Heritability of blood pressure traits and the genetic contribution to blood pressure variance explained by four blood-pressure-related genes

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Objective **To study the heritability of four blood pressure traits and the proportion of variance explained by four blood-pressure-related genes.**

Methods **All participants are members of an extended pedigree from a Dutch genetically isolated population. Heritability and genetic correlations of systolic blood pressure, diastolic blood pressure, mean arterial pressure and pulse pressure were assessed using a variance components approach (SOLAR). Polymorphisms of the α-adducin (*ADD1*), angiotensinogen (*AGT*), angiotensin II type 1 receptor (*AT1R*) and G protein β3 (*GNB3*) genes were typed.**

Results **Heritability estimates were significant for all four blood pressure traits, ranging between 0.24 and 0.37. Genetic correlations between systolic blood pressure, diastolic blood pressure and mean arterial pressure were high (0.93–0.98), and those between pulse pressure and diastolic blood pressure were low (0.05). The *ADD1* polymorphism explained 0.3% of the variance of pulse pressure ($P = 0.07$), and the polymorphism of *GNB3* explained 0.4% of the variance of systolic blood pressure ($P = 0.02$), 0.2% of mean arterial pressure ($P = 0.05$) and 0.3% of pulse pressure ($P = 0.06$).**

Conclusion **Genetic factors contribute to a substantial proportion of blood pressure variance. In this study, the effect of polymorphisms of *ADD1*, *AGT*, *AT1R* and *GNB3* explained a very small proportion of the heritability of blood pressure traits. As new genes associated with blood pressure are localized in the future, their effect on blood pressure variance should be calculated. *J Hypertens* 25:565–570 © 2007 Lippincott Williams & Wilkins.**

Journal of Hypertension 2007, **25**:565–570

Keywords: blood pressure, genes, heritability, variance

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Sponsorship: This research was supported by the Centre for Medical Systems Biology (CMSB), the Netherlands Organisation for Scientific Research (NWO) and the Netherlands Kidney Foundation.

Conflicts of interest: none.

Received 14 July 2006 **Revised** 2 November 2006
Accepted 15 November 2006

See editorial commentary on page 505

Introduction

It has long been recognized that genetic factors play a crucial role in blood pressure regulation. Twin, adoption and nuclear family studies indicated that a substantial proportion of systolic blood pressure (SBP) and diastolic blood pressure (DBP) variance is due to the effect of genes [1–8]. Heritability estimates, however, range widely between different study populations, depending heavily on the type of relative pairs used. Heritability estimates for SBP and DBP vary around 60% in twin studies and around 25% in nuclear family studies [1,3,5,6,9]. Scarcer studies on the heritability of mean arterial pressure (MAP) and pulse pressure (PP) produced estimates that vary from 35 to 60% [10–12]. As these estimates are based on blood pressure correlations between first-degree relatives only, they are likely to be confounded by the effects of a shared familial environment, which causes an overestimation of heritability. Large family-based samples, including second-degree and third-degree relatives, who do not usually share the same household, may therefore generate more accurate heritability estimates of blood pressure.

A number of genes may explain part of the heritability of blood pressure. Genes involved in salt sensitivity and the renin–angiotensin system are known to play a role in

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blood pressure variance [13]. Until now, no studies have addressed the extent to which these genes explain the heritability of blood pressure.

In the present study, we aimed to assess to what extent genes influence blood pressure variance in 1006 inhabitants of a genetically isolated community in the south-west part of The Netherlands. The participants were all related to each other in one extended pedigree, making it less likely that familial effects play a role. Heritability was estimated for four quantitative blood pressure traits: SBP, DBP, MAP and PP. We also estimated what proportion of blood pressure variance could be explained by the following polymorphisms: Gly460Trp of *ADD1*, M235T of angiotensinogen (*AGT*), C573T of the angiotensin II type 1 receptor (*AT1R*) and rs2301339 G/A of *GNB3*.

Materials and methods

Setting

Analyses were performed on phenotypic data collected from Dutch inhabitants of a genetically isolated community in the south-west part of the Netherlands, who participated in the Erasmus Rucphen Family (ERF) study. The ERF study is a family-based cohort study, and is part of an ongoing research program called Genetic Research in Isolated Populations. This program aims to identify genetic risk factors in the development of complex disorders [14]. The study was approved by the Medical Ethics Committee of Erasmus Medical Center Rotterdam. Written informed consent was obtained from all participants.

Participants

Genealogical records demonstrated that almost all of the inhabitants of this isolated population could be traced back to about 150 individuals who founded this community around 1750. For years, minimal inward migration and considerable population growth characterized this population. About 20 000 inhabitants are now scattered over eight adjacent villages. Genealogical information on this population was reconstructed using church and the municipality records, and is currently available in the form of a large pedigree database including over 63 000 individuals.

For the ERF study, 20 couples that had at least six children baptised in the community church between 1880 and 1900 were identified with the help of genealogical records. All living descendants of these couples and their spouses were invited to participate in the study. With the use of the pedigree database, these families could be linked to one founder couple in a large, complex pedigree.

Data collection

Participants were invited for a series of clinical examinations at our research center, located within the community. The blood pressure was measured twice in the sitting position in the right upper arm using an automated device (OMRON 711, automatic IS; Omron Healthcare Inc., Bannockburn, Illinois, USA). The average of these two measurements was used for analysis. The MAP (1/3SBP + 2/3DBP) and PP (SBP − DBP) were calculated. Hypertension was defined as a DBP of 90 mmHg or higher and/or a SBP of 140 mmHg or higher and/or use of antihypertensive medication indicated for the treatment of hypertension [15,16]. Height and weight were measured with the participant dressed in light underclothing and the body mass index (BMI) was calculated (kg/m^2). Finally, a research physician obtained information on medical history, medication use, smoking and alcohol use in a personal interview.

At the start of the clinical examinations, fasting blood samples were drawn for the extraction of DNA and the measurement of lipids, glucose, plasma creatinine, and plasma albumin levels according to a standardized procedure [17,18]. Serum samples were obtained from whole blood after clotting; plasma samples were obtained from whole blood collected in disodium ethylenediamine tetraacetic acid.

Hyperlipidemia was defined as the use of lipid-lowering medication or total cholesterol levels between 6.5 and 9.0 mmol/l and a total cholesterol to high-density lipoprotein (HDL) cholesterol ratio above 5.0, or total cholesterol below 6.5 mmol/l and a ratio above 8.0, or total cholesterol above 9.0 mmol/l, independent of the ratio, or triglycerides above 4.0 mmol/l. These criteria are in accordance with those of the Dutch college of general practitioners. Diabetes mellitus was defined as the use of blood glucose-lowering medication or a fasting serum glucose level above 7.0 mmol/l [19].

Data collection started in June 2002 and was finished in February 2005. In the present study, we focused on the first 1006 participants for whom complete phenotypic, genotypic and genealogical information was available.

Genotyping

We genotyped the following polymorphisms: *ADD1* Gly460Trp, *AGT* M235T, AT1R C573T and GNB3 rs2301339 G/A. Genotyping was performed using TaqMan allelic discrimination Assays-By-Design (Applied Biosystems, Foster City, California, USA) [20]. For *ADD1*, the forward primer sequence was 5′-GAGAAGACAAGATG GCTGAACTCT-3′ and the reverse primer sequence 5′-GTCTTCGACTTGGGACTGCTT-3′. The minor groove binding probes were 5′-VIC-CATTCTGCCCT TCCTC-NFQ-3′ and 5′-FAM-ATTCTGCCATTCC TC-NFQ-3′. We used the reverse strand design for this polymorphism. Forward primer sequences were 5′-GGT TTGCCTTACCTTGGAAGTG-3′ and 5′-TGTGCTT TCCATTATGAGTCCCAAA-3′ for *AGT* and *AT1R*, respectively. Reverse primer sequences were 5′-GCTGT

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GACAGGATGGAAGACT-3′ and 5′-CAGAAAAGGAA ACAGGAAACCCAGTATA-3′ for *AGT* and *AT1R*, respectively. The minor groove binding probes were 5′-VIC-TGGCTCCCATCAGG-NFQ-3′ and 5′-FAM-CTGGC TCCCGTCAGG-NFQ-3′ for *AGT*. The minor groove binding probes were 5′-VIC-CTATCGGGAGGGTTG-NFQ-3′ and 5′-FAM-CTATCGGAAGGGTTG-NFQ-3′ for *AT1R*. We used the reverse strand design for this polymorphism. For *GNB3*, the forward primer sequence was 5′-GGCAGGGCTGCTTCTCA-3′ and the reverse primer sequence was 5′-GCAAGCCGCTGCTCTCA-3′. The minor groove binding probes were 5′-VIC-AAAC CAAGGAAGGGACA-NFQ-3′ and 5′-FAM-ACCAAGG GAGGGACA-NFQ-3′. The assays utilized 5 ng genomic DNA and 5 μl reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 min at 95°C preceded 40 cycles of denaturation at 95°C for 15 s, and annealing and extension at 50°C for 60 s. Allele-specific fluorescence was then analyzed on an ABI Prism 7900HT Sequence Detection System with SDS version 2.1 (Applied Biosystems). Based on the analysis of blind duplicates, there was a 98% concordance in genotyping of *ADD*, 99.4% for *AGT* and 100% for *AT1R* and *GNB3*.

Statistical analysis

Baseline characteristics were compared using univariate analysis of variance or chi-squared statistics. Univariate analyses of variance were used to assess the relation between the *ADD1* Gly460Trp, the *AGT* M235T, the AT1R C573T and the GNB3 rs2301339 G/A polymorphisms and blood pressure traits (SPSS 11.0; SPSS Inc., Chicago, Illinois, USA). A variance component maximum likelihood approach, implemented in the SOLAR software package, was used to estimate heritability and genetic correlations for SBP, DBP, MAP and PP [21]. Heritability (h^2) was estimated as the ratio of the variance of the trait explained by additive polygenic effects to the total phenotypic variance of the trait. We identified significant (environmental) covariates for each blood pressure trait in order to estimate the contribution of environmental factors to blood pressure variance. Significant effects of each covariate were tested using a likelihood ratio test with one degree of freedom (SPSS 11.0). Covariates that were included in the final model were significant at the 0.10 level. These were age, sex, total cholesterol, HDL cholesterol, glucose levels, alcohol intake, antihypertensive medication, and BMI. In order to satisfy distributional assumptions, the SBP, DBP, MAP and PP were natural log-transformed to ensure normally distributed residuals. Heritability models included age, sex, antihypertensive medication, BMI, total cholesterol, HDL cholesterol, alcohol intake and glucose levels as covariates.

Bivariate analysis was performed to estimate the genetic and environmental correlations between the four blood pressure traits [22,23]. The phenotypic correlations between the blood pressure traits were then calculated by the following formula [24,25]: $\rho_P = \sqrt{h_1^2}\sqrt{h_2^2}\rho_G + \sqrt{(1-h_1^2)}\sqrt{(1-h_2^2)}\rho_E$, where h_1^2 and h_2^2 are the heritability estimates of the two blood pressure traits, for which the phenotypic correlation is calculated, and ρ_G and ρ_E are the genetic and environmental correlations between these two traits (as estimated in the bivariate analyses). Significance of the phenotypic, additive genetic and environmental correlations was determined using a likelihood ratio test. To test whether a given correlation between two blood pressure traits was significantly different from zero, the likelihood of a model in which this correlation was constrained to zero was compared with a model in which the same correlation was estimated. Twice the difference in ln-likelihoods of these models yields a test statistic that is asymptotically distributed as a chi-squared statistic with degrees of freedom equal to the difference in number of parameters estimated in the two models. All bivariate analyses were adjusted for age and sex.

Next, we assessed the proportion of variance explained by the following polymorphisms: Gly460Trp of ADD1, M235T of AGT, C573T of AT1R and rs2301339 G/A of GNB3. The significance of these polymorphisms was tested using the likelihood ratio test, where the likelihood of a model including the polymorphism is estimated, then compared with the likelihood of a model without the polymorphism. Twice the difference in the natural ln-likelihoods values of these models yields a test statistic that is asymptotically distributed as a chi-squared statistic with degrees of freedom equal to the difference in number of estimated parameters in the two models being compared [26]. In the output files, the proportion of variance explained by all covariates is presented. The absolute increase in this proportion by adding the polymorphism to the model equals the proportion of variance explained by the polymorphism.

Results

In total, 1006 participants were available for analysis, including 907 first-degree relative pairs, 659 second-degree relative pairs and 2370 third-degree relative pairs.

Table 1 presents the general descriptives of the total study population stratified by sex. The mean age was 56 years for men and 54 years for women, but as participants were ascertained from three generations the age range was very broad (18–92 years). Most characteristics were higher in men, with the exception of HDL cholesterol and smoking.

The heritability estimates for SBP, DBP, MAP and PP are presented in Table 2. Heritability estimates for SBP, DBP, MAP and PP were significant ($P < 0.001$), and ranged from 0.24 for PP to 0.37 for DBP.

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Table 1 General characteristics of the study population

	Men	Women	*P* value
Number	407	597	
Age (years)	55.7 ± 14.4	53.5 ± 15.6	0.02
Systolic blood pressure (mmHg)	145.7 ± 20.3	139.5 ± 22.4	< 0.001
Diastolic blood pressure (mmHg)	82.6 ± 10.4	78.7 ± 9.6	< 0.001
Mean arterial pressure (mmHg)	103.6 ± 12.4	98.6 ± 12.5	< 0.001
Pulse pressure (mmHg)	63.2 ± 16.0	59.8 ± 18.0	< 0.01
Antihypertensive medication (%)	29.1	22.4	0.02
Total cholesterol (mmol/l)	5.5 ± 1.1	5.6 ± 1.2	0.11
High-density lipoprotein cholesterol (mmol/l)	1.1 ± 0.3	1.4 ± 0.4	< 0.001
Low-density lipoprotein cholesterol (mmol/l)	3.7 ± 1.0	3.7 ± 1.0	0.94
Triglycerides (mmol/l)	1.6 ± 1.0	1.3 ± 0.6	< 0.001
Glucose (mmol/l)	5.0 ± 1.1	4.6 ± 1.1	< 0.001
Diabetes mellitus (%)	7.3	6.4	0.61
Hyperlipidemia (%)	35.7	27.5	< 0.01
Body mass index (kg/m^2)	27.7 ± 4.2	26.8 ± 4.8	< 0.01
Current smokers (%)	33.2	48.2	< 0.001
Alcohol use (units/week)	8.4 ± 12.8	1.7 ± 4.0	< 0.001

Data are unadjusted and presented as the percentage or the mean ± SD.

Table 2 Heritability estimates of systolic blood pressure, diastolic blood pressure, mean arterial pressure and pulse pressure

Phenotype	*n*	Heritability, H^2 ± standard error	*P* value
Systolic blood pressure	1006	0.34 ± 0.08	< 0.001
Diastolic blood pressure	1006	0.37 ± 0.09	< 0.001
Mean arterial pressure	1006	0.40 ± 0.08	< 0.001
Pulse pressure	1006	0.24 ± 0.08	< 0.001

Heritability analyses are adjusted for age, sex, antihypertensive medication, body mass index, total cholesterol, high-density lipoprotein cholesterol, alcohol intake and glucose levels.

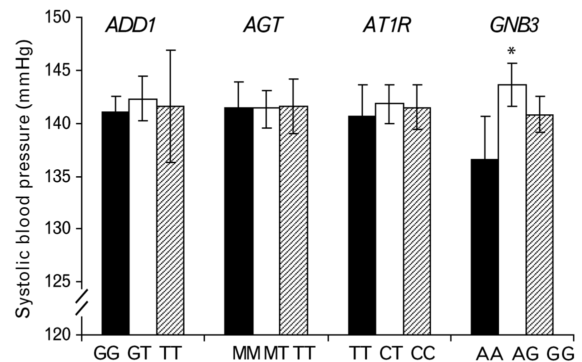
Table 3 presents the results of the bivariate analyses. All correlations were positive and significant, with the exceptions of the phenotypic and genetic correlations between DBP and PP. The phenotypic correlations ranged between 0.15 for DBP–PP (not significant) and 0.90 for SBP–MAP and DBP–MAP. Genetic correlations between SBP, DBP and MAP were significant and very high (0.93–0.98), whereas the genetic correlation between PP and DBP was not significant and was low (0.05). Environmental correlations ranged widely, from 0.19 for DBP–PP to 0.89 for SBP–MAP.

Table 3 Phenotypic, genetic and environmental correlations between systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP)

	Phenotypic correlation, ρ_P	Genetic correlation, ρ_G	Environmental correlation, ρ_E
SBP–DBP	0.68 ± 0.03	0.93 ± 0.13	0.60 ± 0.05
SBP–MAP	0.90 ± 0.01	0.98 ± 0.04	0.89 ± 0.02
SBP–PP	0.80 ± 0.02	0.70 ± 0.11	0.84 ± 0.03
DBP–MAP	0.90 ± 0.01	0.93 ± 0.03	0.88 ± 0.02
DBP–PP	0.15 ± 0.05*	0.05 ± 0.22*	0.19 ± 0.09
MAP–PP	0.52 ± 0.03	0.64 ± 0.21	0.50 ± 0.06

All correlations are significant at $P < 0.001$, except for ρ_P and ρ_G between DBP and PP.

Fig. 1



Mean systolic blood pressure levels in four different genotype groups. *$P < 0.05$ compared with the reference group AA, adjusted for age and sex.

Figure 1 shows that SBP was significantly higher in AG carriers of *GNB3* compared with AA carriers ($P = 0.003$). SBP was also higher in GG carriers, but this was not significant ($P = 0.06$). MAP was also significantly higher in AG carriers ($P = 0.02$) and PP was higher in both AG ($P = 0.002$) and GG carriers ($P = 0.05$, data not shown). No significant differences were found between the other genotype groups and SBP, DBP, MAP or PP. From Fig. 1 we chose the following models for the analyses presented in Table 4; ADD1 dominant model, AGT recessive model, AT1R dominant model and GNB3 dominant model.

Table 4 presents the proportion of variance in SBP, DBP, MAP and PP explained by all covariates and by the polymorphisms in *ADD1*, *AGT*, *AT1R* and *GNB3*. Age and sex explained 25.8% of SBP, 7.4% of DBP, 18.4% of MAP and 24.0% of PP (data not shown). Adding antihypertensive medication, BMI, total cholesterol, HDL cholesterol, alcohol intake and glucose levels to the model only slightly increased the proportion of variance explained by all covariates (with a maximum of 4%), suggesting that age and sex explain most of the blood pressure variance. The Gly460Trp polymorphism of *ADD1* explained 0.3% of the variance of PP ($P = 0.07$). The rs2301339 G/A polymorphism of *GNB3*, explained 0.4% of the variance of SBP ($P = 0.02$), 0.2% of MAP ($P = 0.05$) and 0.3% of PP ($P = 0.06$). The other two polymorphisms also explained less than 1% of the variance of the blood pressure traits, but none were significant.

Discussion

In the present study heritability estimates for blood pressure traits were highly significant and varied between 24 and 40%. High phenotypic, genetic and environmental correlations between SBP, DBP, MAP and PP were also found. The proportion of variance explained by

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Table 4 Proportion of variance of systolic blood pressure, diastolic blood pressure, mean arterial pressure and pulse pressure explained by all covariates and four blood pressure genes

	Proportion of variance explained by								
Phenotype	All covariates (%)	*ADD1* (%)	*P* value	*AGT* (%)	*P* value	*AT1R* (%)	*P* value	*GNB3* (%)	*P* value
Systolic blood pressure	27.8	0.2	0.16	< 0.1	0.45	< 0.1	0.93	0.4	0.02
Diastolic blood pressure	11.0	0.1	0.78	0.2	0.84	0.1	0.95	0.3	0.24
Mean arterial pressure	21.5	< 0.1	0.54	0.1	0.73	0.1	0.99	0.2	0.05
Pulse pressure	24.5	0.3	0.07	< 0.1	0.09	< 0.1	0.15	0.3	0.06

ADD1, α-adducin Gly460Trp; *AGT*, angiotensinogen M235T; *AT1R*, angiotensin II type 1 receptor C573T; *GNB3*, G-protein rs2301339 G/A polymorphism. All analyses are adjusted for age, sex, antihypertensive medication, body mass index, total cholesterol, high-density lipoprotein cholesterol, alcohol intake, glucose levels and pedigree structure.

covariates was mostly determined by age and sex. In the single gene analysis, a small but significant proportion of the variance of SBP and MAP was determined by the rs2301339 G/A polymorphism of *GNB3*.

The strength of our study lies within its population-based nature, embedded in a family-based study design. The members of our extended pedigree therefore represent a random sample of our study population and were not ascertained through persons with extreme blood pressure values.

All of our heritability estimates were significant and ranged between 0.25 for SBP and 0.37 for DBP. They are within the range of those reported in other family-based studies, which range from 0.15 to 0.40 [5,7,8,27–29]. Covariates accounted for about 16% of DBP variance and up to one-third of SBP and PP variance. Other than age and sex, the BMI, fasting glucose levels and alcohol intake were the most important covariates influencing blood pressure in this population.

We observed high genetic correlations between SBP, DBP and MAP, indicating that these traits may share a common genetic background. In other words, the genes that influence SBP variance also influence DBP and MAP variance. In contrast, the genetic correlation between PP and DBP was absent. This suggests the existence of an independent set of genes influencing PP and DBP variance. This is concordant with a previous finding suggesting that DBP and PP map to different loci [30]. The PP is an important measure of arterial stiffness and a strong predictor of cardiovascular morbidity and mortality, independent of blood pressure [31]. Significant heritability estimates for PP and the results of our bivariate analyses therefore make this trait a suitable candidate for future genetic analyses aimed at identifying genes involved in arterial stiffness.

Previous studies found associations between polymorphisms in *ADD1*, *AGT*, *AT1R*, *GNB3* and blood pressure, hypertension and salt sensitivity [13,32–34]. None of these studies, however, addressed the proportion of blood pressure variance that these genes explain. In this study, only the rs2301339 G/A polymorphism of *GNB3* significantly explained a portion of the variance of SBP and MAP. This proportion, however, was less than 1%.

To calculate the association between the four polymorphisms and blood pressure traits, we used the measured genotype approach, comparing the likelihood of a model including the polymorphism with the likelihood of a model without the polymorphism, using the SOLAR software package. This approach was chosen because of the complex nature of our pedigree, which contains numerous loops. The SOLAR software package was able to analyze the complete pedigree, without breaking any of the loops.

In conclusion, our study shows that polymorphisms of *ADD1*, *AGT*, *AT1R* and *GNB3* explain a very small part of the heritability of blood pressure traits. Given the high heritability estimates, many genes remain to be identified.

Acknowledgements

The authors would like to thank all assistants of the ERF research center for their help in data collection, and Petra Veraart, Hilda Kornman and Ed Boeren for their contribution to genealogical research. They would also like to thank all participants of the ERF study for their cooperation.

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