Two Quantitative Trait Loci Affect ACE Activities in Mexican-Americans

Candace M. Kammerer, Nicolas Gouin, Paul B. Samollow, Jane F. VandeBerg, James E. Hixson, Shelley A. Cole, Jean W. MacCluer, Larry D. Atwood

Abstract—Angiotensin-converting enzyme (ACE) activity is highly heritable and has been associated with cardiovascular disease. We are studying the effects of genes and environmental factors on hypertension and related phenotypes, such as ACE activity, in Mexican-American families. In the current study, we performed multipoint linkage analysis to search for quantitative trait loci (QTLs) that affect ACE activities on data from 793 individuals from 29 pedigrees from the San Antonio Family Heart Study. As expected, we obtained strong evidence (maximum log of the odds [LOD]=4.57, genomic P=0.003) that a QTL for ACE activity is located on chromosome 17 near the ACE structural locus. We subsequently performed linkage analyses conditional on the effect of this QTL and obtained strong evidence (LOD=3.34) for a second QTL on chromosome 4 near D4S1548. We next incorporated the ACEIns/Del genotypes in our analyses and removed the evidence for the chromosome 17 QTL (maximum LOD=0.60); however, we retained our evidence for the QTL on chromosome 4q. We conclude that the QTL on chromosome 17 is tightly linked to ACE and is in strong disequilibrium with the insertion/deletion polymorphism, which is consistent with other reports. We also have evidence that an additional QTL affects ACE activity. Identification of this additional QTL might lead to alternate means of prophylaxis. (Hypertension. 2004;43[part 2]:466-470.)

Key Words: ethnic groups ■ blood pressure ■ genetics ■ hypertension, genetic

Serum angiotensin-converting enzyme (ACE) activities are highly heritable, and polymorphisms in the ACE structural locus has been reported to account for 19% to 50% of the variation in serum ACE activity.1 A common Alu repeat insertion/deletion (I/D) in ACE has been associated with increased blood pressure or risk of cardiovascular disease (CVD) in some, but not all, studies.2 Because the I/D polymorphism resides in an intron, these inconsistent associations might imply that the functional polymorphism is located elsewhere within the ACE locus or even in a nearby gene. Alternatively, these observations might be indicative of the presence of additional genes or genotype×environment interactions.

We are studying genes, environmental factors, and their interactions that influence blood pressure regulation as part of the San Antonio Family Heart Study (SAFHS). In this study, we report the results of our multipoint linkage analyses to detect quantitative trait loci (QTLs) that affect ACE activities and the relation between the I/D polymorphism and the QTLs in a group of large Mexican-American families.

Methods

Subjects
We collected systolic and diastolic blood pressure and ACE activity data on 935 Mexican-Americans who had participated in the SAFHS, a population-based, prospective family study of atherosclerosis and its risk factors.3 Proband (aged 40 to 60 years) for each family were identified from a low-income neighborhood by a house-to-house recruitment procedure. All first-, second-, and third-degree relatives of each proband and the proband’s spouse were invited to participate, regardless of medical history. Details of the sampling and recruitment procedures for the SAFHS have been previously described.3

Participating subjects received a physical examination in our clinic in the morning after a 12-hour fast and were interviewed about lifestyle and diet practices. Fasting blood samples were collected for biochemical analysis, and a 2-hour glucose tolerance test was performed. Height and weight were measured after the participant had removed his or her shoes. A questionnaire was administered to obtain information about each subject’s medical history, medication use, dietary habits, physical activity patterns, and smoking and alcohol consumption behaviors, as previously described.3 The Institutional Review Board at the University of Texas Health Science Center at San Antonio approved all procedures, and all subjects gave written, informed consent.

Phenotypes and Genotypes
ACE activity levels (U/L) were measured on a commercially available system (Ciba-Corning Express Plus analyzer) with use of a kit purchased from Sigma Diagnostics.4 The within-run and between-run coefficients of variation for this assay were 1.4% and 4.6%, respectively. ACE activities were transformed by natural logarithms before analysis to reduce skewness. Genotypes on 415

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microsatellite markers from 22 autosomes were available for this study. The distances between markers were computed from our data by using the CRI-MAP software program and verified for consistency with the genetic maps available from the Marshfield (Wis) Medical Research Foundation (www.mfmdlin.edu/genetics) and University of Southampton (UK) (http://cedar.genetics.soton.ac.uk/public_html/gene.html/). The average spacing between markers was 9.5 (Haldane) cM. The I/D genotypes for the ACE locus were assayed as previously described. Information was available for 793 individuals with both ACE activity and genotypic data.

**Statistical Analyses**

The aim of the current analyses was to determine whether QTLs and I/D genotypes at the ACE I/D locus contribute to variation in ACE activity overall. Before the linkage analysis, we used quantitative genetic methods to simultaneously model the total variation in ACE activity trait as a function of the mean value effects attributable to the measured environmental covariates and the proportions of the remaining variation that could be attributed to the residual additive genetic and unmeasured environmental effects, respectively. The purpose of these quantitative genetic analyses was to reduce the amount of unexplained trait variation by accounting for measured effects (e.g., sex, age, ACE genotypes, and other covariates) so that the relative proportion of the variability attributable to the QTL would be maximized. Using data on all 793 individuals with data in 29 pedigrees, we simultaneously estimated all parameters by maximum-likelihood methods. Significance of the residual heritability and the measured covariate effects was assessed by comparing the likelihood of a model in which all parameters were estimated from the likelihood-ratio test as described in detail elsewhere. Because we are primarily interested in detecting genes that affect unmeasured variation, we chose a liberal significance level ($P<0.10$) for inclusion of measured environmental covariates. Details of these analyses are presented elsewhere. After determining significant environmental covariates, we subsequently tested for the effects of the ACE I/D genotypes on lnACE activity by including the ACE genotypes as additional covariates and comparing the likelihood of models with and without incorporation of the ACE genotypic effects.

Multipoint genomic scans were performed by using a variance components method that has been extended for use on full pedigrees, as implemented in SOLAR. In brief, while simultaneously including the effects of covariates in our model, we estimated the genetic variance attributable to the region around a specific genetic marker ($\sigma^2_{\text{a}}$) by specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent relations at a given marker locus that was assumed to be tightly linked to a locus influencing the quantitative trait. We compared the likelihood of the restricted model, in which $\sigma^2_{\text{a}}=0$ (no linkage), with that of a model in which the variance caused by the marker is estimated. After conducting this “first-pass” multipoint linkage analysis to detect QTLs that influence ACE activity, we next performed a second-pass, or sequential, multipoint linkage analysis, in which we included a second QTL effect that was conditional on the effect of the QTL with the highest log of the odds (LOD) score. Sequential multipoint linkage analysis might help eliminate false-positives and/or uncover additional QTLs that might be masked by the marginal effects of other QTLs. To assess the significance of the multipoint LOD scores, we generated an empiric distribution of nominal LOD scores for lnACE activity. This null distribution was based on 10,000 unlinked simulated markers, each evaluated for evidence of linkage. All LOD scores given in the text are empirically adjusted LOD scores. In addition, we calculated genomic probability values according to the suggestion of Lander and Kruglyak.

**Results**

Serum ACE activity and genotypic data were available for a total of 793 individuals in 29 2- and 3-generation pedigrees that ranged in size from 19 to 69 individuals, with a median size of 31. Detailed descriptions of the characteristics of the SAFHS families have been presented elsewhere. In brief, the 472 women and 321 men had mean respective ages of 39.3 and 38.4 years (range, 16 to 92 years) and mean respective body mass indices of 29.5 and 28.3 kg/m$^2$ (range, 16.2 to 55.5 kg/m$^2$) (Table 1). Approximately 14% of the family members were diabetics, 14% of women and 29% of men were smokers, and 22% of the women were postmenopausal at the time of the study. As has been observed in other populations, mean ACE activity was higher in men than in women (38.3 vs 33.6 U/L). The frequency of the ACE $D$ allele was 0.44±0.012, and the frequencies of the $DD$, $DI$, and $II$ genotypes were 0.187, 0.496, and 0.317, respectively, which were not significantly different from those predicted by Hardy-Weinberg equilibrium.

Quantitative genetic analyses of the full SAFHS cohort revealed that 3 external covariates, sex, body mass index, and menopausal status, were significantly associated with lnACE activities and accounted for 4.8% of the total variation. After the effects of these covariates were incorporated simultaneously into our analysis model, the estimated residual heritability for lnACE activity in this Mexican-American population of 793 individuals was 0.649±0.065 ($P<0.00001$).

We next performed multipoint variance components linkage analyses to detect QTLs influencing lnACE activities. As expected, we obtained highly significant evidence that a QTL on chromosome 17q (near D17S1290, which is within 6 Mb of the ACE structural locus; Human Genome Browser Gateway http://genome.ucsc.edu/cgi-bin/hgGateway) affects lnACE activities (maximum multipoint LOD=4.57, genomic $P=0.003$; Figure 1). This QTL accounted for $\approx 50$% of the additive genetic variation in lnACE activity. We also obtained suggestive linkage for a QTL on chromosome 4q (Table 2). To further investigate whether QTLs in addition to the expected chromosome 17 QTL influence lnACE activity, we performed a conditional multipoint linkage analysis by
conditioning on the location of the chromosome 17 QTL for lnACE activity and rerunning the multipoint linkage analyses. These analyses revealed evidence for an additional QTL affecting lnACE on chromosome 4q near D4S1548 (conditional maximum LOD = 3.34 at position 181 cM; Figure 2), and this locus accounted for \( \approx 30\% \) of the additive genetic variation in lnACE activity.

Because the ACE I/D polymorphism is known to be associated with ACE activity in other populations, we tested whether it was associated with lnACE activity in our Mexican-American families. As expected, we found that the I/D genotypes were significantly \((P<0.0001)\) associated with lnACE activity, and the mean of the II genotype was lowest (3.25 \( \pm 0.03 \)), the DD genotypic mean was the highest (3.86 \( \pm 0.04 \)), and the ID genotypic mean was intermediate (3.59 \( \pm 0.04 \)). After the effects of the I/D genotypes were included, the residual heritability was 0.480 \( \pm 0.065 \). Thus, I/D genotypes accounted for \( \approx 59\% \) of the additive genetic variation and \( \approx 25\% \) of the total variation in lnACE activities.

### Discussion

One of the best-studied phenotypes in the renin-angiotensinogen system is ACE activity. ACE activity is central to the control of blood pressure, in that it degrades kinins (vasodilators) and produces angiotensin II, a potent vasoconstrictor. Because of its central role in blood pressure regulation, ACE inhibitors have been successfully used as prophylaxis for hypertension and heart failure. Furthermore, because the ACE I/D polymorphism has been strongly associated with ACE activity in numerous investigations (see the review by Montgomery et al\(^{10}\)), the relation between this polymorphism and hypertension and CVD has also been well studied. However, the relation between ACE activity or ACE genotype and blood pressure or CVD is not straightforward.\(^{10}\)

### Table 2. Maximum Multipoint LOD Scores for lnACE Activity in 29 Mexican-American Families

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>LOD</th>
<th>Position (cM)</th>
<th>lnACE Activity (Conditional Analysis)</th>
<th>lnACE Activity (Incorporating I/D Polymorphisms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.43</td>
<td>272</td>
<td>1.98</td>
<td>1.34</td>
</tr>
<tr>
<td>2</td>
<td>0.17</td>
<td>205</td>
<td>0.13</td>
<td>0.42</td>
</tr>
<tr>
<td>3</td>
<td>1.97</td>
<td>234</td>
<td>0.65</td>
<td>1.17</td>
</tr>
<tr>
<td>4</td>
<td>2.21</td>
<td>181</td>
<td>3.34</td>
<td>2.72</td>
</tr>
<tr>
<td>5</td>
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<td>0</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>6</td>
<td>0.36</td>
<td>210</td>
<td>0.23</td>
<td>1.02</td>
</tr>
<tr>
<td>7</td>
<td>0.12</td>
<td>170</td>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
<td>8</td>
<td>0.69</td>
<td>175</td>
<td>0.58</td>
<td>0.04</td>
</tr>
<tr>
<td>9</td>
<td>1.90</td>
<td>135</td>
<td>1.93</td>
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<tr>
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<td>125</td>
<td>0.19</td>
<td>0.00</td>
</tr>
<tr>
<td>11</td>
<td>0.30</td>
<td>85</td>
<td>0.42</td>
<td>0.02</td>
</tr>
<tr>
<td>12</td>
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<td>27</td>
<td>0.59</td>
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</tr>
<tr>
<td>13</td>
<td>0.38</td>
<td>50</td>
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<td>0.29</td>
</tr>
<tr>
<td>14</td>
<td>0.03</td>
<td>30</td>
<td>0.01</td>
<td>0.26</td>
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<tr>
<td>15</td>
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<td>0</td>
<td>0.59</td>
<td>0.52</td>
</tr>
<tr>
<td>16</td>
<td>0.10</td>
<td>95</td>
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<td>0.22</td>
</tr>
<tr>
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<td>1.54</td>
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<tr>
<td>19</td>
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<tr>
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<td>0.52</td>
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<tr>
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<td>0.27</td>
<td>0</td>
<td>0.65</td>
<td>0.23</td>
</tr>
<tr>
<td>22</td>
<td>0.78</td>
<td>15</td>
<td>0.19</td>
<td>0.19</td>
</tr>
</tbody>
</table>

\( {\text{LOD}} > 2.00 \) are in bold.
Although several small studies in humans have reported relations between ACE activities or ACE genotype and blood pressure and heart failure, larger studies in humans, as well as studies in transgenic mice, have found no relation.

There are several explanations for the seeming paradox between the success of ACE inhibitors on the reduction of cardiovascular mortality and morbidity, the strong relation between ACE I/D genotypes and ACE activity, and the apparent lack of a relation between ACE activity or genotype and blood pressure. First, there could be publication bias, in which small, negative studies are not published, whereas small, positive studies are. Second, Smithies and colleagues present results from a simulation of the renin-angiotensin system pathway that show that because ACE is an intermediate enzyme in the pathway, changes in expression of ACE might not result in changes in blood pressure owing to compensatory changes in angiotensin I, and these simulation results correspond to those in transgenic mouse studies and some studies in humans. Third, there could be genetic and environmental heterogeneity among different groups, especially as manifested by genotype × genotype or genotype × environment interactions. Indeed, considerable research has been done to identify possible genotype × genotype interaction effects within the ACE locus on ACE activity and blood pressure. Furthermore, examples of ACE genotype × environment interaction effects on blood pressure, CVD, or ACE activity have been reported in humans, baboons, and mice.

Another possible explanation for the conflicting reports regarding a possible relation between ACE genotypes, especially the I/D polymorphism, and blood pressure regulation or CVD is the observation that not all of the variation in ACE activity is accounted for by variation in the I/D polymorphism. In fact, studies in families of normotensive French whites and Nigerians indicate that variation in the ACE locus accounts for most of the variation in ACE activity attributable to the ACE locus but only 25% to 49% of the total variation. Given that additive genetic effects account for ≈65% of the variation in ACE activity, it is likely that additional genes, unlinked to the ACE locus, also affect ACE activity.

Consistent with reports in other populations, we also observed that the proportion of total variation in ACE activity attributable to additive genes in our normotensive Mexican-American population is large (65%) and the proportion due to measured environmental covariates is relatively small (4.5%). Furthermore, consistent with previous reports, we also detected linkage to a QTL near the ACE locus and found that the ACE I/D polymorphism is significantly associated with ACE activity. Inclusion of the ACE I/D genotypes as covariates into the linkage analysis model essentially removed the evidence for the chromosome 17 QTL (Figure 1), indicating that, as expected, the chromosome 17 QTL is either the ACE locus or a locus nearby that is in strong disequilibrium with it. Our analyses, and linkage analyses in general, are unable to distinguish between these 2 possibilities. Additional high-resolution analyses are necessary to distinguish between these and other hypothesis, eg, multiply interacting haplotypes, but these analyses are beyond the scope of this report.

In contrast to previous reports, we detected evidence for an additional QTL located on chromosome 4q that influences ACE activities. As would be expected if this additional putative QTL were in fact real, we observed that the strength of this QTL signal increased after accounting for the variation near the ACE locus by using either a conditional oligogenic approach or including ACE I/D genotypic effects (Table 2). This observation increases our confidence that we have detected a QTL on chromosome 4q that affects ACE activity. We are not aware of other whole-genome scans of ACE activity in humans. The initial observation that the major gene for ACE activity was linked to and strongly associated with the ACE I/D polymorphism was reported before the ready availability of molecular and statistical genetic tools that are currently used in QTL linkage analyses. Because the I/D polymorphism was strongly associated with ACE activity in several populations, much subsequent research focused on determining which polymorphism(s) within the ACE locus is responsible for this effect.

Approximately 160 known genes (Human Genome Browser Gateway, http://genome.ucsc.edu/cgi-bin/hgGateway) are located within the 2-LOD support interval for the chromosome 4 QTL (which ranges from 156 to 190 cM pter, or 124 to 159 Mb, on human chromosome 4q), but there are no obvious candidate genes for ACE activity. With the exception of autosomal dominant pseudohypoaldosteronism type I (which is caused by mutations in the mineralocorticoid receptor locus NR3C2), no other QTLs for hypertension have been mapped to this region in humans. In addition, a genome scan of ACE activities in baboons did not reveal any QTLs for ACE activities on the baboon homologue of human chromosome 4q. Several genome screens for QTLs influencing blood pressure have been performed in mice and rats, but a search of the mouse genome with the NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/mapview/) revealed no blood pressure QTLS on mouse chromosomes 3 or 8, the regions that are homologous to human chromosome 4q (http://www.ncbi.nlm.nih.gov/Homology/). In contrast, use of the Virtual Comparative Map tool from the Rat Genome Database revealed several blood pressure QTLS on rat chromosomes 2q, 16p12–14, and 19p12–q12, the regions that exhibit homology to human chromosome 4q (http://rgd.mcw.edu/VCMAP/). A cluster of QTLs that influence systolic, diastolic, and mean arterial pressures is located on rat chromosome 16, and a single blood pressure QTL has been located on rat chromosome 19. However, the most encouraging support for our hypothesis that a novel QTL for ACE activities might reside on human chromosome 4q is that a large cluster of at least 7 blood pressure QTLS, detected in multiple crosses between different inbred lines, have been located on rat chromosome 2q (http://rgd.mcw.edu/tools/qtls/). Thus, identification of this novel ACE QTL could lead to additional insights regarding the regulation of blood pressure in humans and rats.

Perspectives
ACE activity and ACE I/D genotypes have been associated with blood pressure regulation and CVD in some studies but not all. Our study provides evidence that at least 2 QTLS
affect ACE activity levels in humans, 1 of which is highly likely to be the ACE locus, and the other of which is on chromosome 4q, a region that has been predicted to contain blood pressure QTLs, based on studies in rats. Identification of this potential QTL on chromosome 4q might help explain some of the paradoxical results regarding the relation between ACE activity, blood pressure regulation, and CVD and also could lead to further insights regarding blood pressure regulation.

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