Pharmacogenetic Association of the Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism on Blood Pressure and Cardiovascular Risk in Relation to Antihypertensive Treatment

The Genetics of Hypertension-Associated Treatment (GenHAT) Study

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Background—Previous studies have reported that blood pressure response to antihypertensive medications is influenced by genetic variation in the renin-angiotensin-aldosterone system, but no clinical trails have tested whether the ACE insertion/deletion (I/D) polymorphism modifies the association between the type of medication and multiple cardiovascular and renal phenotypes.

Methods and Results—We used a double-blind, active-controlled randomized trial of antihypertensive treatment that included hypertensives ≥55 years of age with ≥1 risk factor for cardiovascular disease. ACE I/D genotypes were determined in 37,939 participants randomized to chlorthalidone, amlodipine, lisinopril, or doxazosin treatments and followed up for 4 to 8 years. Primary outcomes included fatal coronary heart disease (CHD) and/or nonfatal myocardial infarction. Secondary outcomes included stroke, all-cause mortality, combined CHD, and combined cardiovascular disease. Fatal and nonfatal CHD occurred in 3,096 individuals during follow-up. The hazard rates for fatal and nonfatal CHD and the secondary outcomes were similar across antihypertensive treatments. ACE I/D genotype group was not associated with fatal and nonfatal CHD (relative risk of DD versus ID and II, 0.99; 95% CI, 0.91 to 1.07) or any secondary outcome. The 6-year hazard rate for fatal and nonfatal CHD in the DD genotype group was not statistically different from the ID and II genotype group by type of treatment. No secondary outcome measure was statistically different across antihypertensive treatment and ACE I/D genotype strata.

Conclusions—ACE I/D genotype group was not a predictor of CHD, nor did it modify the response to antihypertensive treatment. We conclude that the ACE I/D polymorphism is not a useful marker to predict antihypertensive treatment response.

Key Words: drugs ■ genetics ■ hypertension ■ pharmacogenetics

Essential hypertension is an important threat to public and individual health in the United States. Up to 50 million Americans have hypertension, defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, and/or the use of antihypertensive medication. About one third of those treated have controlled blood pressure.1–3 Although access to medical care is an important correlate of control of hypertension, biological factors may determine blood pressure control. Emerging studies indicate that genetic variation may be a useful marker to assess therapeutic efficacy of antihypertensive medication.4–7 Using genetic variation to predict the clinical response to pharmacological treatment offers a new approach to tailoring antihypertensive treatment. Targeting treatment to the genetic component of hypertension may enhance the efficacy of treatment, resulting in more effective blood pressure control and lower incidence of hypertension-related morbidity and mortality. In addition to improved efficacy, screening for the genetic basis of hypertension may reduce the toxicity profile of antihypertensive drugs and improve the overall outcome and cost-to-benefit ratio.8–10

Candidate genes involved in blood pressure regulation may interact with the type of antihypertensive medication to modify blood pressure response to treatment. The ACE gene is an excellent candidate to evaluate in relation to genotype-treatment interaction because ACE is an important regulator of the renin-angiotensin-aldosterone system. ACE (kininase II) is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation and electrolyte balance by hydrolyzing angiotensin I to angiotensin II, a potent vasocon-

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strictor, and catabolizing bradykinin, a potent vasodilator. ACE is present on the surface of vascular endothelial cells and in circulating plasma; animal studies indicate that tissue-bound ACE is essential for the control of blood pressure and the structure and function of the kidney. In 1990, an insertion/deletion (I/D) polymorphism was detected that accounts for up to 50% of the variation in circulating ACE levels. The frequency of the ACE DD genotype is ≈0.27 in the general population. To determine whether ACE I/D genotype interacts with the type of antihypertensive drug to modify the risk of coronary heart disease (CHD) and other cardiovascular end points in treated hypertensives, the Genetics of Hypertension-Associated Treatment (GenHAT) study examined this association in volunteers who participated in the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). A brief summary follows.

Methods
Details of the methods for both GenHAT and ALLHAT have been presented elsewhere. A brief summary follows.

Hypotheses
We hypothesized that the risk of combined fatal CHD and nonfatal myocardial infarction (MI) for hypertensive patients randomized to an ACE inhibitor as first-line therapy versus hypertensive patients randomized to the other drugs would be lower in individuals with the DD genotype compared with the analogous risk in carriers of the I allele. In February 2000, the doxazosin arm was discontinued because of futility for the primary end point (part of the a priori stopping guidelines for ALLHAT) and a significant 25% increased incidence of cardiovascular disease (CVD) compared with the chlorthalidone arm. Therefore, we examined both the DD versus the aggregate of chlorthalidone and amlopidine and lisinopril versus doxazosin because the 2 comparisons had different lengths of follow-up (mean, 4.9 and 3.1 years, respectively). As secondary hypotheses, we examined (1) the primary outcome with the different individual comparator groups (ie, unaggregated treatments), (2) the primary comparisons using the other secondary outcomes (see below for details), and (3) all of the above within the prespecified subgroups of ALLHAT: black versus nonblack, men versus women, age ≤65 versus >65 years, and presence versus absence of diabetes at baseline.

Study Population
GenHAT is an ancillary study of ALLHAT, a randomized, double-blind, multicenter clinical trial of 42,418 participants from 623 centers designed to determine whether the incidence of fatal CHD and nonfatal MI in high-risk hypertensive patients is lower with treatment with each of 3 newer antihypertensive drug classes—a calcium channel blocker (amlodipine), an ACE inhibitor (lisinopril), and an α-adrenergic blocker (doxazosin)—compared with treatment with a diuretic (chlorthalidone).

About half of the sample subjects were minorities, mostly black and Hispanic, and about half were female. Detailed inclusion and exclusion criteria are published elsewhere. Eligible subjects were required to have at least 1 additional cardiovascular risk factor, including previous MI or stroke, type 2 diabetes, current cigarette smoking, left ventricular hypertrophy by ECG or echocardiography, or low HDL cholesterol level. Informed consent was obtained for each patient, and the protocol was approved by the institutional review board at each participating center.

Intervention
Participants were randomized to 1 of 4 treatments—chlorthalidone, lisinopril, amlopidine, and doxazosin—in a ratio of 1.7:1:1:1, respectively. Randomization was stratified by clinical center. The treatment was given once daily: chlorthalidone 12.5 mg for the first and second titration and 25 mg for the third; lisinopril 10, 20, or 40 mg; amlopidine 2.5, 5, or 10 mg; or doxazosin 2, 4, or 8 mg. All study medications were identical in appearance.

The treatment goal was to achieve systolic and diastolic blood pressures <140 and 90 mm Hg, respectively, on the lowest possible dosage of the first-line drug. Participants were titrated to higher doses to achieve blood pressure control. If blood pressure control was not achieved on the maximum study medication dose, a second-line open-label agent (reserpine, clonidine, or atenolol) and/or a third-line open-label agent (hydralazine) were added. Information about compliance to study medication was collected at each clinic visit. At 6 months of study treatment, 71% were on 1 drug, 26% were on ≥2 drugs, and 3% were on no drugs. Routine clinic visits were scheduled at 3-month intervals during the first year and at 4-month intervals thereafter.

Follow-Up and Outcomes
Randomization to the hypertension component began in February 1994 and concluded January 31, 1998. Follow-up concluded in March 2002. The main outcome for GenHAT is fatal CHD and nonfatal MI. Secondary outcomes included stroke, combined CHD (main outcome, coronary revascularization, or hospitalized angina), combined CVD (combined CHD, stroke, treated angina without hospitalization, congestive heart failure, and peripheral arterial disease), all-cause mortality, and blood pressure at 6 months of treatment.

End points were reported by the clinical investigator. Death certificates and hospitalization records were used to support the clinician-assigned outcomes. Periodic searches through national databases (VA, National Death Index and the Health Care Finance Administration) were performed to ascertain deaths and hospitalization end points that were missed by the clinical sites. MI and stroke were reviewed by an end-point committee to determine whether events met a priori study criteria.

Genotyping Methods
All DNA samples were anonymized as set forth in the Report of the Special Emphasis Panel on Opportunities and Obstacles to Genetic Research in NHLBI Clinical Studies. ACE I/D genotyping was performed using a modification of the procedure described by Kim et al to enhance detection of heterozygotes. This procedure was modified by substituting 0.11 μg/μL BSA for 8-methoxypsoralen in the reaction mixture and using the following cycling conditions: 95°C for 7 minutes, 12 cycles of 95°C for 1 minute, 63°C for 1 minute, and 72°C for 1.5 minute, followed by 30 cycles of 95°C for 45 seconds, 65°C for 45 seconds, 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. Ongoing quality control analyses were conducted with a 5% blind duplicate replicate; agreement between repeated genotypes was 96%.

Statistical Methods
Using the fixed sample size provided by ALLHAT and the available genotypes, the expected frequencies of genotypes (accounting for the known race distribution within ALLHAT when possible) for the I/D variant of the ACE gene, the estimated CHD rates for the treatment groups, and a test of the equality of the ratios of hazard ratios (HRs) in a 2×2×2 design, we had 80% power with a type 1 familywise error of 0.05 to detect a ratio of HRs of 0.65 for the primary hypothesis. The hypothesis was that the relative risk of combined fatal CHD and nonfatal MI for those hypertensive patients randomized to an ACE inhibitor as first-line therapy versus those randomized to the other drugs would be lower in individuals with the DD genotype compared with the analogous relative risk in carriers of the I allele. We initially conducted the analyses using 3 genotypes (DD, ID, and II). However, there were no statistically significant effects of the genotypes coded separately; therefore, all ANCOVA and Cox proportional-hazards model analyses presented here compare the DD genotype with the combined ID and II genotypes (hereafter called the DD genotype group and ID and II genotype groups). Our Cox proportional-hazards analyses would be powered to detect the
interaction if, for the primary outcome, the HR for the ACE inhibitor (lisinopril) versus other drugs was 0.78 for the DD genotype group and the HR for ACE inhibitor (lisinopril) versus other drugs was 1.20 for the II and ID genotype group. The ratio of HRs would be 0.78/1.20 = 0.65.

Baseline characteristics were compared among the 3 genotype groups (DD, ID, and II) using $\chi^2$ tests for categorical variables or ANOVA for continuous variables. Hardy-Weinberg equilibrium was tested with $\chi^2$ tests. Differences between the DD group and the ID and II group in the effects of the treatments on baseline to 6-month blood pressure measurements (visit window, ±3 months) were investigated using ANCOVA with the appropriate interaction terms. Incidence of clinical outcomes between ACE I/D genotypes and treatment groups was investigated with Cox proportional-hazards models. HRs and 95% CIs were calculated. Homogeneity of the effects of treatment among different genotypes was tested by adding interaction terms to the relevant statistical models.19

Results

Study Population and Baseline Characteristics
The ACE I/D genotyped population include 37 939 participants representative of the total 42 418 ALLHAT participants in terms of their principal sociodemographic and clinical characteristics.13 Baseline characteristics according to the ACE I/D genotypes are presented in Table 1. The DD genotype was significantly ($P<0.0001$) more frequent than the ID and II genotypes in black participants. All ethnic group–specific genotype frequencies were in Hardy-Weinberg equilibrium. There were no differences in baseline systolic and diastolic blood pressure levels among the 3 genotypes. This finding was true overall and separately for participants who were and were not taking blood pressure–lowering medications at study entry. However, prevalence of MI, stroke, coronary artery bypass surgery, left ventricular hypertrophy, and estrogen replacement therapy was lowest in the II genotype, whereas mean levels of total cholesterol and HDL cholesterol were highest in the DD genotype. The proportions between the 3 genotypes assigned to each randomized treatment were well balanced (Table 1).

Effects of ACE I/D Genotype Group on 6-Month Blood Pressure Changes
The change from baseline blood pressure to blood pressure after 6 months of study medication according to genotype is listed in Table 2. We hypothesized that the difference in blood pressure response in the DD genotype group versus the ID and II genotype group would be larger in the lisinopril group compared with same relation in the aggregated “other drugs” group. The gene-treatment interaction analysis for blood pressure tested whether the mean blood pressure–lowering medications at study entry. However, prevalence of MI, stroke, coronary artery bypass surgery, left ventricular hypertrophy, and estrogen replacement therapy was lowest in the II genotype, whereas mean levels of total cholesterol and HDL cholesterol were highest in the DD genotype. The proportions between the 3 genotypes assigned to each randomized treatment were well balanced (Table 1).

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Summary of ALLHAT Treatment Effects
There were no treatment differences for the primary outcome of the composite of fatal CHD and nonfatal MI. Likewise, all-cause mortality did not differ between groups. For amlodipine versus chlorthalidone, secondary outcomes were similar except for a higher rate of heart failure with amlodipine (relative risk [RR], 1.38; 95% CI, 1.25 to 1.52). For lisinopril versus chlorthalidone, lisinopril had higher rates of combined CVD (RR, 1.10; 95% CI, 1.05 to 1.16), stroke (RR, 1.15; 95% CI, 1.02 to 1.30), and heart failure (RR, 1.19; 95% CI, 1.07 to 1.31).

Associations of ACE I/D Genotypes With Risk of Clinical Outcomes
The effect of the ACE I/D genotype group was first evaluated for the main outcome and the secondary outcomes. We identified no elevated risk for the DD genotype group compared with the ID and II genotype group (Figure 1) for the primary and secondary outcomes. We then estimated the cumulative event rates by genotype-drug combinations; results are provided by genotype group and drug in Table 3 for 6 years of follow-up for the lisinopril, chlorthalidone, and amlodipine comparisons and in Table 4 for 4 years of follow-up for the lisinopril and doxazosin comparison. The hazard rates were remarkably similar across all genotype-treatment strata, and we found no statistical evidence that the main and secondary clinical outcomes differed across gene-drug strata. Kaplan-Meier survival curves for CHD, all-cause mortality, stroke, and combined CVD according to ACE I/D genotype group and treatment (amlodipine plus chlorthalidone) are illustrated in Figures 2 and 3, confirming the absence of a significant difference in outcomes by ACE I/D genotype group. For all outcomes, there was no statistical evidence that the effects of treatment varied between the ACE DD genotype subgroup and the ID and II genotype subgroup (ratios of HR for the DD versus ID and II genotypes by antihypertensive agents were all close to 1.0; $P=0.26$ for all; Figure 4).

We evaluated gene-drug interactions in relation to the main and secondary outcomes in predefined subgroups based on race, gender, age, and diabetes. The absence of gene-treatment interactions persisted for all ethnic and age subgroups. However, there was evidence for a difference in the risk of the primary outcome, fatal and nonfatal CHD, across gender-gene-drug subgroups. Women with the DD genotype who were treated with lisinopril versus chlorthalidone or
<table>
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<th>DD</th>
<th>Total</th>
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<td>SD or %</td>
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<tr>
<td>DBP</td>
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<td>10</td>
<td>84</td>
<td>10</td>
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<td><strong>Treated at baseline, mm Hg</strong></td>
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<td>16</td>
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<tr>
<td>DBP</td>
<td>83</td>
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<td><strong>Untreated at baseline, mm Hg</strong></td>
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<td>6869</td>
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<td>LDL cholesterol, mg/dL</td>
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SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HDL-C, HDL cholesterol; and LHV, left ventricular hypertrophy. Parameters reported as number and percent are italicized. Percentages represent the fraction of the individuals within a genotype group, except for percentage of overall No., which indicates the proportion each genotype group represents in the total sample.

*Comparisons of baseline characteristics among all 4 treatment groups combined by ACE genotype. Significant results compared with the overall mean were age, in ACE II group: \( P = 0.035 \); age range, in ACE II group: \( P = 0.013 \); history of MI or stroke, in ACE II group: \( P = 0.001 \); history of CABG, in ACE DD group: \( P = 0.025 \); aspirin, in ACE DD group: \( P = 0.021 \); total cholesterol, in ACE II group: \( P = 0.003 \); in ACE ID group: \( P = 0.031 \); potassium, in ACE II group: \( P = 0.035 \); in ACE ID group: \( P = 0.003 \).
amlodipine had an increased risk of CHD (HR, 1.32; 95% CI, 1.02 to 1.69), whereas men with the DD genotype who were treated with lisinopril versus chlorthalidone or amlodipine had a lower RR (HR, 0.87; 95% CI, 0.71 to 1.05). In the ID and II genotype group, there was no difference in CHD risk across the gender-drug categories (men: HR, 0.98; 95% CI, 0.87 to 1.12 for lisinopril versus chlorthalidone or amlodipine; women: HR, 0.95; 95% CI, 0.80 to 1.14 for lisinopril versus chlorthalidone or amlodipine; test for gender-gene-treatment interaction, \( P = 0.023 \)). Similar results were observed for CHD mortality and the lisinopril versus doxazosin comparison (test for gender-gene-drug interaction, \( P = 0.09 \)). Diabetics who had the DD genotype and were treated with lisinopril showed a nearly significant increased risk of end-stage renal disease (HR, 1.58; 95% CI, 0.97 to 2.59) than those treated with chlorthalidone or amlodipine, whereas the comparable risk for nondiabetics was 0.90 (95% CI, 0.37 to 1.43). In the II and ID genotype group, treatment with lisinopril compared with chlorthalidone or amlodipine was associated with a reduced RR for diabetics (HR, 0.90; 95% CI, 0.63 to 1.30), whereas the converse was found for nondiabetics (HR, 1.31; 95% CI, 0.88 to 1.94). The statistical test for the diabetes-by-gene-by-treatment interaction probability value was significant (\( P = 0.02 \)).

**Discussion**

The GenHAT study is the largest pharmacogenetic trial of blood pressure lowering to date. From genotype data collected from \( \approx 40000 \) individuals randomized to 1 of 4 drugs

---

**TABLE 2. Six-month Systolic and Diastolic Blood Pressure Changes by ACE I/D Genotype and Treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ACE DD</th>
<th>ACE ID and II</th>
<th>DD-ID and II†</th>
<th>G-T Intx‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lisinopril</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>2330</td>
<td>5198</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>-3.95±20.57</td>
<td>-4.80±20.20</td>
<td>0.86±0.51</td>
<td>...</td>
</tr>
<tr>
<td>DBP</td>
<td>-2.59±11.24</td>
<td>-3.09±11.22</td>
<td>0.50±0.28</td>
<td>...</td>
</tr>
<tr>
<td><strong>Chlorthalidone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>3787</td>
<td>8942</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>-7.46±18.65</td>
<td>-7.39±18.62</td>
<td>-0.07±0.36</td>
<td>0.100</td>
</tr>
<tr>
<td>DBP</td>
<td>-3.62±10.62</td>
<td>-3.42±10.70</td>
<td>-0.20±0.21</td>
<td>0.098</td>
</tr>
<tr>
<td><strong>Amlodipine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>2244</td>
<td>5261</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>-5.43±19.04</td>
<td>-5.83±18.41</td>
<td>0.40±0.47</td>
<td>0.343</td>
</tr>
<tr>
<td>DBP</td>
<td>-3.61±10.91</td>
<td>-3.88±10.56</td>
<td>0.28±0.27</td>
<td>0.695</td>
</tr>
<tr>
<td><strong>Doxazosin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>2320</td>
<td>5151</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>-5.25±19.22</td>
<td>-4.35±19.47</td>
<td>-0.90±0.48</td>
<td>0.018</td>
</tr>
<tr>
<td>DBP</td>
<td>-3.98±10.99</td>
<td>-3.46±10.83</td>
<td>-0.52±0.27</td>
<td>0.010</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure. *Mean±SE change in blood pressure from baseline to 6 months (mm Hg). †Mean±SE difference of blood pressure change (mm Hg) between the ACE DD genotype group and the ACE ID and II group. ‡Gene-by-treatment interaction (G-T Intx) probability value for the contrast of the mean blood pressure change difference between genotypes (DD minus ID and II) in lisinopril vs other treatments (eg, 0.86 for SBP for lisinopril vs -0.14 for other treatments, \( P = 0.048 \)). §Chlorthalidone, amlodipine, and doxazosin (C+A+D) treatments aggregated.
within the ALLHAT study and followed up for cardiovascular events using standard, well-defined definitions for cardiovascular and renal outcomes, we found no evidence that ACE I/D genotype group is a major modifier of blood pressure response or cardiovascular or renal outcomes. The GenHAT study provides no evidence to support the hypothesis that the ACE I/D genotype group might influence the reduction in blood pressure achieved with ACE inhibitor therapy compared with other commonly used antihypertensive agents. In fact, the opposite was observed: Participants with the DD genotype had a poorer overall response to lisinopril treatment than to any of the other 3 drugs. The ACE I/D polymorphism has been shown to account for up to 50% of the variation in ACE plasma levels.12 Although the effects of ACE inhibitors

### TABLE 3. Total Events, Event Rates, and SE of Primary and Secondary Clinical Outcomes by ACE I/D Genotype and Treatment (6 Years of Lisinopril, Chlorthalidone + Amlodipine, Chlorthalidone, Amlodipine)

<table>
<thead>
<tr>
<th></th>
<th>ACE DD</th>
<th>ACE ID and II</th>
<th>ACE DD</th>
<th>ACE ID and II</th>
<th>ACE DD</th>
<th>ACE ID and II</th>
<th>ACE DD</th>
<th>ACE ID and II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>222</td>
<td>11.5</td>
<td>866</td>
<td>11.1</td>
<td>566</td>
<td>11.2</td>
<td>1369</td>
<td>11.4</td>
</tr>
<tr>
<td>CHF</td>
<td>169</td>
<td>9.0</td>
<td>389</td>
<td>8.8</td>
<td>446</td>
<td>9.3</td>
<td>903</td>
<td>8.6</td>
</tr>
<tr>
<td>Fatal CHF (hospitalized)</td>
<td>136</td>
<td>7.2</td>
<td>299</td>
<td>7.0</td>
<td>365</td>
<td>7.7</td>
<td>819</td>
<td>7.1</td>
</tr>
<tr>
<td>Angina</td>
<td>260</td>
<td>14.0</td>
<td>645</td>
<td>13.5</td>
<td>672</td>
<td>12.3</td>
<td>1629</td>
<td>12.5</td>
</tr>
<tr>
<td>Angina (hospitalized)</td>
<td>190</td>
<td>9.7</td>
<td>429</td>
<td>9.3</td>
<td>451</td>
<td>8.5</td>
<td>1106</td>
<td>8.7</td>
</tr>
<tr>
<td>Coronary revascularizations</td>
<td>181</td>
<td>9.4</td>
<td>448</td>
<td>10.1</td>
<td>480</td>
<td>9.4</td>
<td>1173</td>
<td>9.5</td>
</tr>
<tr>
<td>CHD mortality</td>
<td>100</td>
<td>4.8</td>
<td>194</td>
<td>4.4</td>
<td>221</td>
<td>4.3</td>
<td>542</td>
<td>4.5</td>
</tr>
</tbody>
</table>

**Components of secondary outcomes**

|                |        |               |        |               |        |               |        |               |
| CHF            | 169    | 9.0           | 389    | 8.8           | 446    | 9.3           | 903    | 8.6           | 249    | 8.5           | 543    | 7.5           | 197    | 10.8          |
| Fatal CHF (hospitalized) | 136    | 7.2           | 299    | 7.0           | 365    | 7.7           | 819    | 7.1           | 201    | 6.9           | 453    | 6.3           | 164    | 9.0           |
| Angina         | 260    | 14.0          | 645    | 13.5          | 672    | 12.3          | 1629   | 12.5          | 427    | 12.3          | 1007   | 12.2          | 245    | 12.3          |
| Angina (hospitalized) | 190    | 9.7           | 429    | 9.3           | 451    | 8.5           | 1106   | 8.7           | 287    | 8.6           | 688    | 8.5           | 164    | 8.3           |
| Coronary revascularizations | 181    | 9.4           | 448    | 10.1          | 480    | 9.4           | 1173   | 9.5           | 298    | 9.4           | 695    | 9.0           | 182    | 9.4           |
| CHD mortality  | 100    | 4.8           | 194    | 4.4           | 221    | 4.3           | 542    | 4.5           | 147    | 4.6           | 333    | 4.3           | 74     | 3.8           |

**CHF** indicates congestive heart failure.

*Total events.

†Six-year event rate per 100 persons.

### TABLE 4. Total Events, Event Rates, and SE of Primary and Secondary Clinical Outcomes by ACE I/D Genotype and Treatment (4 Years of Lisinopril and Doxazosin)

<table>
<thead>
<tr>
<th></th>
<th>ACE DD</th>
<th>ACE ID and II</th>
<th>ACE DD</th>
<th>ACE ID and II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>134</td>
<td>7.1</td>
<td>307</td>
<td>7.8</td>
</tr>
<tr>
<td>CHF</td>
<td>212</td>
<td>10.0</td>
<td>457</td>
<td>9.7</td>
</tr>
<tr>
<td>Fatal CHF (hospitalized)</td>
<td>273</td>
<td>14.3</td>
<td>645</td>
<td>16.0</td>
</tr>
<tr>
<td>Angina</td>
<td>83</td>
<td>5.0</td>
<td>186</td>
<td>4.7</td>
</tr>
<tr>
<td>Angina (hospitalized)</td>
<td>512</td>
<td>27.7</td>
<td>1151</td>
<td>27.5</td>
</tr>
<tr>
<td>Coronary revascularizations</td>
<td>24</td>
<td>1.1</td>
<td>49</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Components of secondary outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHF</td>
<td>116</td>
<td>6.5</td>
<td>276</td>
<td>6.9</td>
</tr>
<tr>
<td>Fatal CHF (hospitalized)</td>
<td>86</td>
<td>4.7</td>
<td>195</td>
<td>4.7</td>
</tr>
<tr>
<td>Angina</td>
<td>223</td>
<td>12.1</td>
<td>518</td>
<td>12.8</td>
</tr>
<tr>
<td>Angina (hospitalized)</td>
<td>125</td>
<td>7.1</td>
<td>318</td>
<td>8.0</td>
</tr>
<tr>
<td>Coronary revascularizations</td>
<td>62</td>
<td>3.3</td>
<td>118</td>
<td>2.9</td>
</tr>
</tbody>
</table>

**Abbreviations as in Table 3.**

*Total events.

†Four-year event rate per 100 persons.
on such outcomes have been postulated to depend on the activity of the ACE enzyme and thereby the ACE I/D genotype, this study could find no evidence of any modifying effect of ACE I/D genotype group on the effects of study treatment.

Although not consistently associated with blood pressure or hypertension, the ACE DD genotype has been reported across many studies to be associated with MI, restenosis after coronary stenting, left ventricular hypertrophy, and renal complications in diabetes. Although GenHAT found that the prevalence of MI and left ventricular hypertrophy differed by the ACE I/D genotypes at baseline, the I/D variant did not prospectively identify individuals at risk of fatal and nonfatal CHD or other secondary cardiovascular or renal outcomes. Our prospective results are consistent with findings from the PROGRESS trial, a randomized study of perindopril versus placebo conducted in 6105 individuals with a history of stroke or transient ischemic attack; in that study, hypertension was not an a priori inclusion criterion. The PROGRESS investigators also found no association between the ACE genotype and the long-term risks of stroke, cardiac events, or all-cause mortality. It has been suggested that the ACE DD genotype might be associated with an increased overall death rate. In GenHAT, however, rates of death during the 5-year follow-up were not higher in one ACE I/D genotype group than any other.

The possible interactions between ACE I/D genotype and treatment with antihypertensive agents on blood pressure lowering, total mortality, regression of left ventricular hypertrophy, proteinuria, stroke, CHD and vascular events, and dementia and cognitive decline have been examined. Four of these studies suggested that the ACE DD genotype is more responsive to antihypertensive treatment; 2 studies suggested that the II genotype is more responsive; one study reports differences in blood pressure responses by genotype in men and women; and 4 studies report no significant ACE I/D genotype-by-treatment interactions. However, except for the PROGRESS trial, most studies were small (ie, <100 patients), were observational (ie, subjects were not randomized to a treatment), and were restricted to surrogate markers of cardiovascular risk (ie, blood pressure, left ventricular mass, or proteinuria). GenHAT could find no evidence that the demonstrated lack of beneficial effects of lisinopril-based therapy on cardiovascular and renal outcomes was influenced significantly by ACE I/D genotype group.

Figure 2. Kaplan-Meier plots for lisinopril vs amlodipine plus chlorthalidone by ACE I/D genotype (top, DD; bottom, II and ID) for CHD (left) and mortality (right).
Although no association between ACE I/D genotype group and blood pressure could be identified, it remained possible that postulated blood pressure–independent effects of ACE inhibition might be associated with serum and tissue ACE activity and therefore the ACE I/D genotype. We were able to test for associations between ACE I/D genotype groups and the incidence of predefined cardiovascular and renal outcomes. Overall, the likelihood of these outcomes did not appear to be influenced by the ACE I/D genotype group. Earlier reports of the association of ACE I/D genotype with MI were somewhat variable, and in GenHAT, there was no overall association between these events and ACE I/D genotype group.

The GenHAT study selected individuals on the basis of preexisting coronary risk factors and hypertension; therefore, there is some uncertainty about the applicability of the findings of this study to the association between ACE I/D genotype and risk among individuals without established disease. However, the findings do concur with the prospective analyses of the 348 primary strokes observed over 12 years in the Physicians’ Health Study, suggesting that the absence of association may be consistent across both first and recurrent events, and not a specific feature of our patient group. It is also unlikely that the absences of associations of outcomes with ACE I/D genotype group in GenHAT are a consequence of confounding or the particular ethnic groups studied because participants were randomized and ethnicity was balanced between drug classes.

We did find several differences in cardiovascular outcomes in response to therapy according to gender and diabetes status. Although these results are interesting, given the size of the probability value (0.02) and the number of tests performed (4 subgroups defined a priori times 12 outcomes times 2 genotype groups times 2 treatment comparisons equals 192 tests), these results must be interpreted with caution. Nonetheless, the findings for women were consistent for fatal and nonfatal CHD and CHD mortality and indicated a greater risk of events among DD women treated with lisinopril versus chlorthalidone or amlodipine (HR, 1.32) compared with an HR of 0.86 in DD men. Prior studies have documented the importance of estrogen on the renin-angiotensin system, including increased plasma renin activity, decreased ACE activity, and lower circulating levels of angiotensin II. In general, estrogen inhibits the action of angiotensin II and androgens exacerbate angiotensin II effects. The DD genotype, which is associated with greater...
levels of ACE, may predispose men to a greater benefit of ACE inhibition therapy. The different treatment responses in diabetics versus nondiabetics is interesting in light of the evidence suggesting that ACE inhibitor treatment is renal protective. Although some studies have reported varying response of proteinuria by ACE I/D genotype with ACE inhibition, others have not. No study has yet reported differences in the incidence of end-stage renal disease among hypertensives by ACE I/D genotype and multiple classes of antihypertensive agents. Further research is needed to understand whether these 2 findings are clinically meaningful.

A limitation of our study is the focus on one variant in the ACE gene, the ACE I/D polymorphism. We recognize that there may be additional variants in the ACE gene that may be important in determining the cardiovascular response to antihypertensive therapy, and we plan to add additional genetic variants in the next phase of analysis.

In summary, these analyses of GenHAT are among the most comprehensive assessments of the effects of the ACE I/D genotype group on clinical outcomes to date. The functional implications of variation in ACE enzyme activity in relation to genetic variation in the ACE I/D polymorphism do not appear to translate into significant phenotypic effects. These genotype analyses of GenHAT provide no rationale for using ACE I/D genotype to guide treatment decisions or to provide prognostic information relevant to cardiovascular or renal outcomes.

Acknowledgments
This work was supported by NIH Heart, Lung and Blood Institute grant 5 R01 HL-63082, Genetics of Hypertension Associated Treatment. The ALLHAT study was supported by a contract with the National Heart, Lung and Blood Institute.

Disclosure
Dr Black has served as a consultant to Biovail, First Horizon Pharmaceutical, MSD, Novartis, and Pfizer, and has served on the Speakers’ Bureaus of Biovail, Novartis, and Pfizer.

References


Pharmacogenetic Association of the Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism on Blood Pressure and Cardiovascular Risk in Relation to Antihypertensive Treatment: The Genetics of Hypertension-Associated Treatment (GenHAT) Study
Donna K. Arnett, Barry R. Davis, Charles E. Ford, Eric Boerwinkle, Cathie Leiendecker-Foster, Michael B. Miller, Henry Black and John H. Eckfeldt

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