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Segregation and Linkage Analysis of Serum Angiotensin I–Converting Enzyme Levels: Evidence for Two Quantitative-Trait Loci

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Summary

Human serum angiotensin I–converting enzyme (ACE) levels vary substantially between individuals and are highly heritable. Segregation analysis in European families has shown that more than half of the total variability in ACE levels is influenced by quantitative-trait loci (QTL). One of these QTLs is located within or close to the ACE locus itself. Combined segregation/linkage analysis in a series of African Caribbean families from Jamaica shows that the ACE insertion–deletion polymorphism is in moderate linkage disequilibrium with an ACE-linked QTL. Linkage analysis with a highly informative polymorphism at the neighboring growth-hormone gene (GH) shows surprisingly little support for linkage (LOD score [Z] = 0.12). An extended analysis with a two-QTL model, where an ACE-linked QTL interacts additively with an unlinked QTL, significantly improves both the fit of the model ($P = .002$) and the support for linkage between the ACE-linked QTL and GH polymorphism ($Z = 5.0$). We conclude that two QTLs jointly influence serum ACE levels in this population. One QTL is located within or close to the ACE locus and explains 27% of the total variability; the second QTL is unlinked to the ACE locus and explains 52% of the variability. The identification of the molecular mechanisms underlying both QTLs is necessary in order to interpret the role of ACE in cardiovascular disease.

Introduction

The renin-angiotensin system is critically important in the maintenance of vascular smooth-muscle tone and of salt and water homeostasis in humans (MacGregor et al. 1981; Sealey and Laragh 1990). Renin is a proteolytic enzyme that is released from cells of the juxtaglomerular apparatus in the kidney in response to either a fall in glomerular perfusion pressure or sympathoadrenal activation via the renal nerves. Renin cleaves angiotensinogen that is synthesized in the liver, to the decapeptide angiotensin I (Erdös 1990). Two further residues are cleaved from angiotensin I by the angiotensin I–converting enzyme (ACE) to form angiotensin II, an extremely potent vasoconstrictor that also causes salt and water retention by promoting the synthesis of the mineralocorticoid aldosterone in the zona glomerulosa of the adrenal cortex. ACE also increases vascular tone by its action in degrading the vasodilator bradykinin.

Clinical studies have shown the efficacy of drugs that inhibit ACE, in the treatment of hypertension (Laragh 1989) and in causing the regression of hypertensive left-ventricular hypertrophy (Iriarte et al. 1995). Animal studies have shown an influence of ACE inhibition on left-ventricular remodeling after experimental myocardial infarction (Ertl et al. 1982), and, more recently, studies in humans have shown that ACE inhibition after myocardial infarction produces survival benefit (reviewed in Cleland 1994).

Both serum and membrane-bound isoforms of ACE show activity, although their relative importance in the maintenance of cardiovascular homeostasis is unknown (Wei et al. 1991). In adult life, serum levels are generally constant within an individual over time and are highly heritable (Alhenc-Gelas et al. 1991); segregation analysis suggests that levels are influenced by major genetic factors (Cambien et al. 1988).

Rigat et al. (1990) reported that a 287-bp insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene was associated with serum ACE levels in Caucasian subjects. Subsequent segregation and linkage analysis using this polymorphism in a series of French nuclear families suggested that a quantitative-trait locus (QTL), accounting for 44% of the interindividual variability of ACE levels, is in strong linkage disequilibrium with the I/D polymorphism (Tiret et al. 1992).

Cambien et al. (1992) proposed the I/D polymor-

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phism as a marker of increased risk for myocardial infarction. This association has been confirmed by several follow-up studies (for a review, see Soubrier et al. 1994), with some exceptions (Bohn et al. 1993; Lindpainter et al. 1995). Recently, it has been suggested that the I/D association with myocardial infarction is more striking when considered in conjunction with a polymorphism at the angiotensin II AT1 receptor subtype (Tiret et al. 1994). The I/D polymorphism has also been claimed to be associated with left-ventricular hypertrophy (Schunkert et al. 1994) and has been implicated as a risk factor for death in patients with dilated cardiomyopathy (Raynolds et al. 1993). Thus, there is considerable interest in developing a better understanding of the factors that determine ACE levels in humans, in order to explore and resolve the role of ACE in a range of pathological cardiovascular conditions.

Here we report a combined segregation and linkage analysis of serum ACE levels in 237 members of 44 Jamaican families of African Caribbean descent. We explore the role of the ACE-linked QTL in single- and two-QTL models, using both the ACE I/D polymorphism and a neighboring, highly informative, simple-sequence-repeat polymorphism (SSRP). We also examine the relationship between blood pressure and serum ACE levels, using a population-based, case-control study to compare a series of unrelated hypertensive and normotensive individuals living in Jamaica.

Subjects and Methods

This study was approved by the ethics committee of the University Hospital of the West Indies (UHWI), Kingston, Jamaica.

Hypertensive Cases

One hundred nineteen individuals with essential hypertension were identified as part of a project to investigate the genetic basis of blood-pressure variability in an African Caribbean population. Patients were selected from clinical records after attendance at either the hypertension clinic or medical outpatients' clinic at UHWI. The diagnosis had been established on the basis of three diastolic readings >90 mm Hg when the patients were ages 15–49 years. The hypertensive cases comprise (1) subjects with a long-standing clinical diagnosis of hypertension who were taking antihypertensive medication and (2) subjects with a clinical history of hypertension but who were not currently taking medication and who had diastolic blood pressures >95 mm Hg when examined for this study. Diabetic subjects were excluded. Ninety-eight hypertensive cases who were not taking ACE-inhibitor medication were selected for a case-control analysis of the relationship between hypertension and serum ACE levels.

Control Subjects

Control subjects were sought in the proximate neighborhoods of the cases. A health questionnaire was administered, and the subject's seated blood pressure was measured with a mercury sphygmomanometer at the subject's home. One hundred fifty-six control subjects were measured; all were >30 years old, with both systolic and diastolic blood pressures <50th percentile point ($<P_{50}$) for their age and sex (National Health Survey 1981), both at home and when confirmed at the Tropical Metabolism Research Unit (TMRU), University of the West Indies, Mona. By selecting control subjects with blood pressures $<P_{50}$ and excluding young subjects who may develop hypertension in the future, we aimed to amplify any differences between hypertensive cases and normotensive controls.

Genotype-Association Study

The subjects in this analysis (90 males and 153 females) include all control subjects, 7 hypertensive cases who were "off treatment" at the time of sampling, and 80 subjects who were identified during the search for cases and controls but who were excluded from these two groups. This latter group comprises (1) subjects with a clinical history of hypertension who were not receiving antihypertensive medication and who were found to be normotensive when examined for this study and (2) subjects with no previous diagnosis of hypertension and with either (*a*) systolic or diastolic blood pressures $>P_{50}$ and $<P_{90}$ at the TMRU ($<P_{90}$ = <90th percentile point) or (*b*) a diastolic blood pressure $<P_{50}$ and an age <30 years. Thus a wide range of blood-pressure values for individuals taking no medication are included in this analysis (systolic blood pressure range 89–217 mmHg; diastolic blood pressure range 45–142 mmHg).

Jamaican Families

From the 119 hypertensive patients described above, 44 probands were selected who reported multiple first- and second-and/or third-degree relatives living in the immediate or neighboring parishes. Relatives were then invited to participate in the study and to attend the TMRU for blood pressure measurement, anthropometry, and venesection.

Anthropometry and Blood-Pressure Measurement

All measurements were performed at the TMRU. Height was measured by a Harpenden portable stadiometer. Weight was measured by a Weighlux beam balance. Body-mass index (BMI) was calculated as the ratio of weight (in kg) divided by height (in m) squared. Triceps and subscapular skinfolds were measured with a Harpenden skinfold caliper. Waist circumference was measured in the horizontal plane at the midpoint between

the subcostal margin and the iliac crest. Hip circumference was measured at the level of the greater trochanter. Both circumferences were measured with a fiberglass-coated tape measure. All equipment for the anthropometric measurements was obtained from CMS Weighing Equipment Ltd.

Blood pressures were recorded by a single observer using an automated blood-pressure recorder (Takeda TM2420 monitor; A & D Instruments, Japan) or a mercury sphygmomanometer in those subjects who for technical reasons were unsuitable for measurement by the TM2420. Measurements were taken after subjects had emptied their bladders and had been seated in a quiet air-conditioned room for 5 min. Three readings were taken, separated by 4 min; the mean of the three readings was used in subsequent analyses. The TM2420 monitors were checked for static accuracy at the beginning of the project and at 6-mo intervals.

Serum ACE Levels and DNA Genotyping

After the blood-pressure measurements were completed, 50 ml of blood was drawn. Plasma and serum were separated at 4°C and then were frozen at −80°C. Genomic DNA was extracted from cell pellets (stored at −80°C) by standard methods (Sambrook et al. 1989).

Measurement of ACE activity in serum was performed at the TMRU, by a modification of the method of Beneteau et al. (1986), with the synthetic substrate N-[3-(2-furyl)-acryloyl]-L-phenylalanylglycylglycine (FAPGG) in an automated spectrophotometric assay. ACE levels for 21 individuals in the family study who were taking ACE-inhibitor medications were excluded from subsequent analysis.

The I/D polymorphism at the ACE locus was genotyped by PCR (Rigat et al. 1992). Reactions were carried out in 100 ng DNA in a total volume of 25 μl containing 1 × *Taq* extender buffer (Stratagene), 60 μM dNTPs, and 0.5 μM oligonucleotide primers. After a denaturation step of 95°C for 4 min, there were 35 cycles consisting of 1 min at 95°C, 1 min at 60°C and 2 min at 72°C. Amplification was performed with an Omnigene™ thermal cycler (Hybaid). The 490- and 190-bp alleles were separated by electrophoresis on a 2.5% agarose gel.

Alleles at a simple-sequence-repeat polymorphism (SSRP) at the growth hormone (GH) locus (Jeunemaitre et al. 1992) were amplified in a total volume of 10 μl, with 50 ng DNA, 1 × NH_4^+ buffer (Bioline), 1.5 mM $MgCl_2$, 210 μM dNTPs, and 0.6 μM oligonucleotide primers. An initial denaturation step of 95°C for 4 min was followed by 35 cycles of 95°C for 1 min, 64°C for 1 min, and 72°C for 30 s. The forward primer was fluorescently labeled. Electrophoresis was performed with a 373A DNA Sequencer (Applied Biosystems). Samples were loaded on a 6% denaturing polyacrylamide gel and were run at 30 W for 4 h. Analysis was performed by GeneScan™ 672 (version 1.0) and Genotyper™ (version 1.1b 20) software (Applied Biosystems). This SSRP was found to be highly polymorphic in this set of families, and many alleles differed by only 1 bp. Alleles were classified into 25 groups according to their size. Genotypes for both the I/D and GH polymorphism were assigned "blind" to individuals' phenotypes.

Statistical Methods

Statistical tests for the case-control and genotype-association studies were performed with the BMDP (BMDP Statistical Software) and GLIM 3.77 (The Numerical Algorithms Group) computer programs.

Segregation analysis.—Likelihood calculations were computed by the Pedigree Analysis Package (PAP version 4.0; Hasstedt 1994), which was obtained by anonymous FTP (server: corona.med.utah.edu; directory: pub/software/pap). PAP is written in FORTRAN 77 in a modular form that allows a flexible range of models to be fitted to the data that encompass the unified or general model (Lalouel et al. 1983), which is an extension of the mixed model (Morton et al. 1974). Thus, PAP directly tests Mendelian segregation proportions by incorporating transmission probabilities, consequently reducing false claims of detecting major-gene effects. It is well known that nonnormality (especially skewness and/or kurtosis) may lead to spurious evidence for a major-gene effect (Maclean et al. 1976; Demenais et al. 1986). Unfortunately, mathematical transformation of the data to induce normality prior to analysis reduces the power of detection of major-gene effects (Demenais et al. 1986), and we therefore elected to analyze untransformed data.

The 44 families had been ascertained through probands selected with essential hypertension, without any direct selection or reference to their serum ACE levels. If serum ACE levels were correlated with blood pressure, then it would be important to allow for this in the likelihood calculations, since inadequate correction for potential sources of ascertainment bias can inflate the evidence for a major-gene effect (Morton and Rao 1979). No significant evidence for a correlation between serum ACE levels and blood pressure was found in either the case-control or family-based analysis (for details, see Results). We therefore chose to make no correction for ascertainment.

Model parameters specified two alleles, for low (l) and high (h) serum ACE levels, respectively; Hardy-Weinberg equilibrium was assumed in the computation of genotype frequencies. The PAP subroutine papfqh, which calculates genotype frequencies from haplotype frequencies, was modified for a two-QTL combined segregation/linkage analysis, so that variable disequilibrium was specified between an ACE-linked QTL and the ACE

I/D polymorphism and equilibrium between the resultant haplotypes and a second unlinked QTL. Three mean ACE levels (μ_1, μ_2, and μ_3) are defined, one for each genotype (l/l, l/h, and h/h). Homoscedasticity was assumed by associating each mean with a single parameter, the within-genotype SDs. The total within-genotype variability is partitioned into random nongenetic and polygenic components. The fraction of the within-genotype variability attributed to polygenes is termed the "polygenic heritability" (h^2); this parameter is modeled by an approximate algorithm in large pedigrees (Hasstedt 1993). Three transmission probabilities (τ_1, τ_2, and τ_3) define the probability that a parent with a genotype l/l, l/h, or h/h will transmit an allele 1 to an offspring (Elston and Stewart 1971). These probabilities are 1.0, .5, and .0 for a Mendelian trait, and allowing them to vary freely in the general model eliminates a possible bias toward selecting a major-gene model. PAP allows both single-QTL and two-QTL models to be evaluated. For the latter analysis, the quantitative major-locus-parameter routine (qmlprmv) was modified to fit an additive model so that the joint genotype means (μ_{ij}) were equal to the sum of the two-QTL genotype-specific means (i.e., $\mu_{ij} = \mu_i + \mu_j$). The potential confounding effects of age, sex, systolic and diastolic blood pressure, and BMI were evaluated by simultaneously fitting these covariates with the other parameters in each model.

The total variability of serum ACE levels in the families is therefore modeled in terms of three independent and additive components, attributable to major gene(s), polygenes, and random individual specific factors. The variance due to major genes, (V_G), is

$$\sum_{i=1}^{n} f_i(\mu_i - \mu)^2 ,$$

where f_i and μ_i denote the frequency and mean for the ith genotype, respectively,

$$\mu = \sum_{i=1}^{n} f_i\mu_i ,$$

and where n denotes the number of genotypes.

Maximum-likelihood estimates of parameters were solved iteratively by the GEMINI function (Lalouel 1979). Pairs of models were evaluated with likelihood-ratio tests, in order to select the most parsimonious model that adequately fitted the data. The most complex model (with the maximum number of parameters) was initially fitted, and submodels (with fewer parameters) were then compared. Differences in $-2 \times \log_e$ likelihood are approximated by a χ^2 distribution with df equal to the difference in the number of parameters fitted in each model. The most favorable model was selected as the model with fewest parameters that was not rejected when compared with the most general model. Models were also compared by Akaike's (1974) information criterion (AIC) (AIC = $-2 \times \log_e$ likelihood + twice the number of parameters), where the best fitting model has the minimum AIC.

Table 1

Mean ± SD Values for Age, Blood Pressure, Anthropometric Measures, and Serum ACE Levels, for Hypertensive Cases and Normotensive Controls

	Cases	Controls
N [males, females]	98 [41, 57]	156 [54, 102]
Age (years)	50.6 ± 11.5	47.7 ± 11.3
Systolic blood pressure (mmHg)	152.9 ± 26.7	118.8 ± 11.6**
Diastolic blood pressure (mmHg)	91.7 ± 15.3	70.0 ± 8.4**
BMI (kg/m^2)	28.2 ± 5.4	25.2 ± 4.5*
STR[a]	1.70 ± .57	1.43 ± .49*
WHR[b]	.90 ± .08	.85 ± .07*
Serum ACE (U/liter)	150.3 ± 72.1	159.3 ± 61.7

[a] Subscapular skinfold:triceps skinfold ratio.
[b] Waist circumference:hip circumference ratio.
* $P < .01$.
** $P < .0001$.

Combined segregation/linkage analysis.—The resolution of genetic parameters by segregation analysis can be markedly improved when information from a linked marker is included in the analysis (Bonney et al. 1988). Single-QTL and two-QTL models were fitted by PAP (Hasstedt 1994), with marker data provided by the ACE I/D polymorphism or the GH SSRP. Genotypes for GH were recoded (to a maximum of 11 alleles/family) in order to reduce both the memory requirements and computational speed of the analysis to a manageable extent. GH allele frequencies were subsequently assumed to be equally frequent.

LOD score (Z) values were computed to evaluate the support for linkage between a putative ACE-linked QTL and the GH polymorphism. Genetic-model parameters were derived from either segregation analysis or combined linkage/segregation analysis using the GH polymorphism.

Results

Case-Control Study

The clinical characteristics of the study population are shown in table 1. The hypertensive subjects (98 cases) are slightly older and heavier than the normotensive subjects (156 controls). Blood-pressure differences between cases and controls are highly significant ($P < .0001$) after adjustment for age, gender, and BMI.

Table 2

Mean ± SD Values for Age, Systolic and Diastolic Blood Pressures, BMI, and Serum ACE Level, by I/D Genotype

	II	ID	DD
N [males, females]	55 [23, 32]	100 [29, 71]	88 [38, 50]
Age (years)	47.5 ± 12.9	46.7 ± 14.3	48.1 ± 12.3
Systolic blood pressure (mmHg)	130.1 ± 26.5	124.6 ± 20.0	130.4 ± 21.5
Diastolic blood pressure (mmHg)	74.4 ± 15.0	73.4 ± 11.0	75.6 ± 14.6
BMI (kg/m^2)	24.6 ± 4.6	25.2 ± 4.3	25.0 ± 4.5
Serum ACE (U/liter)	122.9 ± 39.9[a]	166.2 ± 59.3	181.2 ± 64.3

[a] Statistically significant difference (one-way ANOVA: F = 16.8; df = 2,240; $P = 1.5 \times 10^{-7}$).

There was no significant difference in serum ACE levels between cases and controls ($P = .28$), and an analysis of covariance with age, sex, and BMI as covariates also failed to detect any significant difference ($P > .2$). A supplementary regression analysis did not find any significant correlations or differences between serum ACE levels and either age, BMI, or sex (data not shown).

Genotype-Association Study

Table 2 shows the results from an analysis of variance (ANOVA) of age, systolic and diastolic blood pressures, BMI, and serum ACE levels, grouped by the ACE I/D genotypes. There were no significant differences, in age, systolic or diastolic blood pressure, or BMI, between the three groups. There was no significant sex difference between the three groups ($\chi^2 = 4.73$, 2 df, $P = .09$). There were highly significant differences in serum ACE levels between groups defined by I/D genotypes ($P = 1.5 \times 10^{-7}$). There was significant heteroscedasticity of serum ACE levels between ACE I/D genotypes, and we therefore performed the ANOVA of ACE levels after log transformation. Inclusion of subjects taking antihypertensive medication (both including and excluding patients taking an ACE inhibitor) produced similar results (data not shown). We also directly examined the relationship between blood pressure and serum ACE level, using an analysis of covariance with sex, age, and BMI as covariates; no significant relationship was found (data not shown).

Family Studies

Serum ACE levels were measured in 96 men and 141 women. The ages of the family members were 15–82 years (mean 38.5 years, SD 15.2 years); 3 individuals were <18 years of age. The distribution of ACE levels was significantly and positively skewed (skewness parameter = .96, standard error = .12; $P < .001$).

Complex segregation analysis.—The results from the segregation analysis of serum ACE levels in 237 members of 44 Jamaican pedigrees are shown in table 3. The major-gene model is the simplest model (i.e., the one with the fewest parameters) that is not rejected when compared with the general model ($\chi^2 = 2.39$, 4 df, $P = .67$) and is therefore selected as the most favorable model and predicts that 64% of the total variability in ACE levels can be accounted for by a major gene. There is no evidence for a residual polygenic component once a major gene has been fitted. The genotype means are separated by 1.8 (μ_1 and μ_2) SD and 3.2 (μ_2 and μ_3) SD, respectively. Recessive ($P = .00001$), codominant ($P = .003$), and dominant ($P = .0005$) major-gene models were rejected when compared with the major-gene model in table 3, where all three genotype specific means were free to vary. The environmental model restricts the transmission probabilities to be equal to the gene frequency (i.e., $p = \tau_1 = \tau_2 = \tau_3$) and provides a model where a trait can be skewed but not transmitted in a Mendelian pattern; this model was rejected when compared with the general model ($\chi^2 = 36.28$, 4 df, $P = 2.5 \times 10^{-7}$). A two-QTL additive model did not significantly improve the fit ($P = .67$) when compared with the major-gene model. Results are tabulated for likelihood calculations with no allowance for possible confounding effects of age and/or sex.

We were unable to demonstrate any consistent or significant age and/or sex effects in this data set and have therefore chosen to disregard these factors in subsequent analyses. For example, a major-gene model including covariate terms for sex and age did not significantly improve the fit ($\chi^2 = 4.3$, 2 df, $P = .12$). We were also unable to detect any significant effects of blood pressure and/or BMI on ACE levels. For example, a major-gene model including covariate terms for diastolic blood pressure did not significantly improve the fit ($\chi^2 = 0.13$, 1 df, $P = .72$). We therefore disregarded these factors in subsequent analyses and made no ascertainment correction.

Combined linkage/segregation analysis.—Table 4 shows the results from a combined linkage/segregation analysis modeling a single QTL influencing serum ACE levels

Table 3

Unmeasured Segregation Analysis of Serum ACE Levels

Model	p[a]	τ_1	τ_2	τ_3	μ_1	μ_2	μ_3	σ[b]	h^2	$-2 \times \log_e$(likelihood)	No. of Variables	AIC	χ^2	P
General	.75	.97	.61	.00[c]	119.03	192.16	304.40	33.05	.31	599.89	9	18.00	...	...
τ_2	.73	1.00	.59	.00	119.99	186.99	300.79	34.61	.00	601.51	6	13.62	1.62	.654
Mixed	.76	1.00	.50	.00	121.14	185.73	300.19	35.75	.00[c]	602.28	6	14.39	2.39	.495
Major gene	.76	1.00	.50	.00	121.14	185.73	300.19	35.75	.00	602.28	5	12.39	2.39	.664
Environmental	.77	.77	.77	.77	117.97	187.85	303.65	33.58	.00	636.17	5	46.28	36.28	<.001
Polygenic	...	...	...	...	155.38	...	...	60.45	.71	634.45	3	40.56	34.56	<.001
Sporadic	...	...	...	...	152.38	...	...	60.09	.00	671.58	2	75.69	71.69	<.001

[a] Allele frequency.
[b] Residual SD for each genotype.
[c] Parameter maximized at a boundary.

that is completely linked ($\theta = .0$) to the ACE I/D polymorphism. Model 1 is the most general model in this analysis, with variable disequilibrium between the QTL alleles (l and h) and the ACE polymorphism (I and D). Model 2 specifies linkage equilibrium between alleles at the QTL and the ACE I/D polymorphism and is rejected when compared with model 1 ($\chi^2 = 11.18$, 1 df, $P = .0008$). Model 3 specifies complete disequilibrium between the low QTL allele and the I allele and specifies variable disequilibrium between the high and D alleles; this model is strongly rejected when compared with model 1 ($\chi^2 = 34.45$, 1 df, $P = 4.4 \times 10^{-9}$). Model 4 specifies complete disequilibrium between the low and I alleles and between the high and D alleles; this model is strongly rejected when compared with model 1 ($\chi^2 = 62.26$, 2 df, $P = 3.0 \times 10^{-14}$). The variable disequilibrium model (model 1) is therefore selected as the most favorable model; the maximum-likelihood estimates for the haplotype frequencies are low I = .396, low D = .382, high I = .034, and high D = .188. Model 1 was extended to a mixed model by fitting an additional source of variability, a polygenic component. This model did not significantly improve the fit ($P > .05$), although h^2 was moderately high (29%).

Table 5 shows the results from a combined linkage/segregation analysis where two unlinked QTLs are modeled that jointly influence serum ACE levels. The most general model fitted in this analysis (Model 1 + unlinked QTL) specifies a QTL completely linked to, and in variable disequilibrium with, the ACE I/D polymorphism, which interacts additively with a second unlinked-ACE QTL. Model 1 (ACE-linked single-QTL model) is rejected when compared with the general two-QTL model ($\chi^2 = 10.66$, 4 df, $P = .03$). Model 4 + unlinked QTL (a two-QTL model where complete disequilibrium is assumed between the ACE-linked low and I alleles and between the high and D alleles) is also rejected ($\chi^2 = 8.19$, 3 df, $P = .02$).

Table 6 shows the results from a combined linkage/segregation analysis where one or two QTLs are modeled that jointly influence serum ACE levels; one of these QTLs is tightly linked ($\theta = .008$) to the GH SSRP. In this set of families, the ACE I/D polymorphism was tightly linked to the GH SSRP ($Z = 19.5$; $\theta = .008$),

Table 4

Combined Linkage/Segregation Analysis with ACE I/D (Single-QTL Models)

Parameter	Model 1	Model 2	Model 3	Model 4
Frequency of QTL low allele | I	.92	.79	1.00	1.00
Frequency of QTL low allele | D	.67	.79	.77	.00
Frequency of I allele	.43	.43	.43	.43
μ_1	122.46	122.94	134.59	138.08
μ_2	192.14	191.80	213.67	148.17
μ_3	309.80	307.88	292.98	173.11
σ	37.25	37.61	47.77	58.58
$-2 \times \log_e$(likelihood)	992.13	1,003.31	1,026.58	1,054.39
χ^2	...	11.18	34.45	62.26
P	...	.0008	4.4E-09	3.0E-14
AIC	14	23.18	58.54	72.26

Table 5

Combined Linkage/Segregation Analysis with ACE I/D (Single- and Two-QTL Models)

Parameter	Model 1 + Unlinked QTL	Model 1	Model 4 + Unlinked QTL
Frequency of QTL low allele | I	.84	.92	1.00
Frequency of QTL low allele | D	.52	.67	.00
Frequency of I allele	.44	.43	.43
Frequency of unlinked QTL	.82	...	.75
ACE-linked μ_1	135.27	122.46	138.38
ACE-linked μ_2	180.09	192.14	152.98
ACE-linked μ_3	247.53	309.80	163.93
Unlinked QTL μ_1	114.24	...	121.50
Unlinked QTL μ_2	185.46	...	178.34
Unlinked QTL μ_3	303.08	...	293.37
σ	24.14	37.25	36.33
$-2 \times \log_e$(likelihood)	981.47	992.13	989.36
χ^2	...	10.66	7.89
Significance vs. model 1 + unlinked QTL	...	.0307	.0194
AIC	22	24.66	25.89

and the GH SSRP was highly informative; 93% of individuals were heterozygous for this polymorphism. The ACE-linked single-QTL model is rejected when compared with a two-QTL additive model (χ^2 = 16.88, 4 df, P = .002). Linkage equilibrium is assumed between alleles at both the ACE-linked and -unlinked QTLs and the GH SSRP. The two-QTL model predicts that 79% of the total variability of ACE levels can be attributed to QTLs (27% to the ACE-linked QTLs and 52% to the ACE-unlinked QTLs). The single-QTL model may be extended to a mixed model, but this did not improve the fit ($P > .05$), although the maximum-likelihood estimate for the h^2 parameter was 36%. One further two-QTL model was evaluated without the restriction that the two QTLs interact additively; this did not significantly improve the fit (χ^2 = 6.85, 3 df, P = .08).

Linkage analysis.—Table 7 shows the results from a linkage analysis of the GH polymorphism and an ACE-linked QTL, for single- and two-QTL models and parameter estimates derived from the segregation (table 3) and combined linkage/segregation analyses (table 6). All Z values were computed with the recombination fraction (θ) between the GH polymorphism and the ACE-linked QTL fixed at .008. There is very little support for linkage between GH and the ACE-linked QTL (Z = 0.12) when the parameters derived from segregation analysis are used. When segregation parameters estimated under the explicit assumption of homogeneous

Table 6

Combined Linkage/Segregation Analysis with GH (Single- and Two-QTL Models)

Parameter	ACE-Linked + Unlinked QTLs	ACE-Linked QTL
Frequency of ACE-linked QTL low allele	.33	.77
Frequency of unlinked QTL low allele	.67	...
ACE-linked μ_1	126.45	122.05
ACE-linked μ_2	145.21	190.77
ACE-linked μ_3	202.43	310.17
Unlinked QTL μ_1	109.48	...
Unlinked QTL μ_2	140.90	...
Unlinked QTL μ_3	254.29	...
σ	27.52	38.03
$-2 \times \log_e$(likelihood)	1,839.39	1,856.27
χ^2	...	16.88
Significance vs. two-QTL model	...	.0020
AIC	18.00	26.88

Table 7

Testing for Linkage between an ACE-Linked QTL and GH

No. of QTLs	Model	Table[a]	Z
1	Major gene	3	.12
1	ACE-linked QTL	6	1.36
2	ACE-linked + unlinked QTLs	6	4.99

[a] Source of parameters used to compute Z value.

linkage (i.e., combined linkage/segregation analysis) were used, the support for linkage was increased. The Z calculated with the parameters derived from the two-QTL analysis was 4.99.

Discussion

There have been reports of significant relationships between blood pressure and circulating ACE level (Alhenc-Gelas et al. 1991; Watt et al. 1992). However, the correlations between blood pressure and ACE level have not consistently been in the same direction and have been of small magnitude. In our study of 98 subjects with essential hypertension who were compared with 156 normotensive controls, we were unable to demonstrate any differences in serum ACE levels, between hypertensive and normotensive subjects, despite the large and significant differences for blood pressure that are apparent between the groups; this was also true after adjustment for potential confounding effects of age, sex, and BMI. These findings were confirmed in the segregation analysis of serum ACE levels in 237 members of 44 Jamaican families. We were particularly interested to investigate for potential correlations, since the results from segregation analysis may be unwittingly distorted if cryptic ascertainment biases are not allowed for.

There were no significant differences in either systolic or diastolic blood pressure when subjects were grouped by ACE I/D genotype. Serum ACE levels did show a highly significant "codominant" relationship to I/D genotype ($P = 1.5 \times 10^{-7}$). The mean ACE level for the DD genotype is 50% greater than that for the II genotype. In this sample the I/D polymorphism accounts for ~9% of the total variance of serum ACE levels, which is less than has been reported previously for Caucasian subjects, where estimates of the contribution to the variance of the trait have been 15%–47% (Rigat et al. 1990; Harrap et al. 1993).

The results from the segregation analysis of the Jamaican families confirms the previous findings that circulating-ACE levels show substantial interindividual variability and are highly heritable. Major-locus inheritance is supported, since the environmental nontransmission model was strongly rejected whereas the Mendelian segregation model was not rejected. The major-gene model predicts that QTLs determine 64% of the total variability in serum ACE levels. Combined segregation and linkage analysis with the ACE I/D polymorphism shows that there is a significant association between the ACE QTL low and I alleles and between the high and D alleles. This is a finding similar to that reported by Tiret et al. (1992); however, the strength of the disequilibrium in the Jamaican families is considerably weaker than that reported in the French families. The relative strengths for the disequilibrium may be assessed from the relative risk for haplotype frequencies for the ACE-linked QTL and I/D polymorphism (which is 5.7 in the Jamaican families and 70.6 in the French families). In the French families, model 3 (complete disequilibrium between the ACE QTL low and I alleles) was not rejected when compared with the more general variable-disequilibrium model (model 1). Model 3 was strongly rejected ($P = 4.4 \times 10^{-9}$) when a similar test was made in the Jamaican families. Model 4 (complete disequilibrium between an ACE-linked QTL and the I/D polymorphism) was rejected in both the Jamaican and French samples.

We directly tested for linkage between the putative ACE-linked QTL and the neighboring highly informative GH SSRP and were surprised to find virtually no support for linkage ($Z = 0.12$). Z values were not reported in the analysis of ACE in the earlier French study (Tiret et al. 1992). Simulations using SLINK (Ott 1989; Weeks et al. 1990) for an informative polymorphism tightly linked ($\theta = .008$) to a QTL with major-gene parameters as reported in table 3 suggest that, under a similar analytic strategy, the 44 Jamaican families would generate a Z value >3 in 84% of replicates. We therefore decided to examine further models, in order to resolve this apparent paradox of an association that was not confirmed by linkage. The addition of a second QTL that was unlinked to the ACE locus markedly improved both the goodness of fit of the segregation model ($P = .002$) and the support for linkage between the ACE-linked QTL and GH ($Z = 4.99$).

The two-QTL model specifying complete disequilibrium between the ACE-linked QTL and the I/D polymorphism was rejected ($P = .02$) when compared with a more general variable-disequilibrium model (table 5). This result and the rejection of the complete-disequilibrium single-QTL model (model 4; table 4) suggests that the ACE-linked QTL is unlikely to be directly attributed to this polymorphism in this population.

We were not able to detect significant support for a second unlinked QTL in the unmeasured segregation analysis. Power calculations suggest that it may be difficult to distinguish a two-QTL from a single-QTL model by means of segregation analysis. For example, the power of distinguishing a true two-QTL model from a single-QTL model is ~15%, when simulated trait phe-

notypes for the measured individuals in the present study (using parameters derived from the two-QTL analysis reported in table 6) and a 5% significance level are used. The results from unmeasured segregation analysis suggested that a major-gene model fitted the data adequately, that the major-gene component was supported by transmission probability tests, and that a residual polygenic component was insignificant. Similar findings have been reported by Dizier et al. (1993) in their single-major-gene analyses of data generated for two-locus models (i.e., single-gene models may plausibly fit two-locus data). That study also concluded that parameter estimates for putative major genes may be unreliable, which may substantially affect subsequent linkage analysis.

The power to detect linkage between a marker and a multifactorial quantitative trait is increased if all sources of family correlations are taken into account (Martinez et al. 1991; Demenais et al. 1992; Demenais and Lathrop 1993; Thein et al. 1994). Our results show that modeling unlinked genetic factors can significantly influence the results of linkage analysis.

We conclude from our analysis that ACE levels are strongly influenced by multiple QTLs. One of these QTLs is located within or close to the ACE gene itself. The pattern of disequilibrium detected by the I/D polymorphism suggests that this polymorphism is unlikely to be the causative variant and that a search for additional DNA variants within the ACE gene is indicated. There is evidence that a second QTL contributes substantially to the genetic determination of serum ACE levels in this population. Using the parameters derived from the analysis with the GH SSRP, we estimate that 79% of the total variability of serum ACE levels is jointly influenced by two QTLs; the ACE-linked QTL determines 27% of the total variability, and a second, unlinked QTL determines 52% of the variability. This conclusion has important implications for the interpretation of the association between the I/D polymorphism and various cardiovascular diseases.

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