].

Most studies have examined genetic and environmental effects in the variation of blood pressure in cross-sectional samples of data. The main reason for the discrepancies in the heritability estimates has been the age of the individuals in the different samples. Hong *et al.* [3] reported lower heritabilities in middle-aged adults than in elderly twins in a cross-sectional analysis of the data. However, a longitudinal study allows conclusions to be drawn about effects on the variation of blood pressures over time.

Efforts to date have identified several candidate genes involved in blood pressure or primary hypertension. Special attention has been paid to the study of genes implicated in the renin–angiotensin–aldosterone axis, including the angiotensin-I-converting enzyme gene

(ACE), important for circulatory homeostasis, and the angiotensin II type 1 receptor (AT_1R) gene, that seems to mediate the major cardiovascular effects of angiotensin II, an important effector controlling blood pressure and volume in the cardiovascular system (OMIM). However, for specific polymorphisms in these genes, associations with blood pressure have been somewhat contradictory [9–13]. The angiotensin-I-converting enzyme gene has been either linked and/or associated to elevated blood pressure levels in samples of Afro-Caribbeans, Japanese and some Caucasians [10,13–16]. On the other hand, Chinese and most European studies have shown no associations between the angiotensin-I-converting enzyme gene or angiotensin II receptor 1 gene and blood pressure [11,12,17]. The results of these studies suggest that there are possible underlying ethnic differences in the regulation of blood pressure, which have also been emphasized in studies comparing black and whites [9,18].

Variance-components techniques are robust for assessing both linkage and association [19]. In 1999, Fulker *et al.* [20] presented a structural equation modelling approach to obtain maximum-likelihood estimates for the allelic effect based on within-family and between-family differences. It is a flexible method that can combine both sib-pair linkage and association analysis for quantitative traits simultaneously.

The aim of the current study is to evaluate genetic and environmental effects in repeated measures of diastolic and systolic blood pressure in a sample of Swedish twins. Also investigated will be the association between these measured phenotypes and two known polymorphisms: the angiotensin-I-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and the angiotensin II receptor type 1 (AT_1R–A1166C).

Methods

Subjects

This study utilizes data from the Swedish Adoption Twin Study of Aging (SATSA) [3,21,22], which is based on the Swedish Twin Registry [23]. Twins in SATSA were identified as having been reared apart, along with matched pairs of twins who had been reared together [21]. SATSA is a longitudinal study with a 3-year interval between measurement occasions, each wave containing both questionnaire and in-person-testing (IPT) components. The present study consists of only like-sexed twins, who were above the age of 50 years when contacted for the first wave of in-person testing. However, new pairs of twins that turned 50 years of age during the second or third wave of testing were included in the study at subsequent waves as well. There are data on blood pressure from 645 individuals from the first wave of testing, 595 from the second wave and 569 from the third wave. Measures of blood pressure from individuals that have taken medication at a specific time point (wave 1, 2 or 3), including use or combined use of blood pressure-lowering medication, betablockers, diuretics, calcium antagonists and ACE inhibitors, were excluded from the analysis (173 measures from wave 1, 137 from wave 2 and 140 from wave 3). Univariate and multivariate outliers were identified by using estimated *Z*-scores, based on the measure of the Mahalanobis distance [24]. Pairs with *Z*-scores in excess of three for diastolic and 2.7 for systolic blood pressure were excluded from further analyses for that variable. In total, there were 10 univariate outliers for diastolic blood pressure, six for systolic blood pressure, four multivariate outliers for diastolic and 10 for systolic blood pressure. In the final sample, phenotypic information was available from 298 twin pairs (106 monozygotic (MZ) and 192 dizygotic (DZ) pairs) and 86 singletons, where information from only one twin was available.

This study was approved by the Ethics Committee of the Karolinska Institute, the Swedish National Data Inspection Authority and the IRB of the Pennsylvania State University.

Blood pressure measurement

Blood pressure was measured twice by trained nurses using a mercury sphygmomanometer with a cuff size of 17×67 cm with subjects in a supine position after 5 min of rest and after 1 min standing. The fifth phase Korotkoff sound was used as the diastolic reading. The supine measurement was used in the present study. At the beginning of the third wave of testing, measurement devices were changed to digital recorders. Normality of the blood pressure measurements was checked through Q-Q plots in SAS [25].

Genotyping

Individuals were genotyped for two polymorphisms: the insertion/deletion (I/D) polymorphism of the gene that codes for angiotensin-converting enzyme (ACE) located on chromosome 17q23 and the angiotensin II receptor type 1 (AT_1R–A1166C) located on chromosome 3. Genotypes of the ACE I/D and AT_1R–A1166C markers were determined according to Rigat *et al.* [26] and Doria *et al.* [27], respectively.

For the ACE I/D and the AT_1R–A1166C polymorphisms: 86 MZ and 113 DZ twin pairs and 87 MZ and 105 DZ twin pairs were genotyped, respectively; another 53 and 58 genotypes, respectively, were available from only one twin belonging to DZ pairs. A total of 26 individuals from the singletons were genotyped. A total of 46 pairs from the first polymorphism and 48 from the latter were not genotyped at all. Lack of genotyping was either due to depleted blood samples or uncertain genotyping. The latter refers to assay amplification that

is dependent on DNA amount and quality. The assays were the most commonly used at the time. Only pairs where both twins were genotyped and had phenotypic information on at least one measurement occasion (84 MZ, 112 DZ for ACE; 85 MZ, 105 DZ for AT_1R–A1166C) were used for the linkage and association analysis.

Analysis

Twin studies are ideal for estimating genetic and environmental effects of traits and diseases [28]. Identical (MZ) twins share the same genes, whereas fraternal (DZ) twins share on average half of their segregating genes. A broad measure of the similarity between twins is gained from calculations on the intraclass correlations [29]. Comparisons between the intraclass correlations for MZ and DZ twins provide information about the effects that are present.

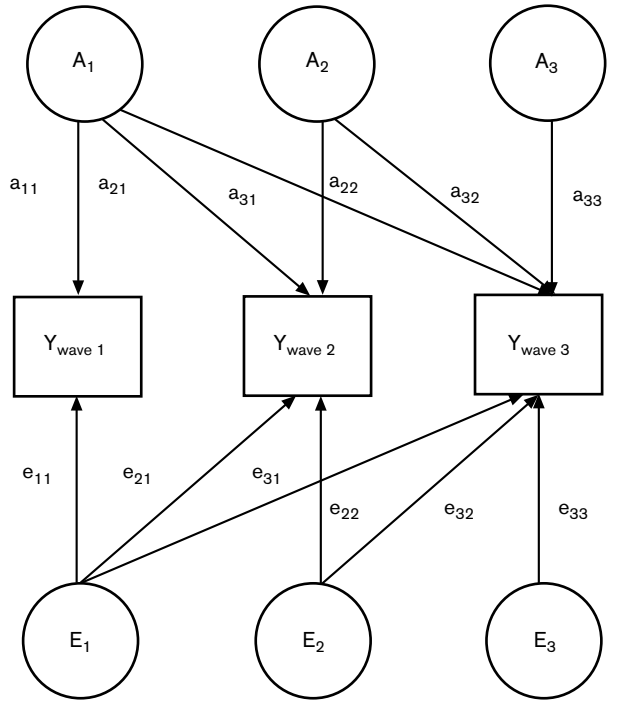
In general, the phenotypic variance is assumed to be due to three latent factors: additive genetic factors (A), shared environmental factors (C), and non-shared environmental factors (E, which also include measurement error): $\mathrm{Var}(Y) = A + C + E$

The correlation for MZ twin pairs is due to additive genetic and shared environmental factors (A + C). The correlation in DZ twins is assumed to be due to the sum of half the genetic, plus shared environmental factors ($\frac{1}{2}A + C$). A genetic effect is indicated if twin similarity is greater among MZ than DZ pairs. Heritability is defined as the proportion of total phenotypic variation directly attributable to genetic effects [30].

Cholesky decomposition

The longitudinal aspect of each phenotype was modelled by means of the Cholesky decomposition [29]. Figure 1 illustrates a path diagram of the model. According to this, the first latent factor, for instance the genetic (A_1), is loading on all three phenotypes. The second latent factor (A_2) loads on the second and third phenotype. Finally, the third latent factor (A_3) loads only on the third phenotype. If several loadings from one factor are significant this indicates that the latent factor (genetic or environmental) influences the trait over time. On the other hand, if each factor only loads on one of the phenotypes, this indicates that separate (genetic or environmental) influences are operating at each time. First, the significance of the genetic and environmental effects were tested by fixing all genetic, shared environmental, or non-shared environmental loadings, separately. Then, the Cholesky model was reduced to a common factor model. That is, one common factor (genetic or environmental) loads on measures at each time point and is therefore proposed to affect the trait over time. Also tested was whether specific (genetic or environmental) effects were important at each time point by allowing only for separate factor loadings at each time point. Further, it is of interest to estimate the portion of the phenotypic correlation between waves that is due to genetic or environmental effects. For instance, in the absence of shared environmental effects, the portion of correlation between phenotypes in wave 1 and 2 due to genetic effects can be calculated as $(a_{11} \times a_{21}) / (a_{11} \times a_{21} + e_{11} \times e_{21})$ and between phenotypes in wave 2 and 3 as $((a_{21} \times a_{31}) + (a_{22} \times a_{32})) / ((a_{21} \times a_{31}) + (a_{22} \times a_{32}) + (e_{21} \times e_{31}) + (e_{22} \times e_{32}))$ (Fig. 1).

Fig. 1



Cholesky decomposition model for three time points, wave 1, 2 and 3. A_1, A_2 and A_3 and E_1, E_2 and E_3 are the genetic and non-shared environmental components at first, second and third wave of testing, respectively. Y is the phenotype under study.

Linkage and association

Models have been presented that jointly perform tests of both linkage and association controlling for spurious associations due to population stratification and admixture [20,31]. Testing linkage, while simultaneously modeling association, would provide a test of whether the Quantitative Trait Locus (QTL) is a candidate, or whether it is in linkage disequilibrium with a trait locus. Linkage is modeled in the covariance structure, while the association parameters along with other covariates are modeled in the means. The weighted likelihood approach is used for linkage, that is the estimated 'identity by descent' (IBD) probabilities of a pair at a particular chromosomal location are used as

weights [32]. The IBD probabilities were estimated from the GENEHUNTER program [33]. In the association part, the genetic effect of a QTL is partitioned into between and within sib-pair components. A robust test for association may be obtained by computing the difference between a model with the within sib-pair parameter free and a model with the same parameter set to 0, while the between sib-pair parameter is free in both models. A model utilizing phenotypic information from all three time points simultaneously was used by constraining the between parameter to be equal at all three time points and similarly for the within parameter.

Structural equation modelling

In all models, the phenotypic means were adjusted for the covariates age and sex. For the models fitted, the degrees of freedom and twice the log-likelihood probability were computed by means of the structural equation-modelling package MX [34]. Models were applied to raw data and a maximum likelihood approach was used to estimate the genetic and environmental components. To compare two models a likelihood ratio test was used. The difference between twice the log-likelihood can be interpreted as a χ^2 statistic. A significant difference indicates that the model with fewer parameters to be estimated fits data worse.

Results

Descriptive statistics

Table 1 presents descriptive statistics of the sample. Age in both men and women was slightly higher in the third wave of testing compared with the ages in the first and second wave of testing. There were no differences in systolic blood pressure between men and women for any of the waves of testing. However, diastolic blood pressure was consistently higher in men compared with women in all three waves of testing. Diastolic and systolic variance is consistently higher in the third wave of testing.

Maximum-likelihood estimates of twin correlations by rearing status adjusted for age and sex are shown in Table 2. In general, MZ correlations were higher than DZ correlations, indicating the importance of genetic effects. Studying twins reared apart and together allows for the evaluation of the importance of shared rearing environmental effects. Correlations in MZ reared together twins were higher than the MZ correlations for the reared apart twins, except in the second wave, for diastolic blood pressure (DBP). In DBP, DZ correlations showed almost the same pattern as the MZ, but not in systolic blood pressure (SBP). This could indicate the importance of rearing effects in DBP, but not necessarily in SBP. However, due to small sample sizes in each category, subsequent analyses used the merged data with respect to rearing.

Table 1 **Mean (SD) for measurements at each wave of clinical testing for men and women**

	Wave 1 mean (SD)	Wave 2 mean (SD)	Wave 3 mean (SD)
Men ($n = 184–198$)			
Age (year)	64.7 (7.6)	64.7 (8.6)	68.00 (8.3)
DBP (mmHg)	88.4 (9.6)	87.2 (9.2)	89.7 (11.5)
SBP (mmHg)	150.9 (18.9)	144.8 (18.8)	148.9 (21.1)
Women ($n = 227–281$)			
Age (year)	64.8 (9.1)	65.3 (9.6)	69.3 (9.7)
DBP (mmHg)	85.3 (8.9)	84.6 (7.9)	84.5 (11.3)
SBP (mmHg)	151.6 (22.1)	143.3 (19.9)	146.2 (22.6)

DBP, diastolic blood pressure; SDP, systolic blood pressure; SD, standard deviation.

One of the model assumptions in structural equation modelling of twin samples is that the means and variances of MZ and DZ are equal. Violations of these assumptions are reflected in the fit of the models under study. Tests based on structural equation modelling showed that there is no difference in means and variances between MZ and DZ pairs (data not shown), except for a significant difference in variances for systolic blood pressure in the first wave of testing, which is probably due to chance.

Cholesky decomposition

We fitted a Cholesky decomposition model to the repeated measures of blood pressure adjusting for age and sex. The results of the best fitting Cholesky decomposition models are presented in Figure 2a for DBP and Figure 2b for SBP. For the models in this sample, the shared environmental factor loadings could be eliminated and the genetic part of the model could be reduced to a common factor. That is, a common genetic factor is loading on all three measurements, indicating that probably the same set of genes are important for blood pressure over this 6-year period of time. For DBP, the genetic factor loading is quite high (0.66) for the first wave of testing (Fig. 2a). It continues to load highly in the second (0.68) and third wave (0.62). Genetic components with 95% confidence intervals (CI) explained 44% (26%, 59%), 46% (29%, 60%) and 39% (22%, 55%) of the total phenotypic variance at wave 1, 2 and 3, respectively. High genetic loadings are also seen for SBP, although the loading is smaller for the second wave (Fig. 2b). The genetic components with 95% CI explained 63% (48%, 75%), 38% (22%, 52%) and 52% (37%, 65%) of the phenotypic variance at wave 1, 2 and 3, respectively. The genetic stability is also evident when looking at estimates of the portion of phenotypic correlation due to genetic effects. It was estimated at 78% for DBP between waves 1 and 2 and 74% between waves 2 and 3. Similarly for SBP, it was estimated at 78% between waves 1 and 2, and 64% between waves 2 and 3.

Table 2 Maximum-likelihood estimates (95% CI) of twin correlations for diastolic and systolic blood pressure adjusted for age and sex by rearing status

	MZ A	DZ A	MZ T	DZ T
n	37–48	94–108	47–55	83–89
DBP (mmHg)				
Wave 1	0.14 (−0.28 to 0.49)	0.24 (−0.02 to 0.47)	0.57 (0.29–0.75)	0.11 (−0.18 to 0.37)
Wave 2	0.55 (0.16–0.77)	0.03 (−0.21 to 0.28)	0.33 (0.03–0.57)	0.39 (0.11–0.60)
Wave 3	0.20 (−0.23 to 0.55)	0.06 (−0.27 to 0.38)	0.45 (0.05–0.70)	0.16 (−0.12 to 0.41)
SBP (mmHg)				
Wave 1	0.48 (0.11–0.71)	0.45 (0.20–0.63)	0.76 (0.57–0.86)	0.13 (−0.17 to 0.40)
Wave 2	0.24 (−0.36 to 0.65)	0.22 (−0.02 to 0.43)	0.26 (−0.06 to 0.52)	0.18 (−0.11 to 0.44)
Wave 3	0.36 (−0.11 to 0.67)	0.25 (−0.10 to 0.52)	0.49 (0.12–0.72)	0.13 (−0.16 to 0.39)

A, reared apart; T, reared together; DBP, diastolic blood pressure; SBP, systolic blood pressure; CI, confidence intervals.

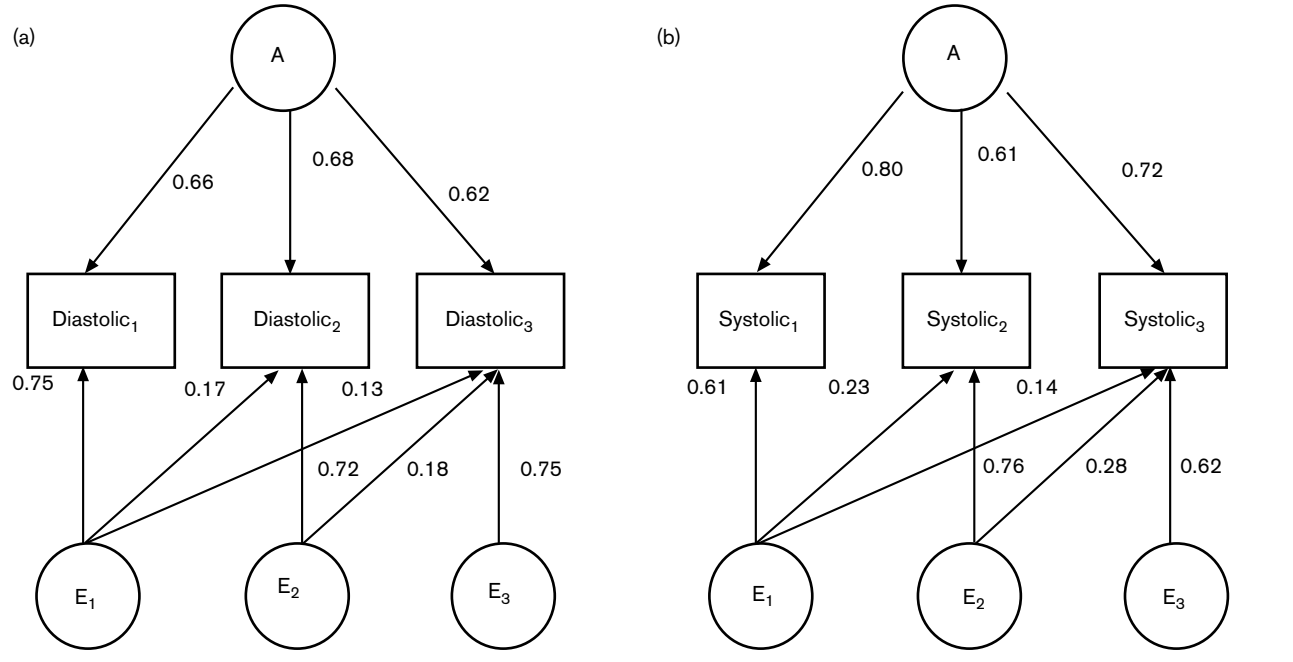
The non-shared environmental loadings could not be reduced to a common factor, or to separate non-shared environmental loadings that act at each time point, without a significant loss in the fitness of the model. Most of the non-shared environmental variance in DBP at waves 2 and 3 comes from time-specific influences, indicating that separate non-shared environmental effects are operating at each time point, with small effects loading from the first to the second (0.17) and third (0.13) and from the second to the third (0.18) (Fig. 2a). A similar pattern was seen in SBP (Fig. 2b).

Linkage and association

The results of the linkage and association tests are shown in Table 3. A χ^2 larger than 3.84 with 1 degree of freedom implies significance at the 0.05 significance level. For the covariance part of the model, the best fitting Cholesky decomposition model from the longitudinal analysis was used, modelling the QTL as a common factor loading on the observations at all three waves. It was possible to equate the QTL paths loading on all three waves of testing without significant loss in the fit of the variance component model.

None of the markers indicated significant linkage. The differences in log-likelihood between the model with the QTL and the model without the QTL are presented in the second last column in Table 3, and is approximately distributed as a χ^2 with 1 degree of freedom.

Fig. 2



Cholesky decomposition model for (a) diastolic and (b) systolic blood pressure, at three time points (wave 1, 2 and 3). Latent factor loadings are standardized to unit variance.

Table 3 **Mean values (± SD) by genotype for the angiotensin-I-converting enzyme insertion/deletion and angiotensin II type 1 receptor markers**

ACE I/D	DD	ID	II	Linkage ($\Delta\chi_1^2$)	Association ($\Delta\chi_1^2$)
DBP (mmHg)					
n	68–88	145–180	83–95		
Wave 1	86.16 (9.31)	87.12 (8.59)	85.69 (9.64)	1.02	0.11
Wave 2	86.12 (8.97)	85.61 (8.83)	86.06 (8.43)		
Wave 3	86.74 (11.17)	86.74 (11.90)	87.40 (11.56)		
SBP (mmHg)					
n	67–89	144–178	71–87		
Wave 1	148.63 (20.14)	149.61 (18.69)	149.47 (20.40)	< 0.01	0.02
Wave 2	143.69 (19.43)	142.54 (19.33)	141.37 (18.43)		
Wave 3	146.99 (21.04)	145.79 (20.89)	146.68 (21.59)		
A1166C	AA	AC	CC		
DBP (mmHg)					
n	110–151	110–135	27–32		
Wave 1	85.84 (8.92)	87.14 (9.88)	85.81 (7.34)	0	2.20
Wave 2	85.99 (8.42)	85.75 (9.53)	85.10 (7.55)		
Wave 3	86.81 (10.77)	87.18 (12.69)	85.84 (10.55)		
SBP (mmHg)					
n	108–152	108–130	26–31		
Wave 1	149.07 (19.35)	152.20 (20.14)	142.96 (15.66)	< 0.01	0.21
Wave 2	141.55 (19.32)	144.71 (19.02)	138.97 (17.06)		
Wave 3	144.97 (21.15)	148.01 (21.69)	144.55 (19.91)		

A $\chi^2 > 3.84$ with 1 degree of freedom implies significance at the 0.05 significance level. $\Delta\chi^2$ is the difference in log-likelihoods between models for test of linkage (df = 1) and association (df = 1). DBP, diastolic blood pressure; SBP, systolic blood pressure.

The robust tests of association showed no significant results (Table 3). Mean values (± standard deviation) are also presented by genotype for both markers. There are no substantial deviations in mean values between genotypes in each wave of testing for DBP and SBP for both angiotensin-I converting enzyme insertion/deletion and angiotensin II type 1 receptor polymorphisms.

An estimate of the contribution of each marker to the total phenotypic variance is evaluated by comparing the total phenotypic variances between a model with between and within sib-pair parameters in the means, with a model without those parameters [35]. The angiotensin-I converting enzyme insertion/deletion and angiotensin II type 1 receptor polymorphisms explained from 0% to $< 1\%$ of the total phenotypic variance in DBP and SBP.

Discussion

This study has quantified genetic and environmental sources of variance in repeated measures of DBP and SBP across a 6-year period and also investigated their association with two known polymorphisms, in a Swedish sample of twins from the population-based Swedish Twin Registry [23]. It showed genetic stability over time with specific non-shared environmental factors acting at each time point. There were no indications of an association between any of the polymorphisms and blood pressure.

In the current study, the repeated measures of DBP and SBP showed a genetic stability over a 6-year period of time. The genetic component of the variance contributed up to 46% of the total phenotypic variance in diastolic blood pressure. For systolic blood pressure, the genetic variance component was estimated up to 63%, indicating a relatively high genetic effect. A measure of stability is the portion of phenotypic correlation due to genetic effects, which was equally high for DBP and SBP between waves of testing. This may imply that the underlying genetic mechanisms of blood pressure regulation do not change appreciably during the 6-year period of time.

Correlations between MZ and DZ pairs reared together and apart showed that a rearing effect could perhaps be of importance for DBP. However, these models could not capture such an effect, probably due to low sample size. Previous studies have also shown that shared effects on the variation of blood pressure are negligible or of minor importance [36,37]. The non-shared environmental component was characterized by time-specific influences, but very little transmission, suggesting non-shared environmental influences having a small long-term effect. The specific non-shared environmental effects could imply the importance of lifestyle habits, such as smoking. However, it could also reflect measurement error. In variance component models, measurement error is included in the non-shared environmental component of variance, which reflects differences among MZ and DZ twin pairs.

Similar results have been reported from other twin and family studies on repeated measures of DBP and SBP

[36,37]. Colletto *et al.* [36] used a different longitudinal modelling approach, a so-called simplex model, in a middle-aged male twin sample followed for 15 years. In agreement with these results, they found genetic stability between the second (twins with an average age of 57 years) and third time point. In contrast, some cross-sectional studies reported age differences in the genetic effects [3,38]. However, to differentiate between true age-specific effects and cohort effects, longitudinal studies are essential.

With the use of structural equation modelling, also tested for was the linkage and association between two known polymorphisms in genes comprising the renin–angiotensin–aldosterone (RAS) pathway. Both of the polymorphisms explained less than 1% of the total phenotypic variance. In general, statistical tests based on means, such as in the association tests, are more powerful than tests based on higher-order moments such as in linkage [31]. Especially in linkage, small sample sizes can also produce biased estimates of the QTL [39]. Another limitation was that only one marker was genotyped for each gene, decreasing the power to conduct linkage in the present study. Therefore the results of linkage and the variance components estimates related to that should be taken with caution in this study.

A recent summary of the association of the angiotensin-I-converting enzyme insertion/deletion polymorphism to risk factors for cardiovascular diseases showed minimal evidence to support its significance. O'Malley *et al.* [40] grouped the studies according to geographical regions and found that most European countries showed lack of an association with cardiovascular disease risks. In contrast, African and Jamaican populations have showed an association [13,16]. Ethnic differences may suggest that a different mix of genes is important for blood pressure regulation in different ethnic groups.

The influence of the angiotensin II type 1 receptor polymorphism on essential hypertension and its higher prevalence among high-risk groups was suggested by previous studies [41,42]. However, we could not support evidence for association in the current study. This could be due to the fact that our sample consisted of normotensive subjects and not strictly hypertensive as in previous studies [41,43]. It could also be due to the small sample size and hence low power for the statistical analysis. Other Nordic studies have not shown an association between the angiotensin II type 1 receptor polymorphism and blood pressure in agreement with our results [12]. However, a Finnish study found an association in subjects with early onset of hypertension and normal body weight [43]. As the sample was selected from a uniform geographical region, it could be suspected that it could have a different genetic background compared with other Nordic countries.

Variance in the DBP and SBP was consistently higher in the third wave of testing, compared with the first and second. This could be due to the fact that nurses changed to digital devices for measuring blood pressure and the readings were imprecise and inaccurate compared with the old method. Recently, a review on blood pressure measurements and devices highlighted the fact that automated devices are known for their inaccuracy and noted that one of the possible reasons could be that most automated devices were initially designed for self-measurement of blood pressure and should not be assumed suitable for clinical use [44]. The effects of that could imply poorer fit in this model testing.

Conclusion

Longitudinal analysis showed that a single latent genetic factor influences variation in blood pressure over time. We could not support any evidence for association between the two central polymorphisms in the renin–angiotensin–aldosterone system and blood pressure. Considering the complexity underlying a phenotype such as blood pressure, genetic variation is probably explained by the influence of minor genetic effects and their interactions. With the completion of a draft of the human genome sequence there is a need to look at more dense maps close to the regions of these polymorphisms in order to identify the genes involved in the regulation of blood pressure. Interactions between these polymorphisms and the environmental backgrounds in which they are expressed are certainly of importance. These must be evaluated in order to disentangle the complexity in blood pressure variation.

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