The Angiotensin I Converting Enzyme Gene and Predisposition to High Blood Pressure


Phenotypic abnormalities of the renin-angiotensin system have been associated with the predisposition to high blood pressure. The angiotensin I converting enzyme (ACE) gene has been implicated as a candidate gene. We examined the distribution of common alleles of the ACE gene and measured circulating components of the renin-angiotensin system and urinary sodium excretion in 170 young Caucasian adults with contrasting genetic predisposition to high blood pressure. Predisposition was defined on the basis of personal and parental blood pressure levels by using the four corners sampling method. Young adults with greatest predisposition who had high blood pressure and two parents with high blood pressure did not show any significant difference in the distribution of the markers of the ACE gene, either as genotype or allele frequencies, when compared with young adults with least predisposition who had low blood pressure and two parents with low blood pressure. Offspring with urinary sodium excretion above the median (143.4 mmol per day) also showed no significant differences in the distribution of ACE alleles or genotype between groups. Different genotypes were associated with different average serum ACE concentrations (p < 0.0001), but plasma angiotensin II and aldosterone showed no significant variation with ACE genotype. These results suggest that in a group of Caucasians selected from the general population, the ACE gene is not associated with genetic predisposition to high blood pressure. In this population common ACE gene allelic markers would not be useful indexes of susceptibility to hypertension. (Hypertension 1993;21:455-460)

KEY WORDS • blood pressure • angiotensin converting enzyme • genetics • family characteristics

ACE (kininase II, EC 3.4.15.1) is widely distributed. It converts angiotensin I to the vasoconstricting and aldosterone-stimulating peptide Ang II and inactivates bradykinin. Both effects might be expected to elevate blood pressure. In clinical practice, drugs that inhibit ACE are used to lower blood pressure in patients with essential hypertension. The insertion/deletion ACE gene polymorphism has been shown to influence the serum concentration of ACE and may affect blood pressure through changes in Ang II, aldosterone, or other vasoactive substances.

Support for the role of the ACE gene as a determinant of blood pressure levels also comes from animal studies. In cross-breeding studies of the stroke-prone spontaneously hypertensive rat, genetic markers of the ACE gene and closely related markers on chromosome 10 are linked to the inheritance of high blood pressure in genetically segregating F₂ rats. These studies also indicate that the phenotypic expression of the hypertensive locus linked to the ACE gene could be augmented by a high sodium diet. Other studies of young spontaneously hypertensive rats show that brief treatment with ACE inhibitors has an antihypertensive effect that persists long after treatment is stopped.

To assess the role of the ACE gene in the determination of blood pressure in humans, we examined the association between structural variation of the ACE gene and the genetic predisposition to high blood pressure. Here we report a comparison of ACE gene polymorphisms in healthy young Caucasian adults with genetic predisposition to either high or low blood pressure.
pressure. In addition, we report the relation between common ACE gene alleles, urinary sodium excretion, and levels of circulating components of the renin-angiotensin system.

**Methods**

**Ascertainment and Sampling Design**

In this study, the four corners approach was used to select from the general population young adults with contrasting genetic predisposition to high blood pressure. All participants were Caucasian and drawn from an area served by two group general practices based at the Ladywell Medical Centre in the west of Edinburgh, Scotland. Two hundred and one young adults attended the medical center for measurements of blood pressure, weight, and height. After 10 minutes of recumbent rest, blood pressure was measured twice with the Hawksley random zero sphygmomanometer by nurses who were unaware to which offspring group individuals belonged. Subjects were allowed to rest supine for 25 minutes with a butterfly needle in situ before blood was taken for estimations of angiotensinogen, plasma renin activity, ACE, Ang II, aldosterone. Additional blood was taken for DNA studies. Subjects were provided with a container and detailed instructions to collect a 24-hour urine sample. Informed consent was obtained from all participants, and all procedures were carried out according to the guidelines of the ethics committees of the Ladywell Medical Centre and Western Infirmary.

**Determination of Angiotensin I Converting Enzyme Gene Marker Alleles**

Lymphoblastoid cell lines were established, and high molecular weight DNA was isolated from peripheral blood leukocytes by routine methods. DNA amplification by polymerase chain reaction using primers flanking the polymorphic region was used to demonstrate the 250-base pair insertion/deletion polymorphism of the ACE gene. DNA analysis was successful in 170 of the 201 offspring.

**Statistical Analyses**

Data are expressed as mean with the 95% confidence interval for the mean except for hormonal variables where the medians and the interquartile ranges are given. Differences in the distribution of ACE genotypes and alleles were analyzed using $\chi^2$ tests. Differences between the groups were analyzed by parametric one-way analysis of variance or, in the case of hormonal variables, by the Kruskal-Wallis nonparametric one-way analysis of variance.

**Results**

Clinical details of the 170 offspring and their parents are summarized in Table 1. These characteristics are similar in all respects to those reported previously for the entire group of 201 individuals. Each group is of similar average age, but there are relatively large differences in personal and parental blood pressures as a result of the selection process. The difference in average offspring blood pressure between the groups at greatest and least genetic predisposition to high blood pressure was 12/6 mm Hg, and the difference between their

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**FIGURE 1.** Schematic representation of a scatter diagram with combined parental blood pressure scores on one axis and offspring blood pressure scores on the other. Elliptical distribution reflects the familial aggregation of blood pressure. The corners contain individuals with different combinations of low and high parental and personal blood pressure scores. Among the four corners, offspring in the upper right hand corner have the greatest genetic predisposition to high blood pressure and those in the lower left hand corner have the least, being predisposed to low blood pressure.
TABLE 1. Characteristics of 170 Young Adults With Contrasting Predisposition to High Blood Pressure

<table>
<thead>
<tr>
<th>Offspring BP</th>
<th>Low parental BP</th>
<th>High parental BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental systolic BP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental diastolic BP (mm Hg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Offspring were selected into four groups on the basis of low or high combined parental blood pressure (BP) and low or high personal levels of BP scores. Data are mean with 95% confidence intervals of the mean in parentheses.

TABLE 2. Distribution of Angiotensin I Converting Enzyme Gene Alleles in Offspring With Contrasting Predisposition to High Blood Pressure

<table>
<thead>
<tr>
<th>Offspring BP</th>
<th>Low parental BP</th>
<th>High parental BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>DD</td>
<td>11 (25.0)</td>
<td>18 (42.9)</td>
</tr>
<tr>
<td>DI</td>
<td>22 (50.0)</td>
<td>16 (38.1)</td>
</tr>
<tr>
<td>II</td>
<td>11 (25.0)</td>
<td>8 (19.0)</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>44 (50.0)</td>
<td>52 (61.9)</td>
</tr>
<tr>
<td>I</td>
<td>44 (50.0)</td>
<td>32 (38.1)</td>
</tr>
</tbody>
</table>

BP, blood pressure; D, deletion allele; I, insertion allele. Offspring were selected into four groups on the basis of low or high combined parental BP and low or high personal levels of BP scores. Percentage of column totals for genotypes and allele frequencies are given in parentheses. None of the differences were significant statistically.
### Table 3. Distribution of Angiotensin I Converting Enzyme Gene Alleles in Offspring With Contrasting Predisposition to High Blood Pressure on High Sodium Intake

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Offspring BP</th>
<th>Low parental BP</th>
<th>High parental BP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>DD</td>
<td>5 (31.3)</td>
<td>9 (45.0)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>DI</td>
<td>8 (50.0)</td>
<td>7 (35.0)</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>II</td>
<td>3 (18.7)</td>
<td>4 (20.0)</td>
<td>2 (14.3)</td>
</tr>
</tbody>
</table>

Alleles
- D: 18 (56.3), 25 (62.5), 17 (60.7), 23 (52.3)
- I: 14 (43.7), 15 (37.5), 11 (39.3), 21 (47.7)

BP, blood pressure; D, deletion allele; I, insertion allele. Offspring who had 24-hour urinary sodium excretion greater than the overall study group median of 143.4 mmol per day were selected into four groups on the basis of low or high combined parental BP and low or high personal levels of BP scores. Percentage of column totals for genotypes and allele frequencies are given in parentheses. None of the differences were significant statistically.

### Discussion

In the present study, we used a novel design to investigate a genetic hypothesis in informative subgroups from the general population. The careful selection of high and low risk groups and the measurement of genotype, intermediate phenotypes, and environmental variables such as sodium intake provides a unique framework in which to study genetic questions. The method complements other genetic techniques such as the sib-pair linkage analysis.

Genetic analysis of quantitative traits such as blood pressure presents special problems concerning the definition of "affected" and "unaffected" individuals. This difficulty weakens the power of multigenerational pedigree linkage studies in the analysis of quantitative traits. Genetic association studies offer another approach and involve comparison of hypertensive and normotensive individuals. These operational definitions are equivalent to the upper 10% and the lower 90% of the blood pressure distribution, respectively. If a number of genes have incremental effects on blood pressure, then there is likely to be a degree of overlap of hypertensive alleles between these two groups. The genetic contrast between high versus "the rest" is not likely to be as large as between high versus low.

The four corners approach samples from the top and bottom of the blood pressure distribution and also takes into account the family history. In our study, family history was based on the measured blood pressure of both parents, a strategy that is superior to that based on reported but unmeasured parental blood pressures or the measured pressure of only one parent.

The four corners method differs from hypertensive/normotensive genetic association studies in two other aspects. In the latter, ascertainment is usually based on clinical records rather than screening of the general population as used in the four corners method. Additionally, we used a broader definition of high blood pressure than usually accepted for clinical purposes. Studies based on the clinical definition of hypertension are more likely to include a certain proportion of nongenetic secondary forms of high blood pressure. This is less likely using a broader definition, which also has the advantage that it includes more individuals, thereby increasing statistical power and making the findings more relevant to the population as a whole.

Using the four corners approach, we have reported that the glucocorticoid receptor gene but not the atrial natriuretic peptide gene is associated with the genetic predisposition to high blood pressure. In the same

### Table 4. Biochemical Variables Related to the Renin-Angiotensin System in Offspring Grouped According to Angiotensin I Converting Enzyme Genotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>DD</th>
<th>DI</th>
<th>II</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity (mEq/L)</td>
<td>1,216 (22-36)</td>
<td>1,180 (27-37)</td>
<td>1,220 (19-41)</td>
<td>0.589</td>
</tr>
<tr>
<td>Angiotensinogen (ng/mL)</td>
<td>28 (1,012-1,504)</td>
<td>1,216 (1,008-1,576)</td>
<td>1,220 (1,077-1,592)</td>
<td>0.0001</td>
</tr>
<tr>
<td>ACE (µg/mL)</td>
<td>28</td>
<td>25</td>
<td>29</td>
<td>0.386</td>
</tr>
<tr>
<td>Angiotensin II (pg/mL)</td>
<td>365 (210-450)</td>
<td>*</td>
<td>330 (260-380)</td>
<td>(190-290)</td>
</tr>
<tr>
<td>Aldosterone (µg/100 mL)</td>
<td>10 (9.2-12.6)</td>
<td>7 (8.6-11.1)</td>
<td>10 (9.4-13.3)</td>
<td>0.179</td>
</tr>
</tbody>
</table>

ACE, angiotensin I converting enzyme; D, deletion allele; I, insertion allele of the ACE gene. Data are medians with interquartile ranges in parentheses. Probability values refer to analyses of differences between the groups by the Kruskal-Wallis test. Differences in serum ACE between individual genotype groups were significant.

*p<0.01 by Mann-Whitney U test between adjacent groups.
population we have shown abnormalities of the phenotypic expression of the renin-angiotensin system in young adults with genetic predisposition to high blood pressure.\(^1,2\) The present study suggests that one candidate, the ACE gene, does not appear to play an important role in this process. We could find no association between ACE genotypes and genetic predisposition to high blood pressure nor any evidence that the ACE gene affects plasma angiotensin concentrations. It seems that in our population, the ACE genetic markers would not be useful indexes of susceptibility to hypertension. We cannot generalize our findings to other geographical or racial groups, but our results concur with the results of a recent sib-pair linkage analysis of the ACE gene and hypertension.\(^1,5\) Our observations do not exclude the possibility that the ACE gene might account for high blood pressure in a subset of hypertensive families.

We used the genetic marker that differentiated a 250-base pair insertion/deletion polymorphism located within an intron of the ACE gene.\(^8\) In the context of our population association study, linkage disequilibrium will be detected when a polymorphic genetic marker, in this case within the ACE gene, and the high blood pressure susceptibility locus are situated close on the chromosome (i.e., at a recombination frequency of 0.5% or less).\(^17\) However, it is possible that important mutations related to the ACE gene might be revealed only by a comprehensive genetic haplotype analysis of this region of the chromosome.

Negative results in this study raise concerns regarding statistical power. Because the number of potential variables and the complexity of their interaction are unknown, relevant power calculations based on arbitrary mathematical models are virtually impossible. Nevertheless, if the 10% difference in the ACE gene allele frequencies between offspring with the greatest contrast in predisposition is real, we should require about 180 individuals in each group (at \(p<0.05\) and power of 80%).\(^18\) The allele frequency difference between offspring at contrasting risk on high sodium intake was only 4% and would require 700 subjects per group to attain the same statistical power. However, such small differences in the relative frequency of the ACE gene alleles would be of limited practical usefulness. Our study numbers provide sufficient power to detect differences in allele frequency of 20% or greater between the groups at highest and lowest risk.\(^18\) Differences of this magnitude might be expected to be of practical value in the assessment of predisposition to high blood pressure.

Our findings contrast with those in experimental genetic hypertension, where it has been reported that the ACE gene is linked to high blood pressure in the salt-loaded stroke-prone spontaneously hypertensive rat.\(^5,6\) In the rat, however, the confidence intervals for the placement of the hypertensive gene on chromosome 10 spanned a much larger genetic distance and included not only the ACE gene but many other genes, including growth hormone and fast nerve growth factor receptor genes.\(^5,6\) It is possible that one of these, rather than the ACE gene, is linked to hypertension in the rat, humans, or both.

The apparently increased phenotypic expression of the ACE gene in the rat under conditions of sodium loading has led some to speculate that a locus linked to the ACE gene might confer "salt-sensitivity."\(^19\) However, the specificity of the sodium effect in respect of the ACE gene has not been clarified. Studies of sodium and the ACE gene present special logistic problems in the population setting as the definition of salt sensitivity depends on a reproducible response of individuals to a salt-loading experiment.\(^20\) In our present study the distribution of the ACE alleles was similar in individuals with or without predisposition to high blood pressure irrespective of their sodium intake, suggesting no association between ACE alleles, sodium intake, and predisposition to high blood pressure.

These studies confirm that genotypic variation in the ACE gene is associated with significant variation in the serum ACE phenotype. However, the relation was not as strong as in a previous study.\(^4\) No such genotype-phenotype relation is evident between the ACE gene and other components of the renin-angiotensin cascade. It seems that genetically determined variation in serum ACE has little impact on the circulating levels of Ang II, which relate more closely to plasma renin activity.

In summary, the ACE gene may be a determinant of serum ACE levels, but it does not appear to confer susceptibility to essential hypertension in our population. If genetically determined variation in the activity of the renin-angiotensin system contributes to high blood pressure in this group,\(^1\) then other candidate genes need to be considered.

Acknowledgments

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