

ABSENCE OF ASSOCIATION OR GENETIC LINKAGE BETWEEN THE ANGIOTENSIN-CONVERTING-ENZYME GENE AND LEFT VENTRICULAR MASS

KLAUS LINDPAINNER, M.D., MINAE LEE, M.D., MARTIN G. LARSON, S.D., V. SRINIVAS RAO, PH.D.,
 MARC A. PFEFFER, M.D., PH.D., JOSE M. ORDOVAS, PH.D., ERNST J. SCHAEFER, M.D.,
 ALEXANDER F. WILSON, PH.D., PETER W.F. WILSON, M.D., RAMACHANDRAN S. VASAN, M.D.,
 RICHARD H. MYERS, PH.D., AND DANIEL LEVY, M.D.

Abstract Background. Homozygous carriers of the *D* allele of the angiotensin-converting-enzyme (*ACE*) gene have been reported to be at increased risk for various cardiovascular disorders, including left ventricular hypertrophy. We investigated the potential role of the *ACE* gene in influencing left ventricular mass.

Methods. Quantitative echocardiographic data and DNA samples were available for 2439 subjects from the Framingham Heart Study. *ACE* genotypes were determined by an assay based on the polymerase chain reaction. (The *D* allele of the *ACE* gene contains a deletion, whereas the *I* [insertion] allele does not.) Left ventricular mass and the prevalence of left ventricular hypertrophy, adjusted for clinical covariates, were analyzed according to genotype. Genetic linkage between the *ACE* locus and left ventricular mass was evaluated by quantitative analysis of pairs of siblings.

Results. The *ACE* genotype was associated neither with left ventricular mass nor with the prevalence of left ventricular hypertrophy. Mean (\pm SE) left ventricular mass

(adjusted for sex) among subjects carrying the *DD*, *DI*, and *II* genotypes was 165 ± 1.6 , 165 ± 1.3 , and 166 ± 2.0 g, respectively ($P = 0.90$). The prevalence of left ventricular hypertrophy among the three genotype groups was 15.6 percent, 13.6 percent, and 15.6 percent, respectively ($P = 0.36$), and the adjusted relative risk of left ventricular hypertrophy associated with the *DD* genotype was 1.10 (95 percent confidence interval, 0.86 to 1.19). Linkage analysis in 759 pairs of siblings using both the *ACE D/I* marker and a microsatellite polymorphism at the neighboring locus for the human growth hormone gene failed to support any role of *ACE* in influencing left ventricular mass.

Conclusions. The *ACE* genotype showed no association with echocardiographically determined left ventricular mass, nor did it confer an increased risk of left ventricular hypertrophy. We found no appreciable role of the *ACE* gene in influencing left ventricular mass. (N Engl J Med 1996;334:1023-8.)

©1996, Massachusetts Medical Society.

LEFT ventricular hypertrophy is recognized as a major independent risk factor for morbidity and mortality from cardiovascular causes.¹⁻⁴ Blood pressure, obesity, and age are important determinants of left ventricular mass⁵; however, they account only for part of the observed variance.⁵ Evidence that left ventricular mass is a familial trait⁶⁻¹⁰ suggests the influence of genetic factors; the absence of simple mendelian patterns of inheritance — except in rare syndromes¹¹ — identifies left ventricular mass as a complex phenotype that is influenced by interacting genetic and environmental factors.

In humans the gene for angiotensin-converting enzyme (*ACE*) occurs in two allelic forms, distinguished by the presence (insertion, *I*) or absence (deletion, *D*)

of a 287-base-pair repetitive DNA domain in intron 16. Homozygosity for the *D* allele was recently reported to be an independent risk factor for electrocardiographically defined left ventricular hypertrophy.¹² In a large cross-sectional sample the *DD* genotype was associated with an overall relative risk of 1.76 that was attributable to a fourfold increase in risk in a subsample of 172 normotensive men.

The *ACE D/I* polymorphism is known to account for about half the variance in *ACE* plasma levels, with higher values occurring in persons with two *D* alleles.¹³⁻¹⁵ On the basis of a number of experimental and clinical observations, the renin-angiotensin system was postulated to be in an activated state in *DD* homozygotes, leading to speculation that this genotype was associated with cardiac hypertrophy. Angiotensin peptides exert trophic influences on cardiomyocytes in culture,^{16,17} and the expression of genes encoding components of the renin-angiotensin system is up-regulated in hypertrophy and remodeling.¹⁸⁻²¹ The ability of *ACE* inhibitors to induce the regression of hypertensive left ventricular hypertrophy^{22,23} and prevent ventricular remodeling after myocardial infarction²⁴⁻²⁶ provides an intriguing clinical correlate to these observations.

The identification of a genetic marker that could be used to predict the risk of developing left ventricular hypertrophy has major implications for such factors as prognostication and risk stratification. On the basis of its association with increased plasma *ACE* activity, the *DD* genotype might even represent a modifiable risk factor responsive to treatment with *ACE* inhibitors. Be-

From the Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital (K.L., M.L., M.A.P.); the Department of Cardiology, Children's Hospital (K.L.); the Divisions of Cardiology and Clinical Epidemiology, Department of Medicine, Beth Israel Hospital (D.L.); Harvard Medical School (K.L., M.L., M.A.P., D.L.); the Department of Neurology (R.H.M.) and the Divisions of Epidemiology and Preventive Medicine, Department of Medicine (M.G.L., V.S.R., D.L.), Boston University Medical School; and the Lipid Metabolism Laboratory, U.S. Department of Agriculture Human Nutrition Research Center on Aging, Tufts University School of Medicine (J.M.O., E.J.S.) — all in Boston; the Framingham Heart Study, Framingham, Mass. (M.G.L., P.W.F.W., R.S.V., D.L.); the Department of Biometry and Genetics, Louisiana State University Medical Center, New Orleans (A.F.W.); and the National Heart, Lung, and Blood Institute, Bethesda, Md. (P.W.F.W., D.L.). Address reprint requests to Dr. Lindpaintner at the Division of Cardiovascular Diseases, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

Supported by a Research Career Development Award (K04-HL03138-01) from the National Heart, Lung, and Blood Institute and a Harcourt General Charitable Foundation Young Investigator's Award (to Dr. Lindpaintner), by a contract with the National Institutes of Health (N01-HC-38038), and by a Public Health Service resource grant (RR03655) from the Division of Research Resources.

fore such far-reaching conclusions can be entertained, evidence based on rigorously performed studies using state-of-the-art diagnostic, epidemiologic, and molecular genetic standards is mandatory. We therefore conducted a large-scale investigation based on echocardiographic assessment of left ventricular mass in a well-characterized sample of subjects from the Framingham Heart Study, using association algorithms and, as an additional powerful analytic tool, pedigree-based linkage analyses.

METHODS

Study Population

The details of the design and methods of the Framingham Heart Study have been presented elsewhere.²⁷ Briefly, starting in 1948, 5209 subjects between the ages of 28 and 62 years were enrolled in the cohort study, and beginning in 1971, a total of 5124 of their children and their children's spouses were enrolled.²⁸ Surviving participants have been examined at regular intervals.²⁸ Blood samples for DNA were collected between 1987 and 1991. Of 6214 subjects who underwent echocardiographic studies between 1979 and 1983, the results for 4973 were technically acceptable. Of these 4973 subjects, 2534 were excluded from the present study for the following reasons: age under 20 years (10 subjects), the presence of a systolic murmur of grade 3 or greater or the presence of a diastolic murmur (108), a creatinine level above 2.0 mg per deciliter (177 μ mol per liter) (11), the lack of a DNA sample (1293, 774 of whom had died or did not participate in the 1987 to 1991 examination cycle), inaccessibility of DNA samples (1100), and untypability of blood samples (12). Thus, a total of 2439 subjects were studied.

Clinically relevant variables including age, height, weight, systolic and diastolic blood pressure, blood glucose concentration, and prevalence of diabetes, ischemic heart disease, congestive heart failure, and antihypertensive treatment at the time of echocardiography were determined with the use of previously published definitions and criteria.²⁹

Echocardiography

As previously described for the Framingham Study,³⁰ M-mode echocardiographic measurements were performed at end-diastole, according to the recommendations of the American Society of Echocardiography³¹ and the Penn convention.³² With the use of these measurements, left ventricular mass was determined by the following equation:

$$\text{left ventricular mass (in grams)} = 1.04[(\text{LVIDD} + \text{VST} + \text{PWT})^3 - (\text{LVIDD})^3] - 13.6,$$

where LVIDD is end-diastolic left ventricular internal diameter, VST is ventricular septal thickness, and PWT is posterior-wall thickness. Left ventricular mass was used as an unadjusted variable and was also adjusted for height (the mass in grams divided by the height in meters).³⁰

Sex-specific population-based echocardiographic values derived from a healthy reference sample served to define categorical criteria for the presence or absence of left ventricular hypertrophy.³⁰ Values exceeding the 95th percentile of this distribution (143 in men and 102 in women) were considered to indicate left ventricular hypertrophy.

Determination of ACE Genotypes

DNA was extracted from blood samples according to standard protocols.^{33,34} We used a modification of the published methods for the determination of ACE genotypes.³⁵ Briefly, 5 μ l containing approximately 5 to 20 ng of genomic DNA was covered with oil, denatured at 95°C for three minutes, and cooled to 80°C before 10 μ l of polymerase-chain-reaction (PCR) master mix,³⁵ containing 0.15 U of *Taq* DNA polymerase, was added. The primers used, the thermocycling

protocol, and the approach to electrophoresis, retesting of *DD* homozygotes, replicate scoring, and quality control have been described previously.³⁵

Determination of Genotypes for the Human Growth Hormone Gene Polymorphism

The genotype of a highly polymorphic microsatellite associated with the gene for human growth hormone (*HGH*), known to be located very close to *ACE*,³⁶ was determined in all 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 mM), deoxynucleoside triphosphates (200 μ M each of adenosine triphosphate, cytidine triphosphate, thymidine triphosphate, and guanosine triphosphate), and primers (100 nM). One of the two primers (sense, 5'ACTGCACCTC-CAGCCTCGGAGACAG3'; reverse, 5'AGAGCAGGTGGTGTGGT-GCTACTC3') was labeled at the 5' end with [³²P] γ -ATP. Reaction products were resolved over sequencing gels containing 6 percent polyacrylamide, 8 M urea, and 30 percent formamide and visualized by autoradiography. Parallel sequencing ladders were used for size standardization. Scoring was carried out as described previously.³⁵

Statistical Analysis

Association Algorithms

Multiple linear regression was used to compare quantitative data on left ventricular mass among subjects with the *DD*, *DI*, and *II* genotypes.³⁷ Each group was analyzed separately according to sex and together (after adjustment with weighted least-squares analysis for the differences between the sexes in the variance of left ventricular mass), with and without adjustments for covariates (age, height, weight, systolic blood pressure, and the presence of ischemic heart disease, congestive heart failure, or diabetes mellitus), with the GLM program in SAS software.³⁸ Secondary tests were carried out to test for dominant, recessive, and additive modes of inheritance. Unadjusted means represent simple, empirical values. Adjusted means were estimated by linear models to incorporate sex differences and the effects of other covariates listed previously. Left ventricular hypertrophy (absence or presence) was analyzed as a categorical variable by logistic regression.³⁹ Separate-sex and pooled analyses, without and with adjustment for covariates, and before and after partitioning into four subgroups defined according to body-mass index (above or below the sex-specific median) and the presence or absence of hypertension (defined as diastolic blood pressure \geq 90 mm Hg, systolic blood pressure \geq 140 mm Hg, or the need for antihypertensive pharmacotherapy) were conducted with the Logistic program in SAS software. To exclude the possibility of bias, all tests were also performed on a subgroup of the sample that included only 1 randomly chosen member from each nuclear family (1717 subjects). All tests were two-sided, and a P value of less than 0.05 was considered to indicate statistical significance. The power to test for differences among mean values in the three genotype groups was assessed for each genotype stratified according to sex and with the estimated root-mean-square error of left ventricular mass after modeling with genotypes and risk factors (26.91 in men, 17.82 in women, and 22.88 in the pooled group).

Algorithms for Linkage Analysis in Pairs of Siblings

The Framingham Heart Study includes 2787 extended families ranging in size from 1 to 25 members. Linkage analysis in the present study was performed on 367 families with at least 2 members (254, 74, 32, 4, 2, and 1 families with 2, 3, 4, 5, 6, and 7 siblings each, respectively) with typable DNA and data on left ventricular mass, comprising 897 persons and providing 759 unique pairs of siblings for linkage analysis (there are $n(n-1)/2$ pairs of siblings in a family with n siblings). Algorithms for quantitative-trait linkage analysis^{40,41} of the *ACE* and *HGH* genotypes in pairs of siblings were used with the SAGE SIBPAL programs.⁴² This nonparametric method accommodates continuous rather than categorical phenotypic variables, requires

no genetic model assumptions, allows the incorporation of covariates, and optimizes the informational content of data by deriving power from both trait-concordant and trait-discordant pairs of siblings (in contrast with methods used to analyze pairs of siblings concordant for a categorical disease phenotype): concordant pairs of siblings are expected to share alleles at an implicated locus, whereas the opposite is expected for discordant pairs.

Regression of the squared difference in traits between pairs of siblings on the estimated proportion of alleles shared on the basis of descent (in lieu of actual information, which is available for only a small fraction of the sibships) was used to evaluate linkage. The regression coefficient, β_1 , was estimated as $\beta_1 = -2(1 - 2\theta)^2 \sigma^2$, where θ is the recombination frequency between marker and trait and σ^2 is the genetic variance of the trait. β_1 is zero if θ equals 0.5 (no linkage) and σ^2 equals 0 (no genetic variance), or if both variables equal zero, and β_1 will be negative if θ is less than 0.5 and σ^2 is greater than 0.

The assumption that pairs of siblings are independent and from a randomly mating population was supported by the finding that the extent of linkage disequilibrium between ACE and HGH was identical to that in a large cross-section of the U.S. population (data not shown).

RESULTS

Allele and Genotype Frequencies

Among the 2439 subjects in the study, the frequencies of the ACE D and I alleles were 0.551 and 0.449, respectively. The observed frequencies of 0.296, 0.510, and 0.194 for the DD, DI, and II genotypes, respectively, agree with the frequencies predicted by Hardy-Weinberg equilibrium (chi-square = 0.946; P = 0.62). The frequencies of 0.553 and 0.447, respectively, for the D and I alleles and frequencies of 0.300, 0.506, and 0.194 for the DD, DI, and II genotypes, respectively, in the subgroup that included only 1 member from each nuclear family (1717 subjects) did not materially affect the results, corresponded with maximum-likelihood estimates of allele frequencies⁴³ (0.543 and 0.457 for D and I, respectively), and maintained the frequencies predicted by Hardy-Weinberg equilibrium.

Thirty-five alleles were observed for the HGH marker. The associated polymorphism information content (a measure ranging from 0 to 1 for the least and most informative markers, respectively) was 0.955 (0.373 for ACE).

No significant differences were found between men and women or among the three ACE-genotype groups as regards age and prevalence of ischemic heart disease, congestive heart failure, diabetes mellitus, and antihypertensive treatment. Height, weight, body-mass index, and systolic blood pressure tended to be higher in men than in women, but did not differ significantly among the ACE-genotype groups (Table 1).

Genotype-Phenotype Associations

Subjects with the DD, DI, and II genotypes had remarkably similar mean values for left ventricular mass (Table 2). No statistically significant differences among genotypes were found for unadjusted or adjusted mean left ventricular mass. Whether the analyses were carried out separately for men and women, for pooled data adjusted for sex, or for subgroups classified according to

Table 1. Characteristics of the Subjects According to ACE Genotype and Sex.*

CHARACTERISTIC	ACE GENOTYPE		
	DD	DI	II
Men (no.)	341	558	217
Age (yr)	48 ± 12	47 ± 13	50 ± 13
Height (m)	1.75 ± 0.07	1.76 ± 0.07	1.76 ± 0.07
Weight (kg)	82 ± 12	82 ± 12	82 ± 11
Body-mass index†	26.7 ± 3.4	26.5 ± 3.6	26.4 ± 3.3
Systolic blood pressure (mm Hg)	128 ± 16	127 ± 16	126 ± 16
Ischemic heart disease or congestive heart failure (%)	5	6	8
Diabetes mellitus (%)	4	6	5
Antihypertensive therapy (%)	15	12	12
Women (no.)	382	685	256
Age (yr)	48 ± 13	50 ± 13	49 ± 14
Height (m)	1.61 ± 0.07	1.61 ± 0.06	1.60 ± 0.07
Weight (kg)	64 ± 12	64 ± 12	64 ± 11
Body-mass index†	24.8 ± 4.6	24.9 ± 4.5	25.0 ± 4.9
Systolic blood pressure (mm Hg)	121 ± 18	122 ± 18	123 ± 18
Ischemic heart disease or congestive heart failure (%)	3	4	3
Diabetes mellitus (%)	3	2	4
Antihypertensive therapy (%)	12	16	12

*Plus-minus values are means ± SD.

†The weight in kilograms divided by the square of the height in meters.

body-mass index and hypertension status, and whether they were performed according to dominant, recessive, or additive modes of inheritance, the differences associated with genotype were small and considerably less than the average 27-g difference accounted for by having a body-mass index above rather than below the median or the average 18-g difference observed between hypertensive and normotensive subjects.

Analysis in which left ventricular mass was included as a dichotomous variable (presence or absence of left ventricular hypertrophy) for different modes of inheritance similarly failed to reveal any effect of the ACE genotype (Table 3). Neither in the entire sample nor in any subgroup defined according to sex, body-mass index, or blood-pressure status was the ACE genotype associated with differences in the prevalence of left ventricular hypertrophy.

To ensure that the inclusion of family members did not bias the results (an unlikely possibility in the absence of rare alleles and effectively of no concern if the null hypothesis is accepted), the analyses were repeated in a subgroup that included only 1 randomly chosen member from each nuclear family (1717 subjects). Almost identical results for genotype-specific and sex-specific mean values and variances of left ventricular mass were again documented, as was the absence of an association between the ACE genotype and the prevalence of left ventricular hypertrophy (data not shown).

Linkage Studies

Quantitative analysis of 759 pairs of siblings for the ACE and HGH genotypes with the use of data on left ventricular mass standardized for sex and height and both with and without adjustment for covariates (body-

mass index and blood pressure) revealed regression coefficients of -0.0043 ($P=0.40$) for *ACE* and -0.0037 ($P=0.38$) for *HGH*. These results, which remained unchanged after the analysis was weighted for multiplex sibships,⁴⁰ provided no support for the presence of linkage between the *ACE* gene or the *ACE* locus and left ventricular mass.

DISCUSSION

Left ventricular hypertrophy is an important risk factor for cardiovascular morbidity and mortality.^{1,4} Rare familial forms of cardiac hypertrophy are inherited as classic mendelian traits; the molecular basis of several of them has recently been elucidated.¹¹ More commonly, left ventricular hypertrophy has multiple causes, has a pattern of familial aggregation,^{6,10} and is associated with other risk factors, such as hypertension and obesity.² The genetic underpinnings of these nonmendelian forms of left ventricular hypertrophy are obscure; however, recent observations have suggested that homozygosity for the *ACE D* allele may represent a marker, or risk factor, for this condition.^{12,44} In contrast to these reports, we found no evidence of an association of the *D/I* polymorphism and echocardiographically determined left ventricular mass or left ventricular hypertrophy, or for the role of any other molecular variant of the

ACE gene and locus in an epidemiologically well characterized, large cross-sectional sample.

There are several explanations for the discrepancy between our findings and the earlier observations. Previous studies were limited by small samples,⁴⁴ post hoc analyses of subgroups,¹² the selection of control samples from patient populations,⁴⁴ and suboptimal characterization of phenotypes.¹² Electrocardiographic criteria for the diagnosis of left ventricular hypertrophy are notoriously insensitive as compared with echocardiographic criteria^{31,32,45}; the high prevalence of left ventricular hypertrophy reported by Schunkert et al.¹² — 19.6 percent in their overall sample (249 of 1270 subjects) and of 18.9 percent in normotensive, middle-aged men (113 of 597 subjects) — arouses concern about the methods used or selection bias (among analogous groups of subjects in the Framingham Study, the electrocardiographically determined prevalence of left ventricular hypertrophy was 4.1 percent and 2.1 percent, respectively⁴⁶). A recent study using echocardiography reached conclusions identical to ours.⁴⁷ Also, genetic-association studies are very sensitive to the selection of representative, genetically compatible controls, a problem often compounded by small samples. As we have recently pointed out,³⁵ there is considerable variability in the frequency of the *DD* genotype among so-called controls in many smaller studies and sometimes even within a single study; in fact, Schunkert et al.¹² reported a prevalence of the *DD* genotype among controls ranging from 17.8 percent to 29.8 percent. The large number of subjects examined in the present study reduces the likelihood of selection bias among controls, and the finding of almost identical allele and genotype frequencies in a similarly large population³⁵ gives us considerable confidence in the reliability of our data. In an effort to replicate the subgroup in which the most striking association between the *ACE* genotype and left ventricular hypertrophy had previously been reported,¹² we divided our sample into high- and low-risk subgroups, according to blood pressure and body-mass index, and found no such association.

As with all studies that fail to reject the null hypothesis, assessment of statistical power is important. There was excellent power to detect small differences in left ventricular mass: assuming that the *D* allele has a linear, additive effect on left ventricular mass, our study provided 80 percent power to detect either an overall difference in left ventricular mass (between the *DD* and *II* genotypes) of 3.24 g among all subjects or a difference of 6.20 g in men and 3.46 g in women. Likewise, if the *D* allele is assumed to have a recessive effect (i.e., analysis for the effect of *DD* vs. that of *DI* and *II* combined), there was 80 percent power to detect a difference in left ventricular mass of 4.91 g in the entire sample and of 9.46 g in men and 5.26 g in women. If the *D* allele was assumed to have a dominant effect (i.e., analysis for the effect of *DD* combined with *DI* vs. that of *II*), there was 80 percent power to detect a difference

Table 2. Left Ventricular Mass According to the *ACE* Genotype.*

VARIABLE†	ACE GENOTYPE			P VALUE
	<i>DD</i>	<i>DI</i>	<i>II</i>	
Men (no.)	341	558	217	
Unadjusted LV mass (g)	201±2.9	199±2.3	199±3.6	0.75
Adjusted LV mass (g)	201±2.5	199±2.0	198±3.2	0.66
Women (no.)	382	685	256	
Unadjusted LV mass (g)	131±1.8	131±1.4	132±2.3	0.92
Adjusted LV mass (g)	131±1.4	130±1.1	131±1.7	0.82
All subjects (no.)	723	1243	473	
Unadjusted (except for sex) LV mass (g)‡	165±1.6	165±1.3	166±2.0	0.90
Adjusted LV mass (g)	165±1.3	163±1.0	164±1.6	0.68
Subgroup				
No hypertension, body-mass index below median (no.)	297	508	201	
Unadjusted LV mass (g)	147±1.9	146±1.5	147±2.3	0.91
Adjusted LV mass (g)	144±1.7	144±1.4	145±2.9	0.82
No hypertension, body-mass index above median (no.)	228	382	141	
Unadjusted LV mass (g)	173±2.6	174±2.1	173±3.3	0.95
Adjusted LV mass (g)	173±2.3	173±1.8	173±2.1	0.96
Hypertension, body-mass index below median (no.)	73	105	34	
Unadjusted LV mass (g)	165±5.1	160±4.2	160±7.2	0.76
Adjusted LV mass (g)	168±5.1	163±4.2	163±7.2	0.72
Hypertension, body-mass index above median (no.)	125	247	96	
Unadjusted LV mass (g)	195±4.4	190±3.2	193±5.0	0.62
Adjusted LV mass (g)	195±4.0	189±2.9	191±4.4	0.44

*Plus-minus values are means ±SE. LV denotes left ventricular. Two subjects could not be classified with regard to hypertension.

†Adjusted values were adjusted for sex, age, height, weight, systolic blood pressure, and the presence of ischemic heart disease, congestive heart failure, or diabetes (in subgroup analyses, because of the smaller samples, diabetes was ignored).

‡Weighted least-squares analysis was used to adjust for the differences between sexes in the variance of left ventricular mass.

Table 3. Prevalence of Left Ventricular Hypertrophy According to the ACE Genotype.*

VARIABLE†	ACE GENOTYPE			P VALUE‡
	DD	DI	II	
Men (no.)	341	558	217	
LVH (%)	15.1	12.9	15.2	
Unadjusted odds ratio	1.05	0.83	1.00	0.43
95% CI	0.66–1.68	0.52–1.29	—	
Adjusted odds ratio	1.06	0.81	1.00	0.40
95% CI	0.64–1.76	0.50–1.31	—	
Women (no.)	382	685	256	
LVH (%)	15.5	14.2	16.0	
Unadjusted odds ratio	0.96	0.87	1.00	0.73
95% CI	0.62–1.48	0.58–1.29	—	
Adjusted odds ratio	1.18	0.87	1.00	0.37
95% CI	0.69–2.01	0.53–1.42	—	
All subjects (no.)	723	1243	473	
LVH (%)	15.6	13.6	15.6	
Odds ratio (adjusted for sex only)§	1.00	0.85	1.00	0.36
95% CI	0.73–1.38	0.63–1.14	—	
Adjusted odds ratio	1.10	0.84	1.00	0.18
95% CI	0.76–1.58	0.60–1.18	—	
Subgroup				
No hypertension, body-mass index below median (no.)	297	508	201	
LVH (%)	3.7	3.1	6.0	
Adjusted odds ratio	0.64	0.54	1.00	0.33
95% CI	0.27–1.54	0.24–1.20	—	
No hypertension, body-mass index above median (no.)	228	382	141	
LVH (%)	18.9	17.3	14.9	
Adjusted odds ratio	1.10	1.15	1.00	0.89
95% CI	0.60–2.07	0.64–2.05	—	
Hypertension, body-mass index below median (no.)	73	105	34	
LVH (%)	19.2	7.6	11.8	
Adjusted odds ratio	1.92	0.62	1.00	0.07
95% CI	0.54–6.78	0.17–2.31	—	
Hypertension, body-mass index above median (no.)	125	247	96	
LVH (%)	36.0	32.0	37.5	
Adjusted odds ratio	1.22	0.80	1.00	0.26
95% CI	0.65–2.28	0.46–1.40	—	

*LVH denotes left ventricular hypertrophy, and CI confidence interval. Left ventricular hypertrophy, calculated as left ventricular mass (in grams) divided by height (in meters), was defined as a value above 143 in men and above 102 in women. Two subjects could not be classified with regard to hypertension.

†For the calculation of the odds ratios, the group with the II genotype was used as the reference group. Adjusted odds ratios were adjusted for sex alone or sex, age, height, weight, systolic blood pressure, and the presence of ischemic heart disease, congestive heart failure, or diabetes (in subgroup analyses, because of the smaller samples, diabetes was ignored). Ninety-five percent confidence intervals are for the odds ratios.

‡P values reflect the results of likelihood-ratio tests for the hypothesis that all three genotypes have identical probabilities of left ventricular hypertrophy.

§Weighted least-squares analysis was used to adjust for the differences between sexes in the variance of left ventricular mass.

in left ventricular mass of 5.67 g in the entire sample and of 10.94 g in men and 6.03 g in women.

Although our results show no effect of the ACE gene or locus on left ventricular mass, there is evidence in the Framingham Heart Study of its heritability.⁴⁸ Correlations for left ventricular mass are two to three times higher among siblings and parent-child pairs than among second-degree relatives or unrelated pairs of spouses.⁴⁸ These findings indicate the presence of familial aggregation of the trait and confirm the appropriateness of the population studied.

Unlike previous investigations focusing on the role of

the ACE gene (except for one examining its role in hypertension⁴⁹), ours had the opportunity to analyze the study population using genetic-linkage algorithms. This approach overcomes the main limitation of association studies with the D/I marker: inherently low specificity of a test in which the indicator is more common than the condition studied. The use of genetic-linkage algorithms eliminates concern about incomplete linkage disequilibrium between the D/I marker and a putative disease mutation, addressing the possible contribution of the gene to left ventricular hypertrophy independently of association with any particular marker allele. The two markers used for this analysis, ACE D/I and the HGH microsatellite, are complementary: the former, albeit less informative, is located directly at the gene; the latter, although much more informative, is located 1.9 cM from ACE (according to two-point linkage analysis⁵⁰). The power of this analysis, on the basis of the number of pairs of siblings available, was 78.4 percent to detect a genetic effect of ACE of 20 percent on the variance of left ventricular mass, and 97.6 percent to detect a genetic effect of 30 percent or greater. Whereas quantitative analysis of pairs of siblings, by its very design, cannot be used to exclude linkage positively, our failure to detect it in a sample as large as the one examined, and with the use of two complementary polymorphic markers, suggests that no major gene effects are attributable to the ACE or HGH genes or loci, although this conclusion must be drawn with caution.

We recognize that M-mode echocardiography for the determination of left ventricular mass is valid only if the geometric assumptions of the model are fulfilled. Since fewer than 4 percent of all subjects had congestive heart failure or coronary heart disease, the magnitude of this potential limitation is small. The sample examined is representative of the ambulatory, noninstitutionalized Framingham Heart Study population. Exclusion of a less healthy group of patients from the analysis (persons who had undergone echocardiography but for whom, because of death or failure to attend the subsequent examination cycle, DNA samples were not available) could have potentially biased our results. However, only if we postulate that the effects of the ACE genotype on left ventricular hypertrophy were exclusively manifest and quite pronounced in this group, would this have affected the overall results of the study (by extrapolation) — an unlikely possibility given the observed similar odds ratios for left ventricular hypertrophy associated with the ACE genotype regardless of conventional risk factors, and contrary to the originally reported limitation of the purported association of the DD genotype and left ventricular hypertrophy to young, normotensive men. The exclusion of all 427 subjects who were taking antihypertensive medications or other cardiac drugs (since such drugs may have potential confounding effects) did not affect the results.

The regression of hypertensive left ventricular hypertrophy²³ and the prevention of ventricular remodeling after myocardial infarction²⁴⁻²⁶ observed in patients treat-

ed with *ACE* inhibitors suggest that the renin-angiotensin system has an important role in the determination of cardiac mass; however, the operative mechanisms have thus far remained elusive. The present data fail to support a role of *ACE* (and *HGH*) gene mutations in determining left ventricular mass and establish that the *ACE D/I* polymorphism is not a useful marker to predict the risk of left ventricular hypertrophy.

We are indebted to Mr. Huang Chao for expert technical assistance.

REFERENCES

- Kannel WB, Gordon T, Castelli WP, Margolis JR. Electrocardiographic left ventricular hypertrophy and risk of coronary heart disease: the Framingham Study. *Ann Intern Med* 1970;72:813-22.
- Levy D, Anderson KM, Savage DD, Kannel WB, Christiansen JC, Castelli WP. Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors: the Framingham Heart Study. *Ann Intern Med* 1988; 108:7-13.
- Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990;322:1561-6.
- Sullivan JM, Vander Zwaag RV, el-Zeky F, Ramanathan KB, Mirvis DM. Left ventricular hypertrophy: effect on survival. *J Am Coll Cardiol* 1993; 22:508-13.
- Lauer MS, Anderson KM, Larson MG, Levy D. A new method for indexing left ventricular mass for differences in body size. *Am J Cardiol* 1994;74: 487-91.
- Fagard R, Van Den Broeke C, Bielen E, Amery A. Maximum oxygen uptake and cardiac size and function in twins. *Am J Cardiol* 1987;60:1362-7.
- Landry F, Bouchard C, Dumesnil J. Cardiac dimension changes with endurance training: indications of a genotype dependency. *JAMA* 1985;254:77-80.
- Harshfield GA, Grim CE, Hwang C, Savage DD, Anderson SJ. Genetic and environmental influences on echocardiographically determined left ventricular mass in black twins. *Am J Hypertens* 1990;3:538-43.
- Verhaeren HA, Schieken RM, Mosteller M, Hewitt JK, Eaves LJ, Nance WE. Bivariate genetic analysis of left ventricular mass and weight in pubertal twins (the Medical College of Virginia twin study). *Am J Cardiol* 1991; 68:661-8.
- Bielen E, Fagard R, Amery A. Inheritance of heart structure and physical exercise capacity: a study of left ventricular structure and exercise capacity in 7-year-old twins. *Eur Heart J* 1990;11:7-16.
- Schwartz K, Carrier L, Guicheney P, Komajda M. Molecular basis of familial cardiomyopathies. *Circulation* 1995;91:532-40.
- Schunkert H, Hense H-W, Holmer SR, et al. Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. *N Engl J Med* 1994;330:1634-8.
- Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;86:1343-6.
- Tiret L, Rigat B, Visvikis S, et al. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (*ACE*) gene controls plasma *ACE* levels. *Am J Hum Genet* 1992;51:197-205.
- Costerousse O, Allegrini J, Lopez M, Alhenc-Gelas F. Angiotensin I-converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. *Biochem J* 1993;290:33-40.
- Baker KM, Aceto JF. Angiotensin II stimulation of protein synthesis and cell growth in chick heart cells. *Am J Physiol* 1990;259:H610-H618.
- Sadoshima J, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* 1993;75:977-84.
- Baker KM, Chernin MI, Wixson SK, Aceto JF. Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. *Am J Physiol* 1990;259:H324-H332.
- Lindpaintner K, Lu W, Neidermayer N, et al. Selective activation of cardiac angiotensinogen gene expression in post-infarction ventricular remodeling in the rat. *J Mol Cell Cardiol* 1993;25:133-43.
- Hirsch AT, Talsness CE, Schunkert H, Paul M, Dzau VJ. Tissue-specific activation of cardiac angiotensin-converting enzyme in experimental heart failure. *Circ Res* 1991;69:475-82.
- Fabris B, Jackson B, Kohzuki M, Perich R, Johnston CI. Increased cardiac angiotensin-converting enzyme in rats with chronic heart failure. *Clin Exp Pharmacol Physiol* 1990;17:309-14.
- Pfeffer JM, Pfeffer MA, Mirsky I, Braunwald E. Regression of left ventricular hypertrophy and prevention of left ventricular dysfunction by captopril in the spontaneously hypertensive rat. *Proc Natl Acad Sci U S A* 1982;79: 3310-4.
- Julien J, Dufloux MA, Prasquier R, et al. Effects of captopril and minoxidil on left ventricular hypertrophy in resistant hypertensive patients: a 6 month double-blind comparison. *J Am Coll Cardiol* 1990;16:137-42.
- Pfeffer MA, Braunwald E, Moyé LA, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the Survival and Ventricular Enlargement trial. *N Engl J Med* 1992;327:669-77.
- The SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med* 1992;327:685-91. [Erratum, *N Engl J Med* 1992;327:1768.]
- Rutherford JD, Pfeffer MA, Moyé LA, et al. Effects of captopril on ischemic events after myocardial infarction: results of the Survival and Ventricular Enlargement trial. *Circulation* 1994;90:1731-8.
- Dawber TR, Meadors GF, Moore FE Jr. Epidemiological approaches to heart disease: the Framingham Study. *Am J Public Health* 1951;41:279-86.
- Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham offspring study. *Am J Epidemiol* 1979;110:281-90.
- Shurtleff D. Some characteristics related to the incidence of cardiovascular disease and death: Framingham Study, 18 year follow-up. In: Kannel WB, Gordon T, eds. *The Framingham Study: an epidemiological investigation of cardiovascular disease*. Sect. 30. Washington, D.C.: Government Printing Office, 1974. (NIH publication no. 74-599.)
- Levy D, Savage DD, Garrison RJ, Anderson KM, Kannel WB, Castelli WP. Echocardiographic criteria for left ventricular hypertrophy: the Framingham Heart Study. *Am J Cardiol* 1987;59:956-60.
- Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978;58:1072-83.
- Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man: anatomic validation of the method. *Circulation* 1977;55:613-8.
- Gross-Bellard M, Oudet P, Chambon P. Isolation of high-molecular-weight DNA from mammalian cells. *Eur J Biochem* 1973;36:32-8.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- Lindpaintner K, Pfeffer MA, Kreutz R, et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 1995;332:706-11.
- Jeunemaitre X, Lifton RP, Hunt SC, Williams RR, Lalouel JM. Absence of linkage between the angiotensin converting enzyme locus and human essential hypertension. *Nat Genet* 1992;1:72-5.
- Kleinbaum DG, Kupper LL, Muller KE. *Applied regression analysis and other multivariable methods*. 2nd ed. Boston: PWS-Kent, 1988.
- SAS/STAT users guide, version 6. 4th ed. Cary, N.C.: SAS Institute, 1989: 891-996, 1071-126.
- Hosmer DW Jr, Lemeshow S. *Applied logistic regression*. New York: John Wiley, 1989.
- Amos CI, Elston RC, Wilson AF, Bailey-Wilson JE. A more powerful robust sib-pair test of linkage for quantitative traits. *Genet Epidemiol* 1989;6:435-49.
- Amos CI, Elston RC. Robust methods for the detection of genetic linkage for quantitative data from pedigrees. *Genet Epidemiol* 1989;6:349-60. [Erratum, *Genet Epidemiol* 1989;6:727.]
- S.A.G.E., statistical analysis for genetic epidemiology, release 2.1, SIBPAL version 2.5. New Orleans: Louisiana State University Center, 1991.
- Boehnke M. Allele frequency estimation from data on relatives. *Am J Hum Genet* 1991;48:22-5.
- Iwai N, Ohmichi N, Nakamura Y, Kinoshita M. DD genotype of the angiotensin-converting enzyme gene is a risk factor for left ventricular hypertrophy. *Circulation* 1994;90:2622-8.
- Ganau A, Devereux RB, Pickering TG, et al. Relation of left ventricular hemodynamic load and contractile performance to left ventricular mass in hypertension. *Circulation* 1990;81:25-36.
- Levy D, Labib SB, Anderson KM, Christiansen JC, Kannel WB, Castelli WB. Determinants of sensitivity and specificity of electrocardiographic criteria for left ventricular hypertrophy. *Circulation* 1990;81:815-20.
- Kupari M, Perola M, Koskinen P, Virolainen J, Karhunen PJ. Left ventricular size, mass, and function in relation to angiotensin-converting enzyme gene polymorphism in humans. *Am J Physiol* 1994;267:H1107-H1111.
- Post WS, Larson MG, Myers RH, Galderisi M, Levy D. Heritability of left ventricular mass. *J Am Coll Cardiol* (in press). abstract.
- Iwai N, Inagami T. Identification of a candidate gene responsible for the high blood pressure of spontaneously hypertensive rats. *J Hypertens* 1992; 10:1155-7.
- Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 1984;36:460-5.