Variation in the Region of the Angiotensin-Converting Enzyme Gene Influences Interindividual Differences in Blood Pressure Levels in Young White Males

Myriam Fornage, PhD; Christopher I. Amos, PhD; Sharon Kardia, PhD; Charles F. Sing, PhD; Stephen T. Turner, MD; Eric Boerwinkle, PhD

**Background**—The renin-angiotensin system regulates blood pressure through its effects on vascular tone, renal hemodynamics, and renal sodium and fluid balance.

**Methods and Results**—Using data from a large population-based sample of 1488 siblings having a mean age of 14.8 years and belonging to the youngest generation of 583 randomly ascertained three-generation pedigrees from Rochester, Minn, we carried out variance components–based linkage analyses to evaluate the contribution of variation in four renin-angiotensin system gene regions (angiotensinogen, renin, angiotensin I–converting enzyme, and angiotensin II receptor type 1) to interindividual variation in systolic, diastolic, and mean arterial pressure. We rejected the null hypothesis that allelic variation in the region of the angiotensin-converting enzyme (ACE) gene does not contribute to interindividual blood pressure variability. After conditioning on measured covariates, variation in this region accounted for 0%, 13% \((P = 0.04)\), and 16% \((P = 0.04)\) of the interindividual variance in systolic, diastolic, and mean arterial pressures, respectively. These estimates were even greater in a subset of subjects with a positive family history of hypertension (0%, 29% \([P = 0.005]\), and 32% \([P < 0.005]\), respectively). In sex-specific analyses, genetic variation in the region of the ACE gene significantly influenced interindividual blood pressure variation in males (37% for SBP \([P = 0.03]\), 38% for DBP \([P = 0.04]\), and 53% for MAP \([P < 0.005]\)) but not in females.

**Conclusions**—Although it is possible that variation in a gene near the ACE gene may explain the observed results, knowledge about the physiological involvement of ACE in blood pressure regulation supports the proposition that the ACE gene itself influences blood pressure variability in a sex-specific manner. *(Circulation. 1998;97:1773-1779.)*

**Key Words:** linkage analysis ■ blood pressure ■ angiotensin-converting enzyme ■ whites ■ males

Hypertension, or high BP, is the most common risk factor for myocardial infarction, stroke, end-stage renal disease, and peripheral vascular disease.\(^\text{1,2}\) Despite evidence for a substantial genetic component underlying interindividual BP variation, progress toward identifying specific genes has been slow. Genetic linkage analysis has been the method of choice for identifying genes contributing to human disease. However, conventional linkage approaches based on log of the odds score analysis require precise specification of the genetic model and may, therefore, not be efficient for the study of a complex trait such as BP. Robust “nonparametric” linkage approaches, based on allele sharing, make minimal assumptions regarding the underlying mode of inheritance and are computationally simple. Thus, they are better suited for and widely applied to the study of phenotypes having a multifactorial etiology, such as BP or essential hypertension.

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From the Human Genetics Center (M.F., E.B.) and the Institute of Molecular Medicine (E.B.), University of Texas at Houston Health Science Center; the Department of Epidemiology, University of Texas M.D. Anderson Cancer Center, Houston (C.I.A.); the Department of Human Genetics, University of Michigan, Ann Arbor (S.K., C.F.S.); and the Division of Hypertension, Department of Internal Medicine, Mayo Clinic, Rochester, Minn (S.T.T.).

Guest editor for this article was Suzanne Oparil, MD, University of Alabama at Birmingham.

Correspondence to Eric Boerwinkle, PhD, Human Genetics Center, University of Texas Houston Health Science Center, PO Box 20334, Houston, TX 77225.

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583 pedigrees ascertained without regard to health status from the population of Rochester, Minn, we tested hypotheses about whether or not variation within or very near the genes encoding the four major components of the RAS contributes to interindividual variance in SBP, DBP, and MAP levels.

Methods

Subjects

All individuals were members of 583 three-generation pedigrees taking part in the Rochester Family Heart Study, a population-based study initiated in 1984 to investigate the role of genetic factors in the occurrence of hypertension and cardiovascular disease in Rochester, Minn. Families participating in the study were ascertained through households having two or more children enrolled in the schools of Rochester, Minn, and without regard to the health status of the family members. All individuals provided their informed consent to participate in the Rochester Family Heart Study. Recruitment and examination protocols have been described elsewhere.6,7 From these pedigrees, 1569 full siblings distributed among 587 sibships and belonging to the youngest generation were identified. For a large proportion of the sibships (92%), both parents were available for genotyping. The most common sibship constellation (47%) was two full siblings, with information on both parents. Nonwhite individuals and those taking medications that may have modified BP levels (n=81 individuals) were excluded in the linkage analyses reported here.

For these analyses, SBP and DBP values were the means of three readings taken at least 2 minutes apart with a random-zero sphygmomanometer. For each reading, the pressure at the Korotkoff phase IV sound was taken as the SBP. DBP was determined at the Korotkoff phase IV sound in children <13 years old and at the Korotkoff phase V sound in all other subjects. MAP was calculated from the averaged SBP and DBP values as follows: MAP=(SBP+2DBP)/3.

Marker Data Collection

All 3653 members of the 583 Rochester Family Heart Study pedigrees were genotyped for one polymorphic marker located within or very near the sequence of each of the genes encoding angiotensin (ie, AGT), renin (ie, REN), angiotensin I–converting enzyme (ie, ACE), and the angiotensin II receptor type 1 (ie, AT1) (Table 1). The AGT and AT1 markers were dinucleotide repeat polymorphisms located 12 and 15 kb, respectively, downstream from the 3' end of the coding gene sequence. The REN marker was a tetranucleotide repeat polymorphism located in intron 7 of the REN gene. The ACE marker was a compound tetranucleotide/dinucleotide repeat located between the 18th and 19th Alu sequences of the regulatory region of the human growth hormone gene. This marker showed complete linkage with the ACE gene in the Centre d'études du polymorphisme humain (CEPH) families.4 Each microsatellite repeat polymorphism was amplified by the polymerase chain reaction using flanking primer pairs and temperature conditions as previously reported (References 8 through 11; Table 1). Size variation of the amplified products was resolved by polyacrylamide gel electrophoresis and subsequent autoradiography. Each genotype was scored independently by two laboratory personnel, and any discrepancies were resolved by a third more senior laboratory supervisor.

Variance Component Linkage Analyses

Identical by descent (IBD) calculations on extended pedigree data were carried out using a modified version of the Curtis and Sham12 algorithm. Allele frequencies at each marker locus were estimated directly by gene counting from genotype data obtained in the total sample. Linkage analyses were carried out using both the regression method of Haseman and Elston13 and a variance components approach.14 Similar results were obtained by both methods. The latter method has been shown to provide greater statistical power and more precise estimates of the components of variance attributable to linked and unlinked genetic factors (see References 14 through 16). This article, therefore, focuses on results obtained from maximum-likelihood estimates of the components of variance as described by Amos et al.15,17 This variance component–based linkage method was used to estimate the relative contribution of each of the four RAS loci to interindividual differences in three measures of BP: SBP, DBP, and MAP. The quantitative trait was modeled as an additive function of fixed effects from covariates, random effects from variation at a genetic locus linked to the marker locus, random familial effects, and residual sources of variation, including measurement error. The contributions of genetic loci unlinked to the marker locus and those of nongenetic factors shared among siblings are confounded in the variance component referred to as "familial effects." The covariate effects evaluated were sex (except in sex-specific analyses), age, age squared, and body mass index. If one assumes no dominance at the linked locus and no recombination between this locus and the marker locus, the covariance matrix (Ω) among individuals in a sibship h is given by Ωh=Πσa2+Πσf2,1+Πσr2, where Π is a matrix with elements πij representing the proportion of alleles shared IBD at the marker locus between individuals i and j in sibship h; Π is the matrix of coefficients of relationship; and I is the identity matrix.

The vector of phenotypic values for each sibship was assumed to have a multivariate normal distribution. A likelihood function was numerically maximized18 to yield estimates of the variance components and covariate effects. Robust estimates of the variance of the maximum-likelihood parameters were computed as described in Amos et al.15 The parameter of primary interest is the component of variance, σa2, representing the contribution of the candidate gene region to the phenotypic variance. The null hypothesis that σa2 is equal to zero was tested by the likelihood-ratio test statistic

\[ L_0 = -2(L_0 - L) \]

where L is the value of the log-likelihood function evaluated at the maximum-likelihood estimates of the parameters and L0 is the maximum of the log-likelihood function evaluated when σa2 is constrained to equal zero. Because estimates of the variance components are constrained to be nonnegative, the asymptotic distribution of L, under the null hypothesis, is approximately a 1/2:1/2 mixture of χ^2 with one degree of freedom and a point mass at zero.19 In this situation, the critical value used to assess the significance of the likelihood-ratio test at the alpha=0.05 level corresponds to the critical value associated with a significance level of 2α (0.10) for the usual χ^2 distribution.

Results

Descriptive statistics for BP and covariate measures in the sample of siblings are given by sex in Table 2. All individuals

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**TABLE 1. Description of Genetic Markers**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Observed Heterozygosity*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>(AAAG)6(AAG)8</td>
<td>.93</td>
<td>8</td>
</tr>
<tr>
<td>AGT</td>
<td>(CA)6</td>
<td>.77</td>
<td>9</td>
</tr>
<tr>
<td>REN</td>
<td>(ACAG)6</td>
<td>.35</td>
<td>10</td>
</tr>
<tr>
<td>AT1</td>
<td>(CA)6</td>
<td>.69</td>
<td>11</td>
</tr>
</tbody>
</table>

*For all individuals in the Rochester Family Heart study pedigrees.

Selected Abbreviations and Acronyms

ACE = angiotensin-converting enzyme (gene)  
AGT = angiotensinogen (gene)  
AT1 = angiotensin II type 1 receptor (gene)  
DBP = diastolic blood pressure  
I/D = insertion/deletion  
MAP = mean arterial pressure  
RAS = renin-angiotensin system  
REN = renin (gene)  
SBP = systolic blood pressure
were white, normotensive (ie, SBP ≤140 and DBP ≤90), and not taking medication that might have modified BP levels. The mean ages were 14.55 years for female subjects and 14.96 years for male subjects and were not significantly different between sexes. Males had a significantly higher SBP than did females. DBP and MAP levels and body mass index were not significantly different between the two sexes.

For each marker locus, the amount of interindividual variation in the three measures of BP attributable to genetic variation in each of the four RAS gene regions, to residual familial effects (including genetic effects unlinked to the marker locus), and to nongenetic sources of variation was estimated while jointly accounting for the effects of measured covariates. The maximum-likelihood estimate of each parameter and its robust SE are shown in Table 3. The \( P \) value for the likelihood-ratio test of the significance of genetic variation linked to the marker locus is also given. After conditioning on covariate effects, variation in the \( AGT \) gene region accounted for 13.0%, 11.1%, and 14.1% of the interindividual variance in SBP, DBP, and MAP, respectively, but contribution of this region to BP variability did not reach statistical significance. Similarly, no evidence for a statistically significant influence of the \( REN \) gene region or the \( AT1 \) gene region on interindividual variation in SBP, DBP, or MAP was obtained in this large sample of sibships. Point estimates of the contribution of the \( REN \) gene region to interindividual variance in SBP, DBP, and MAP were 17.5%, 0%, and 2.39%, respectively. These estimates were 11.3%, 10.3%, and 12.7% for the \( AT1 \) gene region. A significant contribution of the \( ACE \) gene region to interindividual variation in both DBP and MAP was detected and accounted for 13.6% and 16.5% of the variance in DBP and MAP, respectively. No significant influence of this region on interindividual variation in SBP was observed.

Because the impact of genetic variation on interindividual variation in BP levels may differ among subsets of individuals in the population, we next examined the subgroup of siblings with a positive family history of hypertension. Family history of hypertension was defined as those individuals having at least one parent or grandparent with definite essential hypertension (ie, requiring antihypertensive treatment or having an SBP >160 mm Hg or a DBP >95 mm Hg). In this subset, the contribution of variation in the \( ACE \) gene region to BP variation was even stronger, accounting for 29.1% and 32.7% of the total variance in DBP and MAP (\( P=0.005 \) and \( P<0.005 \), respectively (Table 4). No evidence for a contribution of variation in the \( ACE \) gene region to interindividual variation in SBP was observed. We did not detect a significant effect of \( AGT \), \( REN \), and \( AT1 \) on interindividual variation in any measure of BP in this subgroup of individuals with a family history of hypertension (not shown).

Because there is ample evidence that BP distributions differ between males and females (eg, see Reference 6) and because genetic variation may have a different effect between the sexes,20 we next estimated the contribution of each of the four marker regions to interindividual variation in BP levels separately by sex. Results for the \( ACE \) marker are presented in Table 4. A significant contribution of the \( ACE \) gene region
was present in males but not in females for all three measures of BP. These analyses indicated that 37.5% of the variance in SBP \((P=0.03)\), 38.4% of the variance in DBP \((P=0.04)\), and 53.5% of the variance in MAP \((P<0.005)\) was attributable to variation in or around the ACE gene in male siblings only. No evidence for an effect of the AGT, REN, and AT1 gene regions on interindividual variation in SBP, DBP, or MAP was detected in either males or females (data not shown).

### Discussion

We have used variance component–based linkage analyses to estimate the contribution of variation in the regions of the RAS genes to interindividual variation in BP in a large sample of white sibships from Rochester, Minn. Sampling from the youngest generation, wherein the prevalence of hypertension is low, enabled us to examine the complete distribution of alleles underlying interindividual variation in the quantitative BP phenotype in the white population of Rochester, Minn. Such a strategy is not subject to the limitations associated with the presence of treated essential hypertension in older generations, which may result in the exclusion of a large proportion of patients because their measures of BP do not reflect untreated levels. The power of detecting a genetic effect on BP variation in a sample lacking these genetically susceptible individuals may be decreased. Unlike previous studies that focused on restricted subsets of hypertensive individuals (eg, those with severe or familial essential hypertension), our strategy was to estimate the impact of variation in the RAS gene regions in the population at large. Indeed, identifying a gene with large effects on BP levels in only a small subset of severely affected individuals does not establish that this gene will have a significant impact on BP variation in the general population.

We did not find significant evidence that genetic variation in the regions of the AGT or REN gene contributes to interindividual variation of BP in our sample. This finding suggests that the impact of variation in these genes on interindividual variability in BP may not be large in the general population of young white individuals. However, this conclusion does not preclude the possibility that the contribution of these genes may be greater in some subset of individuals. Indeed, two studies have demonstrated significant linkage between the (CA) repeat polymorphism of the AGT gene and a locus that contributes to severe essential hypertension in white individuals. No evidence of linkage or association has been reported between variation in the

### Table 4: Maximum-Likelihood Estimates of Robust SEs of the Variance Contribution of the ACE Locus to SBP, DBP, and MAP Levels in Subsets of Siblings

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Marker Locus</th>
<th>Familial</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siblings with a family history of hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>0.00±9.87</td>
<td>44.02±9.86*</td>
<td>49.49±8.43*</td>
</tr>
<tr>
<td></td>
<td>(0.00)</td>
<td>(47.07)</td>
<td>(52.93)</td>
</tr>
<tr>
<td>DBP</td>
<td>36.80±14.33*</td>
<td>35.47±19.94*</td>
<td>54.21±11.26*</td>
</tr>
<tr>
<td></td>
<td>(29.09)</td>
<td>(28.04)</td>
<td>(42.87)</td>
</tr>
<tr>
<td>MAP</td>
<td>27.30±9.40*</td>
<td>24.21±13.27*</td>
<td>32.00±7.38*</td>
</tr>
<tr>
<td></td>
<td>(32.69)</td>
<td>(28.99)</td>
<td>(38.32)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>33.69±14.33*</td>
<td>8.20±16.63</td>
<td>48.03±10.28*</td>
</tr>
<tr>
<td></td>
<td>(37.46)</td>
<td>(9.12)</td>
<td>(53.42)</td>
</tr>
<tr>
<td>DBP</td>
<td>52.24±21.66*</td>
<td>30.18±29.03</td>
<td>53.58±16.19*</td>
</tr>
<tr>
<td></td>
<td>(38.41)</td>
<td>(22.19)</td>
<td>(39.39)</td>
</tr>
<tr>
<td>MAP</td>
<td>47.31±14.28*</td>
<td>8.30±18.30</td>
<td>32.81±10.09*</td>
</tr>
<tr>
<td></td>
<td>(53.50)</td>
<td>(9.38)</td>
<td>(37.12)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>0.00±14.28</td>
<td>37.65±12.58*</td>
<td>48.12±11.67*</td>
</tr>
<tr>
<td></td>
<td>(0.00)</td>
<td>(43.89)</td>
<td>(56.11)</td>
</tr>
<tr>
<td>DBP</td>
<td>5.54±25.58</td>
<td>47.27±29.81</td>
<td>48.46±18.31*</td>
</tr>
<tr>
<td></td>
<td>(5.47)</td>
<td>(46.68)</td>
<td>(47.85)</td>
</tr>
<tr>
<td>MAP</td>
<td>2.13±9.65</td>
<td>37.58±13.69*</td>
<td>29.09±8.24*</td>
</tr>
<tr>
<td></td>
<td>(3.09)</td>
<td>(54.62)</td>
<td>(42.29)</td>
</tr>
</tbody>
</table>

In parentheses, these estimated variance components are expressed as a percent of the total variance. The \(P\) value from the likelihood-ratio test of significance of the marker locus variance component is also provided.

*Effects significant by Wald test.
REN gene and BP or essential hypertension in whites. These results, along with ours, suggest that the REN gene is unlikely to play a major role in BP variation in whites. It should be pointed out, however, that the observed heterozygosity for the REN marker locus was much less than expected on the basis of the original published report. Although the power of our linkage analyses was high (>80%) for detecting genes with moderate effects (contributing 20% of the phenotypic variance), this power was reduced when the marker locus was less informative, such as the REN marker locus.

No linkage was demonstrated between a (CA) microsatellite of the AT1 gene and a locus that influences interindividual BP variation in our study or a variability in risk of essential hypertension in a previous study by Bonnardeaux et al. However, a significant association of an A1166C polymorphism of this gene with severe essential hypertension was reported in a cross-sectional analysis. Association studies are able to detect weaker genetic effects, which may be difficult to be recognized by linkage analysis. Therefore, we cannot exclude the possibility that the AT1 gene may have a small impact on interindividual variation in BP, especially among individuals with clinically manifest hypertension.

We found significant evidence to support a contribution of the ACE gene region to interindividual variation in BP in our sample of young white individuals. This effect was consistent across analyses when both the Haseman-Elston (not shown) and variance component linkage methods were used and was even stronger in a subset of individuals with a family history of hypertension. Sex-specific analyses indicated a significant influence of variation in the ACE gene region on interindividual variability in all three measures of BP in males but not in females. For DBP, for example, the ACE gene region accounted for 38% of the interindividual BP variation in males, whereas this value was only 5% in females.

ACE plays a key role in the generation of the vasopressor angiotensin II and in the degradation of the vasodilator bradykinin. In addition, the efficacy of ACE inhibitors as therapeutic agents in the treatment of essential hypertension is well documented. Early reports of a strong genetic linkage between a marker located in the ACE gene region and salt-loaded DBP in stroke-prone spontaneously hypertensive rats first pointed to the ACE gene as an attractive candidate for the study of human essential hypertension. An I/D polymorphism located in intron 16 of the gene is associated with interindividual variance in plasma ACE activity. Conflicting results have been reported regarding an association between the ACE I/D polymorphism and either BP or essential hypertension. However, it is not known at this time whether the I/D polymorphism is functionally relevant or whether it is a marker in linkage disequilibrium with a nearby functional mutation.

The mechanism by which the effect of the ACE gene on BP is exerted in a sex-specific fashion remains to be more fully investigated. O’Donnell et al. have recently observed a similar significant relationship between ACE gene region variation and BP in males but not in females. In addition, O’Donnell et al. also report that this sex-specific relationship extends to the clinically defined hypertension end point. An association between plasma ACE activity and BP has been demonstrated in males but not in females in a large population-based study and in other groups of men. Interestingly, a similar sexual dimorphism was observed in a mouse model lacking a functional ACE gene sequence. BP was significantly lower than normal in male but not female mice heterozygous for the disrupted ACE gene. In addition, Kreutz et al. suggested that an interaction between a putative locus located on the Y chromosome and a locus mapping very near the ACE gene may partially explain differences in salt-loaded BP levels observed among spontaneously hypertensive rats.

A previous report by Jeunemaitre et al. demonstrated no evidence of linkage between variation at the ACE locus and a locus contributing to essential hypertension in a sample of white patients from Utah. Failure to detect an effect of a particular gene on BP in a moderately sized, clinic-based sample does not preclude an effect of this gene in other groups of individuals in the population. The possibility must also be considered that age modulates the influence of genetic variation in the ACE gene region on interindividual variation in BP levels. As a result, the contribution of this region to BP variability may not be the same in adults and children. The study of Tiret et al., which reported a significant association between plasma ACE activity and BP levels in the offspring but not the parents of 98 healthy, nuclear families of white origin, is consistent with such a hypothesis. In preliminary analyses (data not shown) of the relationship between the ACE I/D polymorphism, plasma ACE activity, and BP in a subset of the pedigrees analyzed here, we detected a significant relationship between plasma ACE activity and BP. Second, there was also a significant relationship between the ACE I/D polymorphism and SBP, but this relationship was found to be complex. In particular, we detected significant interactions between the ACE I/D polymorphism and body size as they combined to influence BP levels, and these effects were sex dependent.

Although the ACE gene is the prime candidate for the locus responsible for the effect on BP variability in our sample, the present study cannot distinguish between a contribution of the ACE gene itself and that of other genes closely linked to the measured polymorphic marker. At least two other genes with possible influences on BP are located in the region of the ACE gene on chromosome 17: the phenylethanolamine-N-methyltransferase and human growth hormone genes. Phenylethanolamine-N-methyltransferase is an enzyme involved in the biosynthesis of catecholamines in the brain. Phenylethanolamine-N-methyltransferase activity and epinephrine levels have been found to be significantly elevated in various hypertensive animal models. Human growth hormone has major effects on body size and body composition by promoting linear growth, increasing lean body mass, and decreasing body fat. The relationship between BP and body size is well known. Effects of growth hormone associated with increased sodium retention and plasma volume expansion have also been well documented. In addition, administration of low-dose recombinant human growth hormone to growth hormone–deficient subjects produces many of the features of syndrome X, including elevated BP. Linkage disequilibrium analysis using DNA sequence variants in and around these logical candidate genes is being undertaken to confirm
the results presented here and to identify the mutations responsible for the observed effect.

Acknowledgments

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References


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