Clinical Investigation and Reports

Evidence for Association and Genetic Linkage of the Angiotensin-Converting Enzyme Locus With Hypertension and Blood Pressure in Men but Not Women in the Framingham Heart Study

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Background—There is controversy regarding the association of the angiotensin-converting enzyme deletion-insertion (ACE D/I) polymorphism with systemic hypertension and with blood pressure. We investigated these relations in a large population-based sample of men and women by using association and linkage analyses.

Methods and Results—The study sample consisted of 3095 participants in the Framingham Heart Study. Blood pressure measurements were obtained at regular examinations. The ACE D/I polymorphism was identified by using a polymerase chain reaction assay. In logistic regression analysis, the adjusted odds ratios for hypertension among men for the DD and DI genotypes were 1.59 (95% confidence interval [CI], 1.13 to 2.23) and 1.18 (95% CI, 0.87 to 1.62), respectively, versus II (χ² P=.02). In women, adjusted odds ratios for the DD and DI genotypes were 1.00 (95% CI, 0.70 to 1.44) and 0.78 (95% CI, 0.56 to 1.09), respectively (P=.14). In linear regression analysis, there was an association of the ACE DD genotype with increased diastolic blood pressure in men (age-adjusted P=.03, multivariate-adjusted P=.14) but not women. Quantitative trait linkage analyses in 1044 pairs of siblings, by using both ACE D/I and a nearby microsatellite polymorphism of the human growth hormone gene, supported a role of the ACE locus in influencing blood pressure in men but not in women.

Conclusions—In our large, population-based sample, there is evidence for association and genetic linkage of the ACE locus with hypertension and with diastolic blood pressure in men but not women. Our data support the hypothesis that ACE, or a nearby gene, is a sex-specific candidate gene for hypertension. Confirmatory studies in other large population-based samples are warranted. (Circulation. 1998;97:1766-1772.)

Key Words: angiotensin ■ trials ■ genetics ■ genes ■ hormones ■ hypertension ■ blood pressure

Hypertension is a major risk factor for cardiovascular disease in adults and is present in approximately two thirds of all persons over age 65 years. Whereas aging, obesity, and environmental factors such as alcohol consumption contribute to the onset of hypertension, genetic factors also determine a substantial proportion of the variance of blood pressure in the general population. The deletion/insertion (D/I) polymorphism in intron 16 of the angiotensin-converting enzyme (ACE) gene accounts for approximately half the variance in ACE plasma levels, and ACE has been postulated as a candidate gene for blood pressure regulation. However, existing data from case-control studies of the association of ACE D/I and blood pressure are conflicting. and prior sib-pair linkage studies have failed to demonstrate genetic linkage between the ACE locus and hypertension. There also is uncertainty regarding the hypothesis raised by animal data that the effects of ACE D/I on ACE levels and blood pressure may be present in males but not females. It is possible that many prior studies have had inadequate power to detect the modest contribution that might be expected from an individual genetic factor to complex traits such as blood pressure. In addition, discordant results among different studies may arise from differences in either the genetic makeup or the environmental exposure status of different populations.

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The Framingham Heart Study is a large, prospective, population-based study containing >2000 extended families ranging in size from 2 to 25 members, so it is possible to test hypotheses regarding candidate gene loci for hypertension by using both association analyses and pedigree-based linkage analyses. We therefore studied the association of ACE D/I with blood pressure level and with hypertension status in men and women in the Framingham Heart Study.

Methods

Study Sample
The selection criteria and study design of the Framingham Heart Study have been detailed previously. Starting in 1948, 5209 subjects between ages 28 and 62 years were enrolled in the original cohort study, and starting in 1971, 5124 cohort offspring and their spouses were enrolled. Participants were invited to attend regular cycles of follow-up examinations (every 2 years for the cohort study, every 4 years for the offspring study). Blood samples for DNA were collected during examination cycles in the period from June 1987 to February 1991. For the current study, the index examination was defined as the examination at which DNA was collected. Of 5545 subjects who attended an examination during the period of DNA collection, 2450 were excluded for the following reasons: DNA was not collected during examination cycles in the period from June 1987 to February 1991. For the current study, the index examination was defined as the examination at which DNA was collected. Of 5545 subjects who attended an examination during the period of DNA collection, 2450 were excluded for the following reasons: DNA samples not collected (n=505), not accessible for genotyping (n=1848), not able to be adequately genotyped (n=17), severe heart failure (n=47), or severe aortic stenosis (n=33). We excluded persons with these clinical conditions because they are associated with low blood pressure. After these exclusions, 3095 subjects (1445 men and 1650 women) with genotyping of the ACE D/I polymorphism were eligible for the present study.

Measurements
Information regarding blood pressure and other clinical characteristics was obtained at study entry (baseline) and at each follow-up examination, including the index examination. Systolic and diastolic blood pressure values were the means of two physician-obtained measurements recorded 5 minutes apart) determined by the first and fifth Korotkoff phases, respectively, in the left arm of the seated subject with a mercury column sphygmomanometer. The pulse pressure was the difference between the systolic and diastolic blood pressures. Body height and weight were used to calculate body mass index (weight in kilograms divided by height in meters squared). Data were collected on clinical variables including age, cigarette smoking, alcohol consumption, blood glucose concentration and the presence of diabetes, ischemic heart disease, and antihypertensive drug therapy.

Definitions of Hypertension
Hypertension was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg or current use of antihypertensive medication. In a secondary analysis, moderate to severe hypertension was defined as systolic blood pressure of ≥160 mm Hg or diastolic blood pressure of ≥100 mm Hg or use of two or more antihypertensive medications.

Determination of the ACE Genotype
The methods of DNA extraction, amplification, and determination of the ACE genotype have been described previously. DNA was extracted from blood samples according to standard protocols. Briefly, 5 µL (~5 to 20 ng) of genomic DNA was covered with oil, denatured at 95°C for 3 minutes, and cooled to 80°C before the addition of 10 µL of polymerase chain reaction (PCR) master mix containing 0.15 U of Taq DNA polymerase. The primers used, the thermocycling protocol, the approach to electrophoresis, the method for retesting of DD homozygotes and replicate scoring, and the procedures for quality control have been described previously.

Determinations of the Genotypes for the Human Growth Hormone (hGH) Gene Polymorphism
The highly polymorphic microsatellite associated with the hGH gene was also selected for study because of its close proximity to the ACE gene and the resultant low rate of recombination. The method of determination of the hGH microsatellite genotype has been described previously. The hGH genotype was determined in 1039 pairs of siblings. Genomic DNA was amplified during a 35-cycle, two-step PCR protocol (95°C for 15 seconds and 72°C for 2 minutes), with a reaction mix that differed from the one used for the determination of ACE genotype in the concentration of magnesium chloride (2.5 mmol/L), deoxynucleoside triphosphates (200 µL each of adenosine triphosphate, cytidine triphosphate, thymidine triphosphate, and guanosine triphosphate), and primers (100 mmol/L). One of the two primers (sense, 5’ACTGACATTCCAGCTGGGAGA GAG3’; reverse, 5’AGAGCCGGTGGTGTCTACT3’) was labeled at the 5’ end with [32P]y-ATP. Reaction products were resolved over sequencing gels containing 6% polyacrylamide, 8 mol/L urea, and 30% formamide and visualized by autoradiography. Parallel sequencing ladders were used for size standardization. Scoring was carried out as previously described.

Statistical Methods and Association Analyses
Evidence for familial aggregation of quantitative blood pressure measures was demonstrated for diastolic blood pressure, systolic blood pressure, and pulse pressure by calculating intraclass correlations in pairs of siblings, consistent with previously published data from Framingham showing familial aggregation of hypertension. Next, we studied correlations among the quantitative blood pressure measures. There was a high correlation between systolic blood pressure and diastolic blood pressure (r=0.83) and between systolic blood pressure and pulse pressure (r=0.83), but there was a low correlation between diastolic blood pressure and pulse pressure (r=0.09). Therefore, our primary quantitative variables were specified to be diastolic blood pressure and pulse pressure, although analyses were also conducted by using systolic blood pressure.

Because the prevalence of hypertension increases with age, all analyses were adjusted for age. The age-adjusted analyses were performed separately for men and women. The prevalence of hypertension was compared among the three ACE genotypes (DD, DI, and II). With multivariable unconditional logistic regression, analyses were performed to compare the prevalence of hypertension among the three ACE genotypes and the prevalence of moderate to severe hypertension among the three ACE genotypes (reference group, II genotype). Analyses were performed without and with adjustment for other covariates (body mass index, diabetes mellitus, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease). A χ² test statistic was calculated to compare for differences among the three genotypes. With multiple linear regression, the mean values of diastolic blood pressure and pulse pressure were compared among subjects with the DD, DI, and II genotypes. Linear regression analyses were performed without and with other covariates (use of antihypertensive treatment, body mass index, diabetes mellitus, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease). Because family members are more likely to share identical alleles than randomly selected subjects, we repeated the analyses by using generalized estimating equations to account for the possible nonindependence of blood pressure observations. For the association of ACE DI/II with dichotomous (hypertension) and continuous (blood pressure) measures, secondary analyses were carried out to test for dominant, recessive, and additive modes of inheritance.

The SAS System (Release 11, SAS Institute Inc, Cary, NC) was used to perform all statistical analysis with the procedures REG and LOGISTIC. All statistical tests were two sided, and a value of P<.05 was considered statistically significant.

Linkage Analyses
Linkage analysis was selected to investigate the linkage of the ACE DI/II polymorphism and blood pressure was performed on 484 families with at
least two siblings (329, 107, 33, 9, 5 families, and 1 family with 2, 3, 4, 5, 6, and 7 siblings, respectively). These families contained a total of 1044 sibling pairs available for analysis. Linkage analysis was performed on 1039 sibling pairs who also had hGh genotyping. Quantitative trait sib-pair analyses of the ACE and hGh genotypes were performed separately for each of the continuous measures, diastolic blood pressure and pulse pressure, and for the deviance residuals of the dichotomous hypertension variable. The SAGE SIBPAL programs were used to perform all linkage analyses.

Linkage was evaluated by the regression of the squared sib-pair difference on the estimated proportion of alleles shared by the sib-pair at the ACE or hGh locus. We estimated the regression coefficient, \( \beta_i \), as follows: \( \beta_i = -2\theta_i \left(2\sigma^2 - r^2\right) \), where \( \theta_i \) is the recombination frequency between the marker locus and the trait, and \( \sigma^2 \) is the genetic variance of the trait. \( \beta_i \) is 0 if \( \theta_i \) equals 0.5 (no linkage) or \( \theta_i \) equals 0 (no genetic variance), and \( \beta_i \) will be negative if \( \theta_i \) is <0.5 and \( \sigma^2 \) is >0. Sex-specific linkage analyses were also performed. The regression models for continuous blood pressure measures were adjusted for age, body mass index, diabetes mellitus, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease. The continuous variables were modeled as linear terms. For dichotomous variables, the logistic regression models were used. The models were adjusted for age, body mass index, diabetes mellitus, and sex. The results were consistent with an association of hypertension with the ACE genotype. The overall frequencies of the genotypes DD, DI, and II were 55.3 and 44.7, respectively, in the entire sample. The observed genotype frequencies are in agreement with Hardy-Weinberg equilibrium. In further analyses restricted to one member per nuclear family, genotype frequencies did not differ from those in the overall group.

### Odds of Hypertension According to ACE Genotype

The prevalence of hypertension according to ACE genotypes at the time of DNA collection was evaluated in sex-stratified logistic regression analyses (Table 2). There were 689 men and 705 women with hypertension. In men, the age-adjusted odds ratio (OR) of hypertension in the DD and DI groups were 1.67 (95% confidence interval [CI], 1.21 to 2.31) and 1.19 (95% CI, 0.88 to 1.61), respectively (Table 2 and Fig 1), with the II genotype used as the reference group (\( \chi^2 = 0.004 \)). After adjustment for other covariates (body mass index, diabetes mellitus, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease), the ORs for hypertension were 1.59 (95% CI, 1.13 to 2.23) and 1.18 (95% CI, 0.87 to 1.62) for the DD and DI groups, respectively (\( P = 0.02 \)). Secondary testing in men favored an additive (\( P = 0.006 \)) or a recessive (\( P = 0.009 \)) mode of inheritance. In women, there was no relation of ACE genotype with hypertension in either the unadjusted or adjusted models (Table 2 and Fig 2). The multivariable adjusted ORs were 1.00 (95% CI, 0.70 to 1.44) and 0.78 (95% CI, 0.56 to 1.09) for the DD and DI groups, respectively (\( P = 0.14 \)). The results of adjusted models with generalized estimating equations were not materially different (Table 2).

There were 276 men and 333 women with moderate to severe hypertension. In the secondary analysis of moderate to severe hypertension, the direction and magnitude of effect were consistent with an association of hypertension with ACE genotype in men but not women (see Figs 1 and 2). For men, the adjusted ORs of DD and DI for moderate to severe hypertension were 1.43 (95% CI, 0.94 to 2.17) and 1.25 (95% CI, 0.85 to 1.85), but differences among genotypes were not statistically significant (\( P = .24 \)). For women, the corresponding adjusted ORs for DD and DI were 1.06 (95% CI, 0.69 to 1.61) and 1.04 (95% CI, 0.71 to 1.52), respectively (\( P = .97 \)).

### Measured Blood Pressure According to ACE Genotype

In Table 3, blood pressure measures at the time of DNA collection are compared in unadjusted and multivariable models (adjusting for age, body mass index, diabetes mellit-
associates, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease) across the ACE genotypes. In men, there was a consistent and statistically significant increase in age-adjusted diastolic blood pressure with increasing number of D alleles: mean diastolic blood pressure was 81.6 (±0.5), 80.9 (±0.4), and 79.6 (±0.6) mm Hg for DD, DI, and II genotypes, respectively (P = .03). The association was no longer statistically significant after adjustment for use of antihypertensive treatment or other covariates (Table 3). The results of adjusted models with generalized estimating equations, adjusted for age, body mass index, diabetes, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease.

TABLE 2. Odds of the Presence of Hypertension in Men and Women by ACE Genotype

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>DD</th>
<th>DI</th>
<th>II</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=1445)</td>
<td>437</td>
<td>719</td>
<td>288</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>HTN, %</td>
<td>53.1</td>
<td>45.8</td>
<td>44.4</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1.67 (1.21–2.31)</td>
<td>1.19 (0.88–1.61)</td>
<td>1.00</td>
<td>.004</td>
<td>.02</td>
</tr>
<tr>
<td>Fully adjusted OR*</td>
<td>1.59 (1.13–2.23)</td>
<td>1.18 (0.87–1.62)</td>
<td>1.00</td>
<td>.02</td>
<td>.02</td>
</tr>
<tr>
<td>GEE†</td>
<td>1.62 (1.15–2.29)</td>
<td>1.20 (0.88–1.62)</td>
<td>1.00</td>
<td>.02</td>
<td>.02</td>
</tr>
<tr>
<td>Women (n=1650)</td>
<td>492</td>
<td>845</td>
<td>313</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>HTN, %</td>
<td>43.3</td>
<td>41.8</td>
<td>44.4</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1.01 (0.72–1.41)</td>
<td>0.80 (0.59–1.09)</td>
<td>1.00</td>
<td>.15</td>
<td>.14</td>
</tr>
<tr>
<td>Fully adjusted OR*</td>
<td>1.00 (0.70–1.44)</td>
<td>0.78 (0.56–1.09)</td>
<td>1.00</td>
<td>.14</td>
<td>.17</td>
</tr>
<tr>
<td>GEE†</td>
<td>1.04 (0.72–1.50)</td>
<td>0.82 (0.59–1.14)</td>
<td>1.00</td>
<td>.17</td>
<td>.17</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; HTN, hypertension; and OR, odds ratio. 95% Confidence limits are shown in parentheses.

*Logistic regression, adjusted for age, body mass index, diabetes, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease.

†Robust generalized estimating equations, adjusted for age, body mass index, diabetes, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease.

Pressure had a negligible effect on the calculated probability values.

Linkage Analyses

Quantitative trait sib-pair linkage analyses for the ACE and hGH genotypes were performed with blood pressure data at the time of DNA collection with adjustment for covariates (age, body mass index, diabetes mellitus, cigarette smoking, alcohol consumption, presence of ischemic heart disease, and use of antihypertensive treatment). These analyses provide support for linkage of ACE D/I with diastolic blood pressure. In SIBPAL linkage analyses restricted to male siblings only (n=271 male sibling pairs), there was evidence of linkage with diastolic blood pressure in men for ACE (\( b_1 = -1.34, P = .02 \)) and hGH (\( b_1 = -0.75, P = .04 \)). There was no evidence of linkage with pulse pressure for either ACE or hGH. In analyses of siblings restricted to women only (n=274 female sibling pairs), there was no evidence of linkage between measures of blood pressure (diastolic or pulse pressure) and either ACE or hGH. In further sex-pooled linkage analyses with diastolic blood pressure (n=1044 total

![Figure 1](image1.png)

**Figure 1.** Odds ratios for hypertension according to ACE genotype in men.

![Figure 2](image2.png)

**Figure 2.** Odds ratios for hypertension according to ACE genotype in women.
TABLE 3. Diastolic Blood Pressure, Systolic Blood Pressure, and Pulse Pressure in Men and Women by ACE Genotype and Sex

<table>
<thead>
<tr>
<th>Blood Pressure Status</th>
<th>ACE Genotype</th>
<th></th>
<th></th>
<th></th>
<th>(x^2) P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD</td>
<td>DI</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (n=1445)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean diastolic BP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>81.6 (0.5)</td>
<td>80.9 (0.4)</td>
<td>79.6 (0.6)</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>Fully adjusted*</td>
<td>81.5 (0.5)</td>
<td>81.0 (0.4)</td>
<td>80.1 (0.6)</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td>GEE†</td>
<td>81.4 (0.5)</td>
<td>80.9 (0.4)</td>
<td>80.0 (0.6)</td>
<td>.14</td>
<td></td>
</tr>
<tr>
<td>Mean systolic BP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>133.9 (0.8)</td>
<td>133.1 (0.6)</td>
<td>131.0 (1.0)</td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td>Fully adjusted*</td>
<td>133.7 (0.8)</td>
<td>133.0 (0.6)</td>
<td>131.7 (1.0)</td>
<td>.32</td>
<td></td>
</tr>
<tr>
<td>GEE†</td>
<td>133.7 (0.8)</td>
<td>133.2 (0.6)</td>
<td>131.9 (0.9)</td>
<td>.32</td>
<td></td>
</tr>
<tr>
<td>Mean PP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>52.3 (0.6)</td>
<td>52.1 (0.5)</td>
<td>51.4 (0.7)</td>
<td>.64</td>
<td></td>
</tr>
<tr>
<td>Fully adjusted*</td>
<td>52.2 (0.6)</td>
<td>52.0 (0.5)</td>
<td>51.7 (0.7)</td>
<td>.86</td>
<td></td>
</tr>
<tr>
<td>GEE†</td>
<td>52.3 (0.6)</td>
<td>52.3 (0.6)</td>
<td>51.9 (0.7)</td>
<td>.90</td>
<td></td>
</tr>
<tr>
<td>Women (n=1650)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean diastolic BP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>76.4 (0.4)</td>
<td>76.0 (0.3)</td>
<td>76.1 (0.5)</td>
<td>.81</td>
<td></td>
</tr>
<tr>
<td>Fully adjusted*</td>
<td>76.6 (0.4)</td>
<td>76.1 (0.3)</td>
<td>76.3 (0.5)</td>
<td>.57</td>
<td></td>
</tr>
<tr>
<td>GEE†</td>
<td>76.6 (0.4)</td>
<td>76.1 (0.3)</td>
<td>76.4 (0.5)</td>
<td>.55</td>
<td></td>
</tr>
<tr>
<td>Mean systolic BP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>130.9 (0.8)</td>
<td>130.3 (0.6)</td>
<td>130.8 (1.1)</td>
<td>.81</td>
<td></td>
</tr>
<tr>
<td>Fully adjusted*</td>
<td>131.1 (0.8)</td>
<td>130.1 (0.6)</td>
<td>130.4 (1.0)</td>
<td>.62</td>
<td></td>
</tr>
<tr>
<td>GEE†</td>
<td>131.5 (0.8)</td>
<td>130.5 (0.6)</td>
<td>131.0 (1.0)</td>
<td>.61</td>
<td></td>
</tr>
<tr>
<td>Mean PP, mm Hg</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>54.5 (0.6)</td>
<td>54.3 (0.5)</td>
<td>54.7 (0.8)</td>
<td>.89</td>
<td></td>
</tr>
<tr>
<td>Fully adjusted*</td>
<td>54.4 (0.6)</td>
<td>54.0 (0.5)</td>
<td>54.1 (0.8)</td>
<td>.86</td>
<td></td>
</tr>
<tr>
<td>GEE†</td>
<td>54.8 (0.6)</td>
<td>54.4 (0.5)</td>
<td>54.7 (0.7)</td>
<td>.87</td>
<td></td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; BP, blood pressure; PP, pulse pressure; and GEE, generalized estimating equation.

Standard errors are shown in parentheses.

*Multiple linear regression, adjusted for age, use of antihypertensive therapy, body mass index, diabetes, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease.

†Robust generalized estimating equations, adjusted for age, use of antihypertensive therapy, body mass index, diabetes, cigarette smoking, alcohol consumption, and the presence of ischemic heart disease.

Discussion

In our large, population-based sample, the frequencies of the D and I alleles of ACE were similar to those reported in other Caucasian populations. We found consistent evidence in men but not in women of association and genetic linkage of the ACE genotype with diastolic blood pressure and of association with hypertension. There was a statistically significant increase in odds of hypertension with the ACE DD genotype, although there was no clear evidence for the precise mode of inheritance.

Hypertension is a complex trait, and among the many potential candidate genes that may mediate its expression are ACE as well as angiotensinogen and other factors related to the renin-angiotensin system. Although there is strong evidence from association and linkage studies that the ACE D allele accounts for almost half the variance in ACE plasma levels,29–32 there is controversy regarding the association of the ACE locus with blood pressure and hypertension. In humans, a positive association of the ACE D allele has been observed in some4–7 but not other8–13 case-control studies of hypertension. Among the negative case-control studies was a subgroup within the Family Heart Study consisting of 118 subjects with moderate to severe hypertension selected from the Framingham Heart Study.13 In our larger unsel ected sample, which included 276 men and 333 women with moderate to severe hypertension, we observed a possible but not statistically significant association of ACE D/I with moderate to severe hypertension in men but not women. Negative associations between ACE and blood pressure have also been reported in a number of case-control studies of persons with clinically evident coronary atherosclerosis or myocardial infarction.33–41 Although our study sample contained a small number of subjects with clinical evidence of ischemic heart disease, the odds of hypertension and of moderate-to-severe hypertension remained elevated after adjustment for potential confounding factors including the presence of ischemic heart disease.

The case-control design used in many candidate gene association studies is efficient for examination of disease hypotheses, but this methodology may be susceptible to selection bias if there is nonrandom ascertainment and selection of cases or control subjects. We have included all persons within a range of normal and abnormal blood pressures in our study cohort, and we have examined both subjects with and without evidence of ischemic heart disease. In contrast, it is difficult to estimate in case-control studies the extent to which the case mix has excluded individuals who succumbed to hypertension-related morbidity (eg, stroke or renal failure) or mortality. In the Framingham Heart Study, the high prevalence of hypertension (45%) offers the opportunity to perform robust comparisons of cases and nonhypertensive control subjects in a large sample. In addition, because our sample is drawn from a population-based cohort, there may be less susceptibility to the types of bias that are inherent in case-control studies.

There are a limited number of sib-pair linkage studies relating the ACE gene with blood pressure or hypertension. Using the highly informative hGH microsatellite, there was no evidence of linkage between the ACE locus and hypertension in a population-based sample (n=169 sib-pairs)35 selected from Utah residents under age 60 years with a reported family history of early hypertension.30 Because all subjects were taking antihypertensive medication, linkage with blood pressure was not examined; furthermore, there were no reported sex differences.14 In contrast, in the 1044 sib-pairs from our study sample, we find consistent evidence of linkage...
between hGH (as well as ACE) and diastolic blood pressure. A recent study of 1488 siblings from a population-based cohort in Minnesota has also found similar evidence for linkage of the ACE gene region to interindividual variation in diastolic blood pressure as well as mean arterial pressure.\textsuperscript{42} Cases for the Utah cohort included subjects with moderate to severe hypertension,\textsuperscript{30} and it is possible that enrichment for this more severely hypertensive subgroup or inadvertent bias in selection of cases in the Utah cohort explain, in part, the absence of linkage in that sample. Population differences in genetic makeup or confounding environmental exposures are also plausible explanations.

Our data demonstrate that the ACE DD genotype is associated with increased risk for hypertension, and we provide supportive evidence that ACE is a sex-specific hypertension candidate gene. We emphasize that the existence of association and/or linkage between ACE and hypertension in a population-based sample is necessary but not sufficient evidence for a causal link between the ACE gene and hypertension. It is possible that hGH or other genes in linkage disequilibrium with ACE are responsible for the observed effects on blood pressure and hypertension. In particular, this possibility is raised by the evidence for linkage of hGH with diastolic blood pressure.

Our study provides evidence that the effect of the ACE locus may be male specific. The hypothesis that there are sex differences in the effect of ACE D/I on blood pressure is supported by gene-targeting experiments resulting in functional inactivation of the ACE gene in mice, in which the blood pressure effect predominates in males.\textsuperscript{15,16} In humans, there is limited evidence of the existence of a male-specific association between ACE levels and blood pressure.\textsuperscript{43} Although no male-specific effect was noted in previous studies reporting an association between the ACE genotype and blood pressure,\textsuperscript{2–7} Fornage et al.\textsuperscript{44} have recently reported that genetic variation in the region of the ACE gene significantly influences interindividual variation in blood pressure in men but not women. The mechanism of this apparent sex specificity is uncertain. We found no evidence of a relation to menopausal status or use of estrogen replacement therapy. Also, in a previous study with direct measurements with M-mode echocardiography, we found no evidence of either association or linkage between ACE genotype and left ventricular hypertrophy in either men or women.\textsuperscript{20}

Our data must be interpreted with caution regarding any potential clinical implications. In our population, the prevalence of the DD genotype is \(\sim 30\%\) in men, in whom there was a 59\% increased risk of hypertension after adjustment for common hypertension risk factors. Our study offers no convincing evidence for an incremental value of screening for ACE genotype compared with blood pressure screening. Because stroke, myocardial infarction, and renal failure are among the most serious sequelae of hypertension, our findings call for further investigation in large populations to clarify the uncertainty regarding the possible association of ACE with these cardiovascular diseases.

There are also other potential limitations to our study. It remains possible that we have not sufficiently controlled for confounding factors that might explain at least some of the residual associations noted in multivariate analyses. Adjustment was not made for dietary factors (salts, electrolytes) and personal habits such as exercise, which may influence blood pressure. It is possible that regression-dilution bias exists due to the use of a mean of only two readings to characterize blood pressure and hypertensive status.\textsuperscript{49} Furthermore, the categorization of hypertension status based on use of antihypertensive drugs may result in misclassification. However, both types of bias would be expected to reduce the magnitude of effect of the observed associations. It should also be noted that our study sample is largely Caucasian. There may be racial differences in ACE D/I allele frequencies, and in particular there may be little or no variation among black persons in the relation of ACE D/I to ACE levels.\textsuperscript{45} Thus caution should be exercised in extrapolating our findings to non-Caucasian populations. Although our study subjects were taken from a general population, we recognize that the presence or absence of an observed association in any ethnic, racial, or geographic population may be related to a number of other factors including gene-gene interactions and gene-environment interactions.

In conclusion, in our large, population-based sample, there is an association of the ACE locus with hypertension and with blood pressure level in men but not women, and these associations are further supported by evidence of linkage. Our data support the hypothesis that ACE, or a nearby gene, is a sex-specific candidate gene for hypertension. The discrepancy between our population-based data and those of smaller case-control studies supports the need for further confirmatory studies of association and linkage in large population-based samples.

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Evidence for Association and Genetic Linkage of the Angiotensin-Converting Enzyme Locus With Hypertension and Blood Pressure in Men but Not Women in the Framingham Heart Study

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