

Evidence That a Single Gene with Gender- and Age-dependent Effects Influences Systolic Blood Pressure Determination in a Population-based Sample

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Summary

A biometrical study was carried out to evaluate the role of genetic variation in determining interindividual differences in systolic blood pressure (SBP) in the population at large. SBP was measured in 1,266 Caucasian individuals in 278 pedigrees ascertained through children enrolled in the Rochester, MN, school system. The sample included 646 males and 620 females 550 years of age and not taking antihypertensive medication or oral contraceptives. Complex segregation analysis was first applied to these data by using a regression model for age, in which the intercept was gender and ousiotype specific but in which the slope was only gender specific. When the slope was independent of ousiotype, neither variation at a single gene combined with polygenic effects (mixed genetic model) nor variation in a single environmental factor combined with polygenic effects (mixed environmental model) explained the distribution of SBP in this sample. However, when the regression model for age allowed both the intercept and slope to be gender and ousiotype specific, the mixed environmental model was rejected whereas the mixed genetic model was not. These results suggest that variability in SBP may be influenced by major effects of allelic variation at a single gene that are both gender and age dependent. This study (1) suggests that particular genotypes determined by a single gene are associated with a steeper increase of SBP with age among males and females 550 years of age in the general population and (2) illustrates the need to consider models that more realistically represent the relationship between genotypic variability and phenotypic variability, to understand the genetics of human quantitative traits.

Introduction

Hypertension has emerged from epidemiological studies as one of the major risk factors in the prediction of coronary heart disease. Family studies in various populations have established that blood pressure aggregates in families. Both genetic and environmental factors contribute to this familial aggregation (for a review, see Rapp 1983; Sing et al. 1986; Camussi and Bianchi 1988). Biometrical strategies used to quantify the relative contribution of genetic and environmental

sources of phenotypic variance have consistently shown that 20%–30% of the interindividual variation in systolic blood pressure (SBP) may be attributable to polygenes (Annest et al. 1979; Ward et al. 1979; Morton et al. 1980; Moll et al. 1983; Pérusse et al. 1989).

Although it is generally recognized that genetic factors contribute to the determination of interindividual differences in blood pressure levels, the characterization of these genetic factors has been the object of heated debate (Swales 1985). Platt (1967) argued that hypertension is a specific disease entity and that the skewed shape of the blood pressure distribution is an indication of the presence of two or more *qualitatively* distinct subpopulations characterized by allelic variations at a single gene. Pickering (1967), on the other hand, argued that hypertension merely represents the upper end of the blood pressure distribution and that

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quantitative deviations from the mean are a consequence of the interaction between the effects of many polygenes with exposures to numerous environmental factors. This model for interindividual differences in blood pressure level does not include major effects of allelic variation at a single gene. One recent study provided some evidence for a very rare single-locus genotype that is expected to occur in 0.03% of the population (Carter and Kannel 1990). Since the rare genotype is associated with low SBP levels in the Framingham cohort, it cannot explain the prevalence of hypertension. All other studies that have used biometrical genetic methods that include the major effects of a single gene support Pickering's view that interindividual variation in blood pressure is associated with the combined effects of polygenes, shared familial factors, and environmental factors specific to the individual (Krieger et al. 1980; Morton et al. 1980; Marazita et al. 1987).

Two reasons may explain the unsuccessful attempts to find evidence for single genes with major allelic effects on blood pressure level. First, blood pressure is a complex trait that is determined by a large number of interacting biological components of the circulatory, nervous, renal, and endocrine systems (Genest et al. 1983). Inherited quantitative variation occurs for most of the traits that define each of these components. Because blood pressure level is determined by the combined action of all components, an allelic effect on any one of these traits is expected to have a relatively small effect on blood pressure level (Sing and Moll 1989). Hence, the power to detect single-gene effects should be enhanced by first considering those intermediate traits that link allelic variation to interindividual variation in blood pressure levels. Several measures of cellular electrolyte-transport systems have been considered as candidate intermediate traits. Evidence that these traits are relevant comes from epidemiological studies showing that their average levels are different between normotensives and hypertensives and, in some cases, between normotensives with and without a positive family history of hypertension (Turner et al. 1985; Motulsky et al. 1987; Williams et al. 1988a, 1988b). Complex segregation analyses have suggested that red blood cell sodium-lithium countertransport (Boerwinkle et al. 1986; Hasstedt et al. 1988b; Rebbeck et al. 1991), red blood cell sodium concentration (Hasstedt et al. 1988a), and urinary kallikrein concentration (Berry et al. 1989) are each influenced by allelic variation at a single gene. Because of the multifactorial basis of blood pressure determination, the major effects de-

tected in these studies probably contribute to the polygenic component of variation in SBP levels.

Second, the influence that genotypic variation at a single locus has on blood pressure may depend on the level of a second risk factor, such as age, body size, or exposure to dietary sodium. Since blood pressure is known to increase with age in most populations, traditional biometrical approaches have adjusted for age, prior to analyses to fit genetic models. This strategy assumes that the relationship between blood pressure and age is not genotype dependent. If the effects of allelic variation at a single gene are dependent on a concomitant such as age, these effects would not be detected by the traditional approach. The only segregation-analysis application that has considered a genotype-dependent relationship between age and a quantitative trait in humans showed that the increase of low-density-lipoprotein cholesterol with age in a large kindred with familial hypercholesterolemia was influenced by genetic variation at a single locus and that the genotype-specific age-regression model fit the data significantly better than did a model in which such an effect was not considered (Moll et al. 1984).

We report here an application of complex segregation analysis to determine whether there is evidence that a single gene has major effects on the increase of SBP with increasing age. Data collected in 278 pedigrees ascertained, without regard to health status, from the Rochester, MN, population were analyzed. A model that defines age- and gender-specific effects for genotypes determined by a single gene fit these data significantly better than did a model in which the genotype means were not age dependent. These single-locus genotypes predicted larger differences in SBP in older individuals than in younger individuals. The relative frequency of the allele at this locus that is associated with higher levels of SBP was estimated to be .09.

Subjects and Methods

Population Studied

The Rochester Family Heart Study was initiated in 1984 to investigate the effects that environmental and inherited traits have on lipid transport and hypertension in a large population-based study. The characteristics of the Rochester population over age 16 years are similar to those of U.S. whites, with the exception of (1) the proportion of the population employed in the health care industry (8.1%, vs. 6.9% for the U.S.

white population) and (2) associated higher education (81.7% with at least 12 years of education, vs. 68.8% for the U.S. population). Extended pedigrees were ascertained through households with children enrolled in primary and/or secondary schools of Rochester (for details, see Turner et al. 1985, 1989; Moll et al. 1989). Letters requesting participation were sent to 5,270 households that had two or more children enrolled in the schools. From a total of 1,812 questionnaires (34.4%) that were returned, 159 households were judged unsuitable for sampling (Turner et al. 1989). Between 1984 and 1988, 436 households were contacted and 300 agreed to participate. A total of 2,004 individuals identified by these households completed a clinic visit. Written consent was obtained from each subject, and the study was approved by the Institutional Review Board of the Mayo Clinic and Foundation.

Before visiting the clinic, individuals were asked to complete a questionnaire about the use of prescribed and nonprescribed medications taken during the preceding month. On the day of the visit to the Mayo Clinic, each subject was seen by a physician who performed a physical examination, reviewed the persons medical history, and reviewed the questionnaire regarding use of medications.

For the study reported here the following exclusions were applied to the sample of 2,004 individuals: First, six non-Caucasians and 14 individuals randomly selected from 14 twin pairs present in the sample were excluded. Second, 643 individuals age 50 years or older were removed from the sample. Prevalence of hypertension in the Rochester population is almost 10 times higher in individuals age 50 years or older (28.3%) than in those under 50 years (3.3%), and most of these individuals are on medication to reduce their blood pressure (Turner and Michels, in press). Inclusion of all individuals over age 50 years would lead to a sample in which many individuals have blood pressure levels lowered by use of antihypertensive medications. However, inclusion only of older individuals not on antihypertensive medication would result in a biased sample of the population over age 50 years. Hence, we chose to consider only that fraction of the total sample that was less than 50 years of age. Of the remaining 1,341 individuals, 43 women taking oral contraceptives and 32 individuals taking antihypertensive medication were excluded. These exclusions led to a final sample of 1,266 individuals distributed among 278 pedigrees. The average number of individuals per pedigree was 4.55. Pedigree size

ranged from one (12 pedigrees) to 19 (one pedigree). The 12 pedigrees with one individual involved six adoptees and six individuals whose parents were over age 50 years. The 266 pedigrees with at least two individuals included 216 nuclear families, 21 extended pedigrees, and, because of the age restriction, 29 full sibships. The size of the nuclear families ranged from two to nine individuals. Both parents and at least one child were included in 164 of the nuclear families.

Blood Pressure Determination

Blood pressure measurements were taken following the recommendations of the American Heart Association (Kirkendall et al. 1981) by using a random zero sphygmomanometer (Hawksley & Sons, Ltd., West Sussex, England). The individuals were asked to remain seated for at least 5 min before three readings were taken in the right arm. Each reading was separated by at least 2 min. SBP was recorded at the Korotkoff phase I sound, while diastolic blood pressure was recorded at Korotkoff phase IV in children under 13 years of age and at Korotkoff phase V in all others. The readings were taken by two internists in the adults and by two pediatricians in the children. No significant interobserver differences were found in the measurements of either SBP or diastolic blood pressure (Turner et al. 1989). Only SBP data were analyzed in the present study.

Statistical Analyses

Model definitions.—Complex segregation analysis (Elston and Stewart 1971; Lalouel et al. 1983) was used to study the gender- and age-dependent influence that allelic variation at a single gene had on SBP level. We first describe the parameters of a general “unified” model (Lalouel et al. 1983) for the distribution of SBP in the population at large and then turn to a description of the hypothesis-testing strategy that was used to establish the contribution of each parameter of this model.

The most general model considered here includes (1) the independent and additive contribution of a single factor with major effects on SBP levels, (2) small additive allelic effects of a large number of independent polygenes, and (3) individual-specific environmental influences. This general model assumes Hardy-Weinberg equilibrium at all loci, no linkage disequilibrium between pairs of loci, no epistasis, and no genotype \times environment interactions. The single factor is modeled as having two alternatives, L (low) and H (high), that combine to determine three classes, or oisiotypes

(Cannings et al. 1978), of individuals denoted LL, LH, and HH in this paper. The relative frequencies of L and H in the population being sampled are p and q ($q = 1 - p$), respectively. Regardless of whether these major effects have a genetic or environmental origin, the analysis assumes that the relative frequencies of the ousiotypes— $f(LL)$, $f(LH)$, and $f(HH)$ in the population at large—are p^2 , $2pq$, and q^2 , respectively. Other parameters of the model define the distribution of SBP within each ousiotype. They include the phenotypic mean of each ousiotype and the phenotypic variance among individuals with the same ousiotype. The distribution of SBP for the o th ousiotype is assumed to be normal, with mean μ_o and a variance σ^2 attributable to segregation of polygenes, individual-specific environmental effects, and measurement error. The variance among individuals having a particular ousiotype (σ^2) is assumed to be the same for all three ousiotypes. The effects of polygenotypes (γ_{pg}) are assumed to be normally distributed about the mean of each ousiotype, with variance σ_γ^2 (the additive genetic variance). The ratio σ_γ^2/σ^2 is the proportion of the variance attributable to the polygenes (h^2). The distribution of SBP for each polygenotype, pg , is assumed to be normal with variance σ_e^2 ($\sigma_e^2 = \sigma^2 - \sigma_\gamma^2$).

The distribution of SBP in offspring, conditional on their parental ousiotypes, is defined in terms of transmission parameters. τ_1 , τ_2 , and τ_3 are the probabilities that an individual of ousiotype LL, LH, and HH, respectively, transmits the L alternative to his or her offspring. In general, $\tau(o|o_f, o_m)$ is the probability that a child has the ousiotype o , given the ousiotypes o_f and o_m of the father and mother, respectively. For the general model (Lalouel et al. 1983), these transmission probabilities are each estimated under the constraint that they be in the interval 0 to 1. The distribution of polygenotype effects in children, conditional on polygenotype effects of their parents— γ_{pgf} and γ_{pgm} —is described by the normal distribution $N[\gamma_{pgc}(\gamma_{pgf} + \gamma_{pgm})/2, \sigma_\gamma^2/2]$.

The likelihood L , under this general model, for a randomly selected pedigree with two parents (individuals 1 and 2) and two offspring (individuals 3 and 4) is

$$L = \sum_{o_1} \dots \sum_{o_4} \int \dots \int \prod_{i=1}^2 N(SBP_i | \mu_{o_i} + \gamma_{pgi}, \sigma_e^2) \\ \times f(o_i) \times N(\gamma_{pgi} | 0, \sigma_\gamma^2) \times \prod_{j=3}^4 N(SBP_j | \mu_{o_j} + \gamma_{pgj}, \sigma_e^2)$$

$$\times \tau(o_j | o_{fj}, o_{mj}) \times N[\gamma_{pgj} | (\gamma_{pgfj} + \gamma_{pgmj})/2, \sigma_\gamma^2/2] \\ d(pg_4) \dots d(pg_1),$$

with summations over the ousiotypes and with integrations over polygenotypes. The subscripts i and j represent individuals with no ancestors (originals) and individuals with ancestors, respectively, in the pedigree.

The general model described above assumes that the effect of an ousiotype is on the mean level of the trait and that this effect is independent of age and gender. We have extended this model by first modifying the penetrance function of the likelihood to include a quadratic age regression. For this purpose, μ_o in the above likelihood equation is replaced by $\alpha_o + \beta_o^1 \text{age} + \beta_o^2 \text{age}^2$, where α_o , β_o^1 , and β_o^2 represent, respectively, the ousiotype-specific intercept and the linear and quadratic terms of the regression. An additional extension of the model allows the regression parameters to be gender specific in addition to being ousiotype dependent. The variance about the regression line (σ^2) was assumed to be the same for each ousiotype and to be equal in males and females. This more complete general model that includes ousiotype- and gender-specific age effects is defined by a total of 24 parameters: the intercepts and the linear and quadratic-regression coefficients for each ousiotype in males and females (18 parameters), the relative frequency (p) of L, the variance (σ^2), the heritability (h^2), and the three transmission probabilities (τ_1 , τ_2 , and τ_3).

Alternative models, parameter estimation, and hypothesis testing.—Five alternatives to the general model were considered. Each represents a restriction of one or more of the parameter values of the general model described above. The values of the transmission parameters determine whether the major effects of a single factor are explained by an environmental or a genetic etiology. Under the *mixed environmental model*, the transmission probabilities are restricted to equal the parameter p ($\tau_1 = \tau_2 = \tau_3 = p$). This model hypothesizes that an individual's ousiotype is independent of the parental ousiotypes and hence that the factors responsible for phenotypic differences are not transmitted from one generation to another. The *mixed genetic model* restricts the transmission probabilities to the values expected under Mendelian transmission ($\tau_1 = 1.0$, $\tau_2 = .5$, and $\tau_3 = 0$). A *polygenic model* explains the distribution of SBP without the

major effects of a single factor. Hence the transmission parameters and separate ousiotype means are not included in this restricted model. A “no-polygenes” model explains the distribution of SBP by considering only the major effects of a single factor and individual-specific environmental effects; i.e., h^2 is restricted to be zero. Finally, the *sporadic model* describes the phenotypic variation entirely by individual-specific environmental effects.

Estimates of the parameters associated with each of the models considered were taken to be those that maximized the value of the likelihood of the model, given the observed data. For the mixed models ($h^2 \neq 0$), the likelihood was computed by using an approximation to the likelihood (Hasstedt 1982). For all other models, the exact likelihood was computed by using the pedigree analysis package (PAP) developed by Hasstedt (Hasstedt and Cartwright 1981) and was maximized by using a quasi-Newton optimization method implemented in the GEMINI computer program (Lalouel 1979).

A two-step hypothesis-testing strategy was carried out to establish whether major genetic effects and polygenes are required to explain the distribution of SBP within and among the 278 pedigrees. In the first step, each of the five restricted models described above was compared with a general model in which the intercept was both gender- and ousiotype-specific but in which the linear and quadratic age-regression terms were defined to be only gender specific. This corresponds to the traditional strategy of carrying out a complex segregation analysis on data that have been adjusted for age variability separately in males and females.

In a second step we compared restricted models with a general model which defined both the intercept and the regression relationship between SBP and age to be both gender and ousiotype specific. Here we first considered restricted models that represented null hypotheses about heterogeneity of regression parameters among ousiotypes. The model that, with the fewest number of linear and quadratic parameters, explained the data as well as the general model was taken to be the most parsimonious regression model. Next, each of the five restricted models that represent null hypotheses about genetic parameters τ_1 , τ_2 , τ_3 , p , and h^2 were compared with the most parsimonious general model. This two-step hypothesis-testing strategy allowed us to establish the validity of the assumption of homogeneity of the regression of SBP on age among ousiotypes, an assumption usually made in the appli-

cation of complex segregation analysis to human quantitative data.

The likelihood ratio test was used for hypothesis testing. Asymptotically, if the null hypothesis represented by a restricted model is true, then minus twice the difference between the log_e likelihoods of the restricted and the unrestricted models is distributed approximately as a χ^2 . df are equal to the number of independent parameters restricted by the particular null hypothesis.

Results

The sample of 1,266 individuals included 646 males age 23.9 ± 13.4 (mean \pm SD) years and 620 females age 24.9 ± 13.7 who had SBP of 106.4 ± 12.3 and 102.3 ± 12.5 mm Hg, respectively. Gender explained 2.1% of variability in SBP. Age and age squared explained 28.1% of variability in males and 17.3% of variability in females. However, since both gender and age were incorporated into the complex segregation analysis models, no adjustment of SBP for gender or age differences was performed.

Table 1 presents the maximum likelihood estimates (MLEs) of the parameters of the general model, as well as those obtained under the five restricted models. A general model that considers the intercepts to be different but the regression slopes to be the same in males and females was rejected ($\chi^2 = 32.21$, df = 2, result not shown). Hence, all restricted models considered in table 1 assumed the age regression to be gender specific. For this analysis we assume that the linear- and quadratic-regression effects of age on SBP were equal among ousiotypes ($\beta^1_{LLM} = \beta^1_{LHM} = \beta^1_{HHM}$; $\beta^2_{LLM} = \beta^2_{LHM} = \beta^2_{HHM}$; $\beta^1_{LLF} = \beta^1_{LHF} = \beta^1_{HHF}$; and $\beta^2_{LLF} = \beta^2_{LHF} = \beta^2_{HHF}$). Such a model is equivalent to the one traditionally used when data are adjusted for age effects within gender before a segregation analysis is considered.

Each of the five restricted models was rejected ($p < .01$) when compared with the general model. Rejection of a model without polygenes supports the presence of polygenic loci. Rejection of polygenic and sporadic models when compared with the general model supports the presence of a single factor with large effects on SBP. Furthermore, both the mixed genetic model and the mixed environmental model were also rejected when compared with the general model. Therefore, no inference can be drawn about the mode of transmission of the single factor, as the data do not

Table 1

Parameter MLEs and χ^2 Statistics Obtained from Complex Segregation Analysis of SBP When Intercept Is Ousiotype Specific and Gender Specific But Slope Is Only Gender Specific^a

PARAMETER	VALUE GIVEN BY MODEL ^b					
	General	No Polygenes	Mixed Environmental	Mixed Genetic	Polygenic	Sporadic
p621 ± .068	.817	.840	.811	1.0	1.0
α_{LLM}	80.562 ± 1.977	78.026	81.135	80.769	81.843	79.404
α_{LHM}	78.858 ± 3.177	89.366	81.179	79.905	81.843	79.404
α_{HHM}	100.649 ± 4.676	104.452	101.667	101.079	81.843	79.404
β^1_{LLM}	2.076 ± .182	1.932	1.927	1.970	1.889	2.119
β^1_{LHM}	2.076 ± .182	1.932	1.927	1.970	1.889	2.119
β^1_{HHM}	2.076 ± .182	1.932	1.927	1.970	1.889	2.119
β^2_{LLM}	-.31 ± .003	-.029	-.028	-.029	-.028	-.032
β^2_{LHM}	-.31 ± .003	-.029	-.028	-.029	-.028	-.032
β^2_{HHM}	-.31 ± .003	-.029	-.028	-.029	-.028	-.032
α_{LLF}	91.654 ± 2.007	87.977	90.672	89.810	92.767	91.303
α_{LHF}	91.353 ± 4.348	99.873	92.832	94.788	92.767	91.303
α_{HHF}	119.764 ± 5.144	125.369	122.920	122.304	92.767	91.303
β^1_{LLF}566 ± .182	.581	.596	.556	.460	.585
β^1_{LHF}566 ± .182	.581	.596	.556	.460	.585
β^1_{HHF}566 ± .182	.581	.596	.556	.460	.585
β^2_{LLF}	-.005 ± .003	-.005	-.005	-.004	-.002	-.004
β^2_{LHF}	-.005 ± .003	-.005	-.005	-.004	-.002	-.004
β^2_{HHF}	-.005 ± .003	-.005	-.005	-.004	-.002	-.004
h^2388 ± .068382	.335	.374	...
σ	9.758 ± .280	8.456	9.929	9.638	10.858	10.869
τ_1	[1.0]	[1.0]	(p)	(1.0)
τ_2866 ± .078	.522	(p)	(.5)
τ_3046 ± .176	.290	(p)	(.0)
$-\log L$	4,749.334	4,758.913	4,762.825	4,756.507	4,786.177	4,816.793
χ^2		19.16*	26.98*	14.35*	73.69*	134.92*
df		1	2	2	7	8

^a The mean μ_o of the o th ousiotype is given by the following regression: $\alpha_o + \beta^1_o \cdot \text{age} + \beta^2_o \cdot \text{age}^2$, where α , β^1 and β^2 are, respectively, the intercept, slope, and quadratic terms of the regression.

^b All models are obtained by restricting the linear and quadratic effects to be equal among ousiotypes in males ($\beta^1_{LLM} = \beta^1_{LHM} = \beta^1_{HHM}$ and $\beta^2_{LLM} = \beta^2_{LHM} = \beta^2_{HHM}$) and to be equal among ousiotypes in females ($\beta^1_{LLF} = \beta^1_{LHF} = \beta^1_{HHF}$ and $\beta^2_{LLF} = \beta^2_{LHF} = \beta^2_{HHF}$). Brackets indicate that the value is at the boundary; parentheses indicate that the value is fixed.

* $P < .01$.

support either genetic transmission or the alternative of no transmission of the effects of this factor from parents to offspring.

The MLEs of the parameters of a general model that includes gender- and ousiotype-dependent intercepts and regressions of SBP on age are presented in the first column of table 2. The comparison of this "complete" general model with the general model of table 1 reveals that the hypothesis of no ousiotype-specific age effects is rejected with a $\chi^2 = 33.75$ ($P < .01$). Hence, we conclude that a model with gender- and ousiotype-dependent age regressions fits the data significantly better than does a model that considers only gender-specific age regressions.

In order to determine the most parsimonious regression relationship between SBP and age within each ousiotype for each gender, a series of tests of the age-regression parameters were then performed. We first considered whether the quadratic terms were needed, by constraining to zero, simultaneously in males and females, the second-order coefficients ($\beta^2_{LLM} = \beta^2_{LHM} = \beta^2_{HHM} = \beta^2_{LLF} = \beta^2_{LHF} = \beta^2_{HHF} = 0$). This reduced model was rejected with a $\chi^2 = 107.03$ ($df = 6$). However, the hypotheses of no quadratic effects in only HH ($\beta^2_{HHM} = \beta^2_{HHF} = 0$) and in both HH and LH ($\beta^2_{LHM} = \beta^2_{HHM} = \beta^2_{LHF} = \beta^2_{HHF} = 0$) ousiotypes were not rejected ($\chi^2 = 1.96$, $df = 2$ and $\chi^2 = 2.48$, $df = 4$, respectively). These results suggest the pres-

Table 2

Parameter MLEs and χ^2 Statistics Obtained from Complex Segregation Analysis of SBP by Using an Ousiotype- and Gender-specific Age-Regression Model^a

PARAMETER	VALUE GIVEN BY MODEL ^b				
	Complete General	Most Parsimonious	Mixed Environmental	Mixed Genetic	No Polygenes
p825	.865	.900	.914 ± .021	.880
α_{LLM}	77.325	78.617	78.824	78.595 ± 2.15	76.524
α_{LHM}	93.144	85.961	87.355	90.439 ± 3.79	101.244
α_{HHM}	131.686	107.524	86.817	103.582 ± 14.17	100.708
β^1_{LLM}	2.478	2.329	2.324	2.265 ± .198	2.269
β^1_{LHM}035	.769	.751	.730 ± .099	.528
β^1_{HHM}	-1.250	.974	1.517	1.156 ± .429	1.305
β^2_{LLM}	-.041	-.038	-.038	-.036 ± .004	-.036
β^2_{LHM}012
β^2_{HHM}034
α_{LLF}	93.654	94.799	96.337	93.887 ± 1.20	92.173
α_{LHF}	77.445	88.412	81.302	92.372 ± 5.22	98.120
α_{HHF}	17.077	108.642	107.207	90.492 ± 17.95	100.702
β^1_{LLF}402	.237	.198	.269 ± .041	.285
β^1_{LHF}	1.860	.921	1.088	.861 ± .124	.609
β^1_{HHF}	9.243	1.402	1.379	1.857 ± .592	1.214
β^2_{LLF}	-.004
β^2_{LHF}	-.018
β^2_{HHF}	-.139
h^2441	.436	.439	.366 ± .077	(0)
σ	9.201	9.415	9.438	9.466 ± .249	8.833
τ_1928	.944	(<i>p</i>)	(1.0)	(1.0)
τ_2921	.891	(<i>p</i>)	(.5)	(.5)
τ_3	[0]	[0]	(<i>p</i>)	(.0)	(.0)
$-\log_e L$	4,730.263	4,734.497	4,739.915	4,737.419	4,749.484
χ^2 : General.....		8.47, df = 5			
Most parsimonious.....			10.84*, df = 2	5.84, df = 2	
Mixed genetic.....					24.13*, df = 1

^a The mean μ_o of the *o*th ousiotype is given by the following regression: $\alpha_o + \beta^1_o \cdot \text{age} + \beta^2_o \cdot \text{age}^2$, where α , β^1 and β^2 are, respectively, the intercept, slope, and quadratic terms of the regression.

^b Brackets indicate that the value is at the boundary; parentheses indicate that the value is fixed.

* $P < .01$.

ence of a significant quadratic effect in either LL males or LL females. The quadratic-regression parameter obtained from the model with no quadratic effects in LH and HH ousiotypes in males and females were smaller in LL females ($\beta^2_{LLF} = -.007$) than in LL males ($\beta^2_{LLM} = -.04$). The second-order coefficient in LL females was then constrained to zero to give the most parsimonious regression model presented in the second column of table 2. This model was found to fit the data as well as did the complete general model ($\chi^2 = 8.47$, $df = 5$). The fact that the ousiotype-specific

regression equations were different in males and females further supports the gender-specific nature of the relationship between SBP and age.

Other reduced models were then compared with this most parsimonious model. Tests regarding the mode of transmission of the gender- and age-dependent single-factor effects were first conducted. The hypothesis of no transmission (mixed environmental model) of the single-factor effects was rejected ($\chi^2 = 10.84$, $df = 2$), while the hypothesis of Mendelian transmission of these effects (mixed genetic model) could not

be rejected ($\chi^2 = 5.84$, $df = 2$). Only 2 df were used to derive the probability levels associated with the χ^2 values of these two models, because the MLE of τ_3 was at the boundary in the most parsimonious model. These results suggest that SBP is influenced by major effects of allelic variation at a single gene that are gender and age dependent. Finally, the hypothesis that the polygenic component contributes to the variation about the regression lines was tested by fixing h^2 at zero. When tested against the mixed genetic model, this hypothesis is rejected ($\chi^2 = 24.13$, $df = 1$).

Although the mixed genetic model was not rejected when compared with the most parsimonious general model in table 2, the estimate of τ_1 was .944, the estimate of τ_2 was .891, and the χ^2 was 5.84 (2 df). We partitioned the χ^2 into two single tests to determine whether the estimate of τ_2 was different from the Mendelian expectation of .5. We estimated the parameter MLEs and $\log_e L$ under a model with τ_1 and τ_3 restricted to their Mendelian expectations and allowed only τ_2 , p , h^2 , σ^2 , and the penetrance parameters to float. The value of $-\log_e L$ under this model (4,736.401) was then compared with (1) that of the most parsimonious general model as a test of τ_3 and (2) that of the genetic mixed model as a test of τ_2 . The χ^2 of the test was 3.81 ($df = 1$) for τ_3 and was 2.03 ($df = 1$) for τ_2 . This partitioning of the χ^2 comparing the mixed genetic model with the most parsimonious general model confirms that neither τ_1 nor τ_2 is significantly different from the Mendelian expectation for it.

The analyses presented in tables 1 and 2 give strong support to the hypothesis that both a single gene with gender- and age-dependent effects and polygenes contribute to the determination of interindividual differences in SBP. Under the mixed genetic model, restricting the effects of the H allele to be either dominant or recessive to the L allele, for both the intercept and slope in both males and females, resulted in a poorer fit of the data, compared with the model in table 2 ($\chi^2 = 17.22$, $df = 4$, $p < .01$ for H dominant; $\chi^2 = 78.84$, $df = 4$, $p < .01$ for H recessive). The estimate of the relative frequency of the H allele in the Rochester population is .09. Approximately 1% of the population will be homozygous, and 16% will be heterozygous for this allele. The regression lines from the best-fitting model (mixed genetic model; table 2) are presented in figure 1. The plots of the regressions show that allelic variation in this single gene is associated with larger differences among older individuals than among younger individuals. This effect is stronger in females.

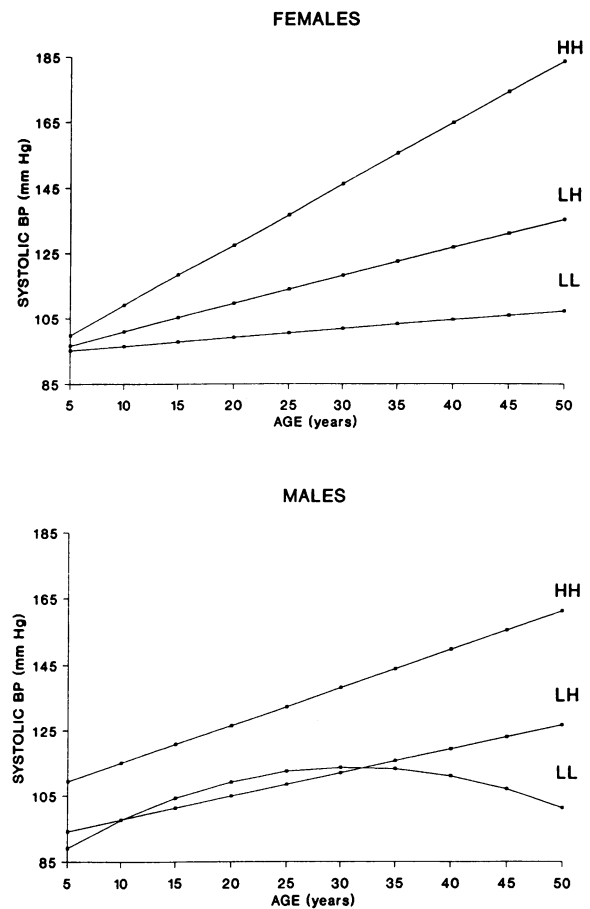


Figure 1 Relationship between SBP and age in LL, LH, and HH genotypes in females and males. Data are derived from the parameter estimates of the mixed genetic model (table 2).

Discussion

The present study represents one of the first applications of complex segregation analysis to blood pressure measurements obtained in a large number of pedigrees ascertained, without regard to health, from the population at large. Furthermore, to the best of our knowledge, this is the first time that a gender- and genotype-specific age-regression model has been used to investigate the genetics of a quantitative trait in humans. Two major findings emerged from the present study.

First, the use of a complex segregation analysis model that considers the effects of the single locus to be gender and age dependent provides a strategy to find more support for a single gene with a large effect on differences in SBP. This is well illustrated when results presented in table 1 and table 2 are compared.

The only interpretation that can be made from the results obtained in table 1 is that both polygenes and a single factor probably contribute to SBP differences in this population. No inference can be made about the mode of transmission of this factor from one generation to the other, because both mixed genetic and mixed environmental models are rejected. However, when the relationship between SBP and age is ousiotype dependent (table 2), the complex segregation analysis provides strong support for the role of a single gene in determining interindividual variation. This finding suggests that the traditional mixed model of complex segregation analysis (Elston and Stewart 1971; Lalouel et al. 1983), a model which specifies the major effects that a single factor has on the level of a trait after adjustment for concomitants, does not reflect adequately the complexity of the relationship between the genotype and the phenotype in determining the multifactorial inheritance of blood pressure. Models that consider the influence of a single locus to be dependent on concomitants such as gender and age are likely to be more realistic and to have the potential to improve our understanding of the genetics of blood pressure, as well as our understanding of other quantitative traits that are involved in determination of the etiology of common human diseases.

The second finding is that, for the first time, blood pressure is shown to be influenced by common allelic variations at a single gene. Although a recent study reported evidence for a rare genotype at a single locus associated with low SBP levels (Carter and Kannel 1990), that study did not consider the mixed environmental model as a reduced model. Furthermore, a very rare gene for low SBP cannot be a gene that contributes to the predisposition to hypertension. The new finding emerging from the present study is that the effects associated with the single-locus genotypes are gender and age dependent. Larger mean differences among genotypes occur in older individuals compared with younger individuals. The results presented in figure 1 and table 2 suggest that about 17% of the population will carry an allele that is predictive of larger differences in older persons. In a 30-year follow-up study of blood pressure in a cohort of 1,050 white males, Harlan et al. (1973) have shown that individuals with the highest values of SBP at age 54 years had higher SBP levels at age 24–54 years and experienced a steep rise of blood pressure in middle age. These results are consistent with those obtained in the present study and suggest a basis for tracking of SBP over time in adults.

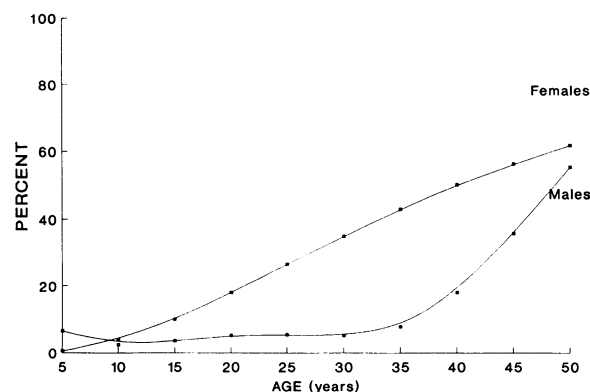


Figure 2 Percent of SBP variation attributable to single-locus genotype in males and females at different ages. Estimates are from the mixed genetic model in table 2. Total phenotypic variance (V_p) in SBP is partitioned as $V_p = V_{MG} + V_{PG} + V_E$, where V_{MG} is the variance attributable to variation at the major-locus genotype, V_{PG} is the variance attributable to polygenes, and V_E is the variance associated with individual-specific environmental factors. In this model, the fraction of variance attributable to polygenes (V_{PG}/V_p , where $V_{PG} = h^2\sigma^2$) and to individual-specific environmental factors (V_E/V_p , where $V_E = \sigma^2 - V_{PG}$) is the same across gender and age. Within each gender and at a given age the variance associated with differences among genotypes is $V_{MG} = p^2(\mu_{LL} - \mu)^2 + 2pq(\mu_{LH} - \mu)^2 + q^2(\mu_{HH} - \mu)^2$, where μ is the weighted mean of adjusted SBP on the ousiotype frequencies ($\mu = p^2\mu_{LL} + 2pq\mu_{LH} + q^2\mu_{HH}$). The proportion of total phenotypic variance attributable to variation at the major-locus genotype is V_{MG}/V_p .

Under the assumption that allelic effects are gender and age dependent in the Rochester population, one can estimate the impact that the single gene has on variation in SBP among individuals at different ages. For this purpose the parameter estimates of the mixed genetic model presented in table 2 were used to compute the percent of SBP variation attributable to the single-locus genotypes. The results are presented in figure 2. Given that the assumptions underlying our model are true (the frequency of the H allele and polygenic variance do not change across ages and genders, and there is homogeneity of the phenotypic variance about the gender- and genotype-specific regressions), this figure suggests that the impact that the single gene has on SBP variation increases with age in both males and females. The major effects of this single gene account for about 1% and 6% of the SBP variation at the age of 5 years and about 61% and 55% of the variation at the age of 50 years in females and males, respectively. Over this age interval the total expected phenotypic variance in SBP increases from 95.8 to 199.7 in males and from 90.0 to 232.7 in females.

Only speculations can be made about the mechanism through which this single gene affects SBP at different ages. The gene may be "switched on" at a certain period in life and may contribute to the elevation of blood pressure independently of the influence of environmental factors. It is also possible that a specific combination of environments occurring at a given time in an individual's life may lead to the expression of the gene.

How can these results improve our understanding of the genetics of essential hypertension? At the heart of the Platt-Pickering controversy was the central question of unimodality versus multimodality—or, in other words, whether blood pressure variation in the population could be represented by a single continuous distribution or by a mixture of two or more distributions (Swales 1985). Platt (1967) argued that individuals with essential hypertension represent a subgroup characterized by an inherited tendency to develop high blood pressure in *middle age*, and he hypothesized that segregation of blood pressure into "normotensives" and "hypertensives" was the result of a single gene with a dominant pattern of transmission. This hypothesis implies that the major effects of this single gene are expected to be age dependent. Greater evidence for mixture of distributions and for an increase in blood pressure variance with age are predicted by this hypothesis. The results of Rice et al.'s (1990) recent study of commingling and segregation analyses of blood pressure in French-Canadian families are consistent with this hypothesis. Their study found evidence for commingling in parents and in the pooled sample of parents and offspring but not when only offspring were considered, suggesting that the factor responsible for admixture in the distribution of SBP is age dependent. These results are also consistent with the results reported here, as we observed larger mean differences among single-locus genotypes as individuals age. On the other hand, our results are also in agreement with Pickering's view, as they support the significant contribution of polygenes in determining interindividual differences in blood pressure. Although the biometrical studies reported here do not identify the single gene responsible for elevated blood pressure, they do suggest that Platt and Pickering were probably both correct, in part, in their explanations of the genetic etiology of essential hypertension.

In summary, results of the present study reveal that major effects of allelic variation at a single gene interact with effects of factors that are associated with gen-

der and age to determined interindividual differences in blood pressure. This gene may be involved in determining a predisposition for development of hypertension in a subset of the population. The validity of this hypothesis will be tested in an independent sample of data that is currently being collected in the Rochester population. The results reported here also emphasize the need to consider models that reflect more adequately the relationship between genotype and phenotype to better understand the influence of genetic factors in complex traits such as blood pressure. The models used in the present study represent a step in that direction.

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References

- Annett JL, Sing CF, Biron P, Mongeau JG (1979) Familial aggregation of blood pressure and weight in adoptive families. II. Estimation of the relative contributions of genetic and common environmental factors to blood pressure correlations between family members. *Am J Epidemiol* 110: 492–503
- Berry TD, Hasstedt SJ, Hunt SC, Wu LL, Smith JB, Ash O, Kuida H, et al (1989) A gene for high urinary kallikrein may protect against hypertension in Utah kindreds. *Hypertension* 13:3–8
- Boerwinkle E, Turner ST, Weinshilboum R, Johnson M, Richelson E, Sing CF (1986) Analysis of the distribution of erythrocyte sodium lithium countertransport in a sample representative of the general population. *Genet Epidemiol* 3:365–378
- Camussi A, Bianchi G (1988) Genetics of essential hypertension: from the unimodal-bimodal controversy to molecular technology. *Hypertension* 12:620–628
- Cannings C, Thompson EA, Skolnick MH (1978) Probability functions on complex pedigrees. *Adv Appl Prob* 10: 26–61

- Carter C, Kannel WB (1990) Evidence of a rare gene for low systolic blood pressure in the Framingham Heart Study. *Human Heredity* 40:235–241
- Elston RC, Stewart J (1971) A general model for the genetic analysis of pedigree data. *Hum Hered* 21:523–542
- Genest O, Kuchel O, Hamet P, Cantin M (1983) Hypertension: physiopathology and treatment, 2d ed. McGraw-Hill, New York
- Harlan W, Oberman A, Mitchell RE, Graybiel A (1973) A 30-year study of blood pressure in a white male cohort. In: Onesti G, Kwan KE, Moyer JH (eds) Hypertension: mechanisms and management. Grune & Stratton, New York, pp 85–91
- Hasstedt SJ (1982) A mixed-model likelihood approximation on large pedigrees. *Comput Biomed Res* 15:295–307
- Hasstedt SJ, Cartwright P (1981) PAP: pedigree analysis program. Tech rep 13, Department of Medical Biophysics and Computing, University of Utah, Salt Lake City
- Hasstedt SJ, Hunt SC, Wu LL, Williams RR (1988a) The inheritance of intraerythrocytic sodium level. *Am J Med Genet* 29:193–203
- Hasstedt SJ, Wu LL, Ash O, Kuida H, Williams RR (1988b) Hypertension and sodium-lithium countertransport in Utah pedigrees: evidence for major-locus inheritance. *Am J Hum Genet* 43:14–22
- Kirkendall WM, Feinleib M, Fries ED, Mark AL (1981) Recommendations for human blood pressure determination by sphygmomanometers. *Hypertension* 3 (4): 509A–519A
- Krieger H, Morton NE, Rao DC, Azevedo E (1980) Familial determinants of blood pressure in northeastern Brazil. *Hum Genet* 53:415–418
- Lalouel JM (1979) GEMINI: a computer program for optimization of a nonlinear function. Tech rep 14, Department of Medical Biophysics and Computing, University of Utah, Salt Lake City
- Lalouel JM, Rao DC, Morton NE, Elston RC (1983) A unified model for complex segregation analysis. *Am J Hum Genet* 35:816–826
- Marazita ML, Elston RC, Nambodiri KK, Hames CG (1987) Genetic analysis of serum lipid levels and blood pressure in a large kindred. *Am J Med Genet* 26:511–519
- Moll PP, Harburg E, Burns TL, Schork MA, Ozgoren F (1983) Heredity, stress and blood pressure, a family set approach: the Detroit project revisited. *J Chronic Dis* 36: 317–328
- Moll PP, Michels VV, Weidman WH, Kottke BA (1989) Genetic determination of plasma apolipoprotein AI in a population-based sample. *Am J Hum Genet* 44:124–139
- Moll PP, Sing CF, Lussier-Cacan S, Davignon J (1984) An application of a model for a genotype-dependent relationship between a concomitant (age) and a quantitative trait (LDL cholesterol) in pedigree data. *Genet Epidemiol* 1: 301–314
- Morton NE, Gulbrandsen CL, Rao DC, Rhoads GG, Kagan A (1980) Determinants of blood pressure in Japanese-American families. *Hum Genet* 53:261–266
- Motulsky AG, Burke W, Billings PR, Ward RH (1987) Hypertension and the genetics of red cell membrane abnormalities. In: Weatherall D (ed) Molecular approaches to human polygenic disease. John Wiley & Sons, Chichester, pp 150–166
- Pérusse L, Rice T, Bouchard C, Vogler GP, Rao DC (1989) Cardiovascular risk factors in a French-Canadian population: resolution of genetic and familial environmental effects on blood pressure by using extensive information on environmental correlates. *Am J Hum Genet* 45:240–251
- Pickering G (1967) The inheritance of arterial pressure. In: Stamler J, Stamler R, Pullman TN (eds) The epidemiology of hypertension. Grune & Stratton, New York, pp 18–27
- Platt P (1967) The influence of heredity. In: Stamler J, Stamler R, Pullman TN (eds) The epidemiology of hypertension. Grune & Stratton, New York, pp 9–17
- Rapp JP (1983) Genetics of experimental hypertension and human hypertension. In: Genest J, Kuchel O, Hamet P, Cantin M (eds) Hypertension: physiopathology and treatment, 2d ed. McGraw-Hill, New York, pp 582–598
- Rebeck TR, Turner ST, Michels VV, Moll PP (1991) Genetic and environmental explanations for the distribution of sodium-lithium countertransport in pedigrees from Rochester, MN. *Am J Hum Genet* 49:000–000
- Rice T, Bouchard C, Borecki IB, Rao DC (1990) Commingling and segregation analysis of blood pressure in a French-Canadian population. *Am J Hum Genet* 46:37–44
- Sing CF, Boerwinkle E, Turner ST (1986) Genetics of primary hypertension. *Clin Exp Hypertens [A]* 8:623–651
- Sing CF, Moll PP (1989) Genetics of variability of CHD risk. *Int J Epidemiol* 18 (Suppl 1): S183–S195
- Swales JD (1985) Platt versus Pickering: an episode in recent medical history. Keynes, Cambridge
- Turner ST, Johnson M, Boerwinkle E, Richelson E, Taswell HF, Sing CF (1985) Sodium-lithium countertransport and blood pressure in healthy blood donors. *Hypertension* 7: 955–962
- Turner ST, Michels VV. Sodium-lithium countertransport and hypertension in Rochester, Minnesota. *Hypertension* (in press)
- Turner ST, Weidman WH, Michels VV, Reed TJ, Ormson CL, Fuller T, Sing CF (1989) Distribution of sodium-lithium countertransport and blood pressure in Caucasians five to eighty-nine years of age. *Hypertension* 13: 378–391
- Ward RH, Chin PG, Prior IAM (1979) Genetic epidemiology of blood pressure in a migrating isolate: prospectus. In: Sing CF, Skolnick M (eds) Genetic analysis of common

- diseases: applications to predictive factors in coronary disease. Alan R Liss, New York, pp 675–709
- Williams RR, Hunt SC, Hasstedt SJ, Berry TD, Wu LL, Barlow GK, Stults BM, et al (1988*a*) Definition of genetic factors in hypertension: a search for major genes, polygenes, and homogeneous subtypes. *J Cardiovasc Pharmacol* 12 [Suppl 3]:S7-S20
- Williams RR, Hunt SC, Wu LL, Hasstedt SJ, Hopkins PN, Ash KO (1988*b*) Genetic and epidemiological studies on electrolyte transport systems in hypertension. *Clin Physiol Biochem* 6:136–149