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Genetic and Environmental Control of Blood Pressure in Twins and Their Family Members

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Abstract. An interdisciplinary study, in adult twins and their family members, of the genetic and environmental determinants of complex physiologic functions is in progress. This report summarizes our initial studies of the control of the level of systolic (K1) and diastolic (K5) blood pressure in 202 monogygotic (MZ) and 121 dizygotic (DZ) twins, their spouses and their children. Correlation coefficients for blood pressure were adjusted for the covariates age, sex, body mass index (wt/ht^2) and screener, all of which significantly augment most correlations. These adjusted correlation coefficients in MZ twins are 0.5 for both K1 and K5 blood pressure. For DZ twins, the adjusted correlation coefficients are 0.21 (K1) and 0.24 (K5). MZ twin-offspring adjusted correlation coefficients are higher than MZ twin-niece/nephew adjusted correlation coefficients (0.12 and 0.06, respectively, for K1; 0.20 and 0.13, respectively, for K5), despite the genetic identity of these relationships. That environmental factors may explain these differences is suggested by other differences in adjusted correlation coefficients that are greater than those predicted by the degree of genetic similarity. In addition, we have assessed the relationship between two biochemical-physiological processes, the urinary excretion of kallikrein and transport of sodium in the erythrocyte (the sodium countertransport and the sodium-potassium-chloride cotransport systems), and blood pressure control, since both have been implicated in the control of blood pressure level. Although we found evidence for substantial genetic control of both phenomena, we were unable to establish any correlation between either function and the level of blood pressure in our normotensive subjects.

These data point to the operation of three broad categories of control of level of blood pressure: constitutional factors (age, sex, body mass), genetic factors and environ-

mental factors. The identities of the genetic and environmental factors are unknown at this time.

Key words: Blood pressure, Constitution, Genetic factors, Environment, Twin half-sib model

INTRODUCTION

Most people believe intuitively that even the most complex physiologic function is controlled in some part by genetic factors. A complex physiologic function (for example, the control of plasma cholesterol concentration) is the result of a number of biochemical reactions, some dependent and some independent. The enzymes, receptors, or other effector agents that are involved in these reactions are coded for by the host genome; thus, genes control the final phenotype. On the other hand, we also believe that genes do not work *in vacuo*, and that they are subject to significant modification by environmental factors in the determination of phenotype. These hypotheses are easily stated, but considerable difficulty arises in attempting to determine the relative contributions of genotype and environment to the final phenotype. This is important for situations in which the ultimate phenotype leads to disease, since we may be able to address issues of prevention and therapy with such information. When we determine that environmental factors are especially important, we can take steps to minimize exposure. If we find that the interactions of major components of both environment and genotype determine the final phenotype, we may devote our efforts to identifying individuals with the susceptible genotype, and to minimizing exposure to the environmental factors in only those individuals. Thus, we might conduct antismoking efforts in populations that are genetically liable for the development of chronic lung disease or lung cancer (should we identify such populations) rather than in the population at large. Moreover, having established the role of genotype and environment, we can then try to identify the specific genetic and environmental factors that are operative.

We at Harvard Medical School have established an interdisciplinary effort to focus on this very area. Our group includes epidemiologists (Drs. Frank Speizer, Susan Redline, Scott Weiss, Ira Tager), geneticists (Peter Tishler and Frances Lewitter), biostatisticians (Bernard Rosner, Mark Segal and Alvaro Munoz), physiologists (Mitzy Canessa, Harry Margolius) and clinicians (many of the above). We try to bring our different points of view to bear on experimental approaches to the understanding of complex physiologic variables. We believe that the twin half-sib model, as popularized by Nance and coworkers [14], is an extremely potent tool for study of these complex phenomena.

This report summarizes our initial studies of the control of level of blood pressure in adult twins and their family members. We believe that the current state of the art justifies several assumptions about blood pressure, and these assumptions underlie our study. First, blood pressure is distributed as a continuous variable. The evidence that blood pressure distributes unimodally, with a progressive skew "to the right" with age, appears to be overwhelming [15]. Second, a wealth of data concerning the mechanism of blood pressure control can be accrued from studies of ambulatory populations. We acknowledge that the many studies that have utilized highly controlled clinical research centers for the

study of a few subjects have yielded important leads about the physiologic control of blood pressure. However, the environments of clinical reasearch wards, by being so highly controlled, are highly artificial. For this reason, it may be impossible to gauge the relative importance, or even the importance at all, of any single control mechanism solely through this type of study. Studies in ambulatory populations, while fraught with other problems such as *lack* of environmental control and limitations on sample collection, nonetheless offer a good approach to understanding this aspect of blood pressure control.

In the late 1970s we established the Greater Boston Twin Registry, consisting of adult twins and their families [10]. Twins were identified primarily through the distribution of fliers to school children in grades K-9 in selected towns in the Greater Boston area. The fliers, which requested the cooperation of all adult twins in a health study, were brought home by the children, completed by twin parents, and mailed directly to study personnel. The eligibility of respondents was determined by the following criteria: each twin had a child aged 5-15 years, the twins were of the same sex, and both members of the pair were available for study. Eligible families were visited at home by a trained technician. Subjects were asked to complete or have completed questionnaires eliciting information on zygosity and blood pressure, as well as information on other risk factors for cardiovascular and pulmonary diseases. Three consecutive blood pressure measurements were obtained on all subjects after 5-10 minutes seated at rest. Readings were obtained on the Hawksley Random Zero, mercury filled sphygmomanometer, using cuff sizes recommended by the American Heart Association [9,27]. The same screener always completed these measurements on all members of the nuclear family, and about 85% of twinships were visited solely by the same screener. A casual urine sample was obtained on the majority (64%) of subjects. Venous blood samples were also obtained from 79 twin pairs.

Subjects were first classified according to zygosity, using a computerized decision tree algorithm that was based upon modifications of the questionnaire of Sarna et al [21]. The validity of this classification scheme was verified with the blood samples, by the analysis of blood groups ABO, Rh, MNs, serum Factor B, complement 3, haptoglobin and group specific component. We found that 39 of the 43 twin pairs labeled as MZ by blood group criteria (total concordance for all erythrocyte and serum factors) had been similarly classified by questionnaire, and 32 of the 36 twin pairs labeled as DZ (any discordance in blood markers) had been so classified by questionnaire. Thus, the sensitivity and specificity of the questionnaire alone in determining zygosity were 91 and 89%. We were unable to classify a small number of twin pairs, and these were omitted from our studies of blood pressure. We also eliminated subjects who were currently under treatment for hypertension, were afflicted with some illness that might distort the blood pressure, or were not compliant. The final population of twins and their family members, enumerated in Table 1, includes 202 MZ and 121 DZ twins, and 163 and 97 spouses and 408 and 266 offspring of MZ and DZ twins, respectively.

RESULTS

Familial Aggregation of Blood Pressure

Our comparisons involve either systolic (K1) or diastolic (K5) blood pressure measurements that have been adjusted for a number of covariates. This is accomplished with a generalized

Table 1 - Greater Boston Twin Registry: Characteristics of sample for blood pressure study

No. MZ twins/twin pairs		202/97
No. DZ twins/twin pairs		121/57
No. MZ spouses		163
No. DZ spouses		97
No. MZ offspring		408
No. DZ offspring		266
Female/Male ratio:	MZ twins	2.6
	DZ twins	2.2

least squares regression analysis that estimates intrapair correlations after adjusting for differences in environmental exposure and anthropomorphic features between cotwins [20]. The regression model is of the following general form:

$$(1)\quad Y_{ij} = a + \beta_1 X_{ij1} + \ldots + \beta_k X_{ijk} + e_{ij}$$

where Y_{ij} is K1 or K5 blood pressure for the jth twin (j = 1,2) from the ith twinship, and the X_{ij}'s are the covariates age, sex, body mass index (Quetelet index; wt/ht^2) and screener [3]. The residual e_{ij}'s are errors that are assumed to be normally distributed with mean 0 and unknown variance. The correlation between e_{i1} and e_{i2} is ρ, the intrapair correlation after adjustment for the aforementioned covariates. For estimation and hypothesis testing purposes, we fitted the model using maximum likelihood methods. Since the model requires that each pair have the same intraclass correlations, which may not be true in a combined cohort of MZ and DZ twins, we have fitted the model separately for each twin type.

The generalized least squares analysis (equation 1) was used for two purposes: 1) obtaining the intraclass correlation coefficients for MZ and DZ twins and for the offspring of twins; and 2) obtaining best estimates of blood pressures in offspring of twins for use in calculating residual scores of blood pressure after adjustment for age, sex, body mass index and screener. These residual blood pressure scores (calculated as observed minus expected blood pressure) were used for obtaining standard interclass correlation coefficients for all other comparisons (twins vs offspring, twins vs nieces/nephews, and offspring of twins vs cousins).

The results of comparisons of blood pressure among twins are presented in Table 2. Adjusted correlation coefficients among MZ twins are 0.5 for both K1 and K5 blood pressure, and both are highly statistically significantly different from zero. Adjusted correlation coefficients among DZ twins are 0.21 for K1 and 0.24 for K5 blood pressures; these approximate or just achieve statistical significance, respectively. The large MZ-DZ differences in correlations suggest the operation of a major genetic component. Indeed, one can calculate the "heritability" to be about 0.5-0.6.

The overall effect of adjustment of K1 or K5 blood pressure for age, sex, body mass index and screener appears to be greater in DZ twins than in MZ twins (Table 2; contrast raw with adjusted correlation coefficients). The effect in the children of twins (both MZ and DZ) is similar to that in DZ twins: the raw and adjusted correlation coefficients for K1 were 0.31 and 0.16, and for K5 were 0.31 and 0.22. Body mass index is the major contributor to this adjustment, and has the most statistically significant effect on blood

Table 2 - Twin-twin intraclass correlation coefficients for systolic (K1) and diastolic (K5) blood pressure

	Raw K1 BP	Raw K5 BP	Adj. K1 BP[a]	Adj. K5 BP[a]
MZ twins	0.53	0.55	0.50***	0.50***
DZ twins	0.31	0.35	0.21*	0.24**

[a]Adjusted for age, sex, wt/ht^2, screener.
*P =0.10; **P =0.05; ***P < 0.0001.

pressure. Clearly, in children and most twin adults, three "constitutional" covariates that influence level of blood pressure are body mass, age and sex.

A second important comparison is of twin-offspring vs twin-nieces/nephews. In these comparisons, the degree of genetic relatedness is constant in MZ twinships (0.5 for each), while that of DZ twin-nieces/nephews is half that of twin-offspring (0.25 vs 0.5; compare Fig. 1 vs Fig. 2). Thus, with significant genetic factors, one would expect to see a smaller difference in correlation coefficients in MZ comparisons than in DZ comparisons. Both correlation coefficients in MZ comparisons might approximate those of DZ twins (Table

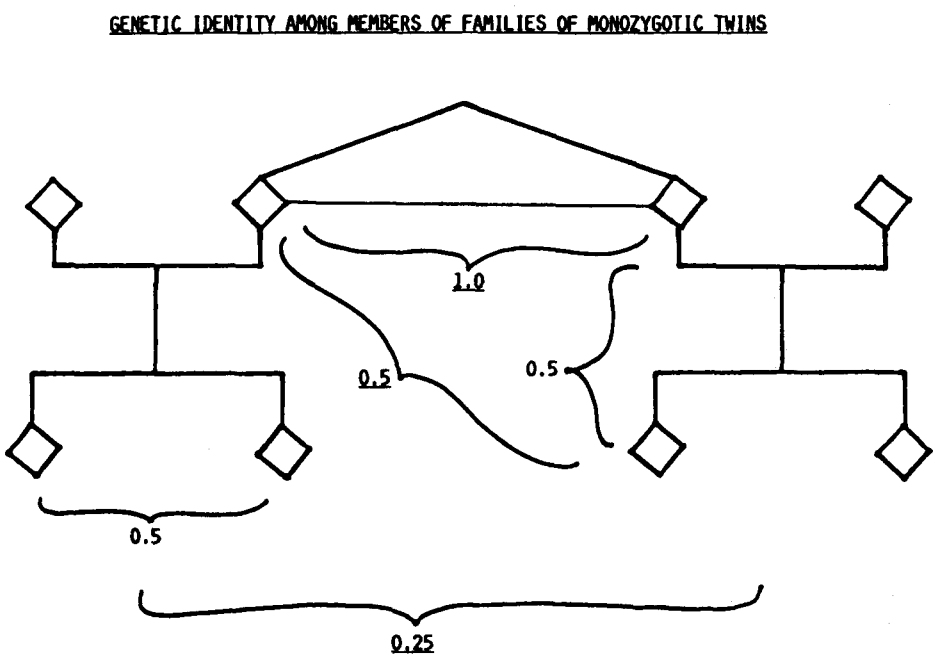

Fig. 1 - Schematic representation of degree of genetic identity among members of families of MZ twins of either sex. The number adjacent a bracket represents the degree of genetic identity for the relationship subtended by the bracket.

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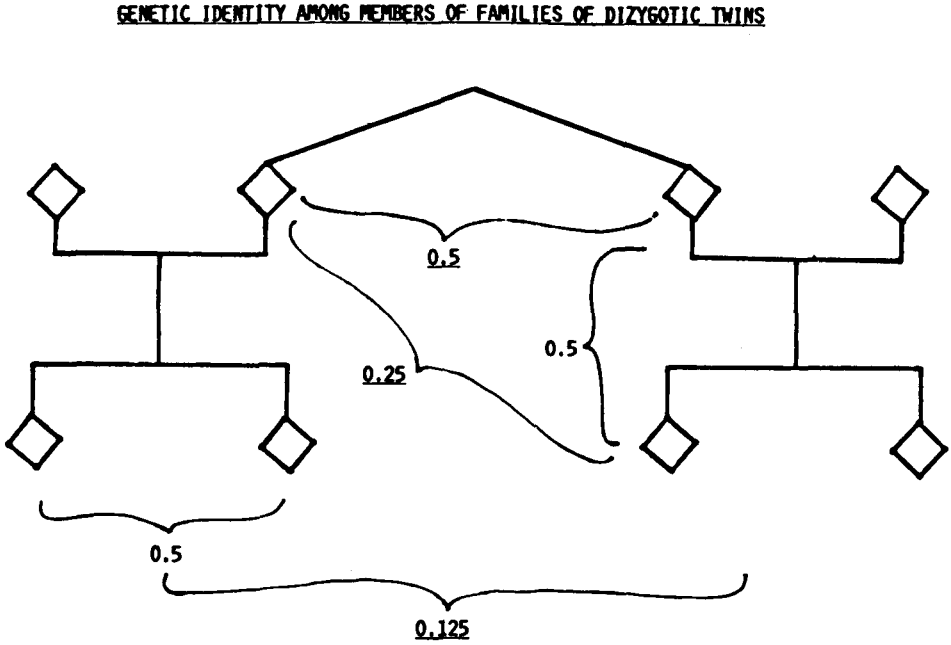

Fig. 2 - Schematic representation of degree of genetic identity among members of families of DZ twins of either sex. The number adjacent a bracket represents the degree of genetic identity for the relationship subtended by the bracket.

Table 3 - Twin-child interclass correlation coefficients (ρ) for blood pressure[a]

	Twin-offspring		Twin-Niece/Nephew	
	ρ	P	ρ	P
Systolic: MZ	0.12	0.02	0.06	NS
DZ	0.16	0.01	0.15	0.03
Diastolic: MZ	0.20	0.0004	0.13	0.01
DZ	0.25	0.0005	0.02	NS

[a]Correlations and significance testing of these correlations were computed by the pairwise method as outlined in refs. 17 and 18.

2), who have the same degree of genetic relatedness. Within the families of MZ twins, the twin-offspring K1 and K5 correlation coefficients are 0.12 and 0.20 (Table 3). The same correlation coefficients for twin-nieces/nephews are lower by 35-50%: 0.06 and 0.13 for K1 and K5 blood pressure, respectively (Table 3). These differences may result from an environmental factor, operating almost coequally with genotype. Within families of DZ twins, the difference in K5 correlation coefficient between twin-offspring and twin-nieces/nephews is much larger: 0.25 vs 0.02. However, there is virtually no difference in correlation coefficient for K1 blood pressure (0.16 vs 0.15).

Table 4 - Correlation coefficients (ρ) for blood pressure among offspring of twins[a]

	Offspring-Sibs		Offspring-Cousins	
	ρ	P	ρ	P
Systolic: MZ	0.19	0.0009	- 0.04	NS
DZ	0.25	0.0003	0.06	NS
Diastolic: MZ	0.22	0.0001	0.06	NS
DZ	0.32	< 0.0001	0.02	NS

[a]Correlation coefficients among offspring were computed using the generalized least squares method (text, equation1). Correlation coefficients among offspring and their cousins were derived using inter-class correlation methods based on residual scores of blood pressure after adjustment for age, sex, body mass index and screener [19].

A third important comparison is of offspring of twins compared among themselves vs offspring of twins and their cousins. Offspring of twins, either MZ or DZ, share 0.5 of their genes. Offspring of MZ twins share 0.25 of their genes with their cousins, while offspring of DZ twins share 0.125 of their genes with their cousins (compare Fig. 1 with Fig. 2). A two-fold difference in correlation coefficient might be anticipated. The intra-class correlation coefficients among offspring of both MZ and DZ twins were within the range that can be predicted on the basis of other data presented thus far (0.19-0.32; Table 4). However, correlation coefficients for all offspring-cousin comparisons are virtually zero, and differ little from each other according to zygosity. Despite this, the correlation coefficients for these comparisons in MZ families are what would be predicted on the basis of comparisons of twins and their children vs twins and nieces/nephews: similar correlation coefficients among offspring-siblings and twin-children, either offspring or nieces/nephews; and a ca. 50% lower correlation coefficient for offspring-cousins than those of twins and their children (compare Tables 3 and 4). These comparisons had suggested an additional environmental component that acted upon genotype to decrease correlations by 35-50%.

Biological Correlates of the Familial Aggregation of Blood Pressure

Urinary Kallikrein Excretion. We have begun studies of specific biochemical processes that might control the level of blood pressure. Our first study was of the renal kallikrein system. An extensive literature in experimental systems has related the production of renal kallikrein, a proteolytic enzyme that produces a potent vasodilator kinin peptide, to vascular resistance and blood pressure [13]. Zinner, Margolius, Kass and associates found a weak but statistically significant inverse correlation between the urinary excretion of kallikrein and blood pressure in a longitudinal study of children and their mothers [13,28]. They also demonstrated a significant familial aggregation of urinary kallikrein excretion. We have assessed the heritability of urinary kallikrein excretion and its relationship to blood pressure [24]. Urine samples from a subset of 12 twin pairs and their family members (24 spouses, 40 offspring) and 13 DZ twin pairs and family members (26 spouses, 55 offspring) were assayed for kallikrein by radioimmunoassay, and results were expres-

sed as the log kallikrein/creatinine for this analysis.

We first examined the data regarding the familial aggregation of kallikrein excretion alone (Table 5). The correlation coefficients for MZ and DZ twins are close to what might be predicted on the basis of their degree of genetic identity: ρ values of 0.82 and 0.34, respectively. Similarly, the correlations among MZ and DZ twin offspring, and those among MZ and DZ twin offspring and their cousins are close to the predictions of the genetic model (0.51 and 0.38 for offspring-siblings, and 0.30 and 0.19 for offspring-cousins of MZ and DZ twins, respectively); and all correlations among members of DZ twin families are close to what is expected by this model. On the other hand, two correlations among members of MZ families are essentially zero: those for twins and their offspring (- 0.07) and for twins and their nieces/nephews (0.02). In sum, 8 of 10 correlations are consistent with those expected if they resulted from genetic determination alone (Table 5). Of the 6 important correlations in which differences in degree of genetic identity exist between MZ and DZ families, 5 are consistent with a significant genetic basis.

Table 5 - Correlation coefficients for urinary kallikrein

Comparison	Correlation coefficient (ρ)			
	MZ twin families		DZ twin families	
	Expected[a]	Observed	Expected[a]	Observed
Twin-Twin[b]	1.0	0.82	0.5	0.34
Twin-Offspring[c]	0.5	- 0.07	0.5	0.40
Twin-Niece/Nephew[c]	0.5	0.02	0.25	0.16
Twin Offspring-Siblings[b]	0.5	0.51	0.5	0.38
Twin Offspring-Cousins[c]	0.25	0.30	0.125	0.19

[a]Expected values of ρ are based upon the degree of genetic identity. See Figs. 1 and 2.

[b]Intraclass correlation coefficients.

[c]Interclass correlation coefficients.

In this subset, we could demonstrate the familial aggregation of blood pressure, with correlation coefficients among twins that were similar to those of the entire population. We attempted to relate log kallikrein/creatinine to blood pressure, using a multiple regression analysis to control for age, sex and weight. We found a *positive* and statistically significant correlation between kallikrein and blood pressure: for K1 blood pressure, the regression coefficient = 2.35 and P = 0.02; and for K5 blood pressure, the regression coefficient = 2.02 and P = 0.04.

Thus, while we have established a case for a strong genetic underpinning for urinary kallikrein excretion, we did not establish a physiologically reasonable causal relation between urinary kallikrein excretion and blood pressure level. Indeed, the relationship would appear from the present data to be exactly the opposite (increased urinary kallikrein excretion with increased blood pressure) and contrary to logic for an enzyme that generates vasodilator materials.

Erythrocyte Sodium Transport. The activity of two erythrocyte systems for the transport of sodium (Na) may be related to hypertension. These systems are the Na countertransport system (which promotes the exchange of Na for Na), and the Na-potassium-chloride cotransport system (which functions under different circumstances as either an Na or a chloride pump). Others have reported finding increased activity of the Na countertransport system in erythrocytes of a large proportion (35-60%) of Caucasian subjects with essential hypertension, and in their adolescent offspring [1,4,26]. A higher Michaelis constant (Km) for the activation by cellular Na of the outward side of the cotransport system has also been reported [6]. Lewitter and Canessa have studied the activity of the countertransport and the cotransport systems in erythrocytes from a subset of 23 MZ and 29 DZ twin pairs [11]. The aim of their study was to provide initial evidence concerning the possible heritability of these Na transport systems, and to relate this activity to level of blood pressure. Adjusting for age, sex and body mass, they derived intraclass correlation coefficients for the countertransport system of 0.71 for MZ and 0.22 for DZ twins. For the cotransport system, these correlation coefficients were 0.78 and 0.36. Level of either systolic or diastolic blood pressure, when added as an additional independent covariate, had no significant effect on the adjusted activity of either transport system. This suggests that activity of the Na transport systems and level of blood pressure (when within the normal range) are independent. Moreover, a family history of hypertension had a significant *negative* effect on activity of the Na cotransport system, a finding that is at variance with the relationship gleaned from other studies of Caucasian families [2].

DISCUSSION

Twin studies of the inheritance of blood pressure have been popular since the original investigation by Stocks in 1930 [25]. Most studies with reasonable sample size have documented a high degree of correlation of blood pressures of MZ twins, and a lesser correlation for DZ twins [5,7,8,12]. Most studies have been limited to the comparison of MZ vs DZ twins, however. Two exceptions are the studies of Rose et al and Nance et al. The former study included comparisons of family members other than twins, but only in MZ twin families and only for systolic blood pressure [16]. Parent-child and parent-niece/nephew correlation coefficients were virtually identical, suggesting the operation of major genetic determinants of systolic blood pressure. Nance et al, also studying MZ twins and their family members, developed evidence for a major maternal effect on systolic blood pressure and for several types of genetic influence on both systolic and diastolic blood pressure [14].

Our study, which is of K1 and K5 blood pressures in families of both MZ and DZ twins, corroborates many of the findings of the aforementioned studies. Blood pressures among MZ twins are highly correlated ($\rho = 0.5$), while those for DZ twins are about half as correlated. Our correlation coefficients are among the lowest found in studies of twins (Table 6). Part of this study may result from the adjustments we have made for covariates: adjusting at all, adjusting with improved covariates such as the body mass index, or adjusting with a new statistical method all differentiate our study from those of others. Indeed, we found that the adjustment for body mass index was of high statistical significance, and for age and sex was also substantial, particularly in DZ twins and children. Of the other

Table 6 - Twin correlations for blood pressure in previous and current studies

Study	Population	Systolic BP Correlation coefficient		Diastolic BP Correlation coefficient	
		MZ twins	DZ twins	MZ twins	DZ twins
Havlik et al [8], Collaborative Perinatal Project	115 MZ, 82 DZ pairs, age 7 yr	0.54	0.40	0.54	0.27
McIlhaney et al [12]	82 MZ, 113 DZ pairs, age 14 ±6.5 yr	0.85	0.50	0.80	0.54
Feinleib et al [5], NHLBI Twin Study	250 MZ, 264 DZ pairs, white males, age 42-56 yr	0.55	0.25	0.58	0.27
Rose et al [16], Indiana Twin Panel	76 MZ pairs, adult	0.72		0.42	
Grim and Cantor [7]	19 MZ, 10 DZ pairs, blacks	0.81	0.28	0.88	0.54
Tishler et al, Greater Boston Twin Registry	202 MZ, 97 pairs; 121 DZ, 57 pairs; adult	0.50	0.21	0.50	0.24

comparisons in these families, that of twin-offspring vs twin-niece/nephew suggests that environmental as well as genetic factors are operative: for these comparisons in MZ twin families, the correlations of twin-niece/nephew are only 50-65% of that of twin-offspring, despite their genetic identity.

At this point, we believe that we can partition the control of blood pressure in these families of twins into three broad categories. The first category includes genetic factors, which may account for as much as 50% of the correlation of blood pressure. The second category is a substantial environmental component, as indicated by the findings in offspring of twins and also in the correlation among twins and spouses in both our study (data not presented) and that of others [23]. This environmental component probably increases with age [22]. Finally, there are the constitutional factors age, sex and body mass. Whether these are markers for purely genetic factors or more complicated genetic-environmental interactions is not clear.

We have studied two potential biochemical mediators of control of blood pressure, but neither appears from our data to have such a role. Both the urinary excretion of kallikrein and the activity of two Na transport systems may themselves be under a high degree of genetic control. However, neither seems to vary in any rational sense with the level of systolic or diastolic blood pressure, at least when blood pressure is in the normal range.

Finally, we urge collaboration among investigators with twin registries, so that analyses can benefit from larger sample size. As our work suggests, large sample sizes may aid in clarifying the significance (in a statistical and biological sense) of small differences in correlation coefficients. Certainly, there are problems concerning the comparability of procedures and measurements among different data sets, but these can be considered in the analyses. Studies of twins and their families offer a powerful tool for beginning to understand how complex functions are regulated. Data made available from multiple sources will only increase this power.

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