Functional Variant of CYP4A11 20-Hydroxyeicosatetraenoic Acid Synthase Is Associated With Essential Hypertension

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Background—The CYP4A11 arachidonic acid monooxygenase oxidizes endogenous arachidonic acid (AA) to 20-hydroxyeicosatetraenoic acid (20-HETE), a metabolite with renovascular and tubular functions. Mice with targeted disruption of Cyp4a14, a murine homologue of CYP4A11, have severe hypertension. We combined molecular and biochemical approaches to identify a functional variant of the CYP4A11 20-HETE synthase and determine its association with hypertensive status in 2 independent human populations.

Methods and Results—A thymidine-to-cytosine polymorphism at nucleotide 8590 resulted in a phenylalanine-to-serine substitution at amino acid 434. Expression of cDNA with serine 434 resulted in a protein with a significantly reduced AA and lauric acid metabolizing activity. In a population of 512 whites from Tennessee, the age, body mass index, and gender-adjusted OR of having hypertension attributable to the 8590C variant was 2.31 (95% CI 1.41 to 3.78) compared with the reference 8590TT genotype. In subjects from the Framingham Heart Study, the adjusted ORs of hypertension associated with the 8590C variant were 1.23 (CI 0.94 to 1.59; n/H110051538) in all subjects and 1.33 (CI 1.01 to 1.77; n=1331) when subjects with diabetes were excluded. No association of the variant with hypertension was detected in a population of 120 blacks.

Conclusions—We identified a variant of the human CYP4A11 (T8590C) that encodes for a monooxygenase with reduced 20-HETE synthase activity. The association of the T8590C variant with hypertension supports its role as a polygenic determinant of blood pressure control in humans, and results obtained from the large population database suggest that the relevance of the variant may vary according to hypertension comorbidity. (Circulation. 2005;111:63-69.)

Key Words: genetics ■ hypertension, renal ■ lipids ■ metabolism
define their role in human hypertension. As a prerequisite, we
addressed the question of whether CYP4A22 had enzymatic
activity or a role in renal fatty acid metabolism. We present
data that CYP4A22 lacks functional activity in the kidney.
Therefore, we combined molecular and biochemical ap-
proaches to identify functional variants in CYP4A11 and test
their relevance to hypertension in 2 independent human
populations.

Methods

Subjects

Tennessee Cohort
Normotensive subjects were volunteers recruited through health
screening examinations performed primarily at Vanderbilt Uni-
versity. Subjects were defined as normotensive if they had seated
systolic and diastolic pressures of less than 140 and 90 mm Hg,
respectively, and no history of identification or treatment for hy-
ertension. Hypertensive subjects were ascertained from the Vanderbilt
Hypertension Center and had either established hypertension, de-
fined as antihypertensive treatment for >6 months, or a seated
diastolic pressure >90 mm Hg on at least 3 occasions. Diastolic
blood pressure was defined by the absence of Korotkoff sounds
(phase V). Secondary hypertension was excluded by history and
physical examination. Ethnicity was self-reported, and inclusion in a
particular ethnic group generally required that a proband’s parents
and grandparents recognized themselves by the same ethnic design-
ation as that of the proband. All subjects gave written consent, and
the Vanderbilt University Institutional Review Board approved the
protocol. All genomic samples were coded to protect donor identity.

Framingham Offspring Cohort
The design of the Framingham Heart Study has been detailed
previously.21 The offspring cohort comprises offspring (and their
spouses) of the original Framingham participants (recruited in 1948).
The Framingham Heart Study cohort is almost entirely white. Those
in the offspring cohort were invited to have follow-up laboratory and
physical and laboratory examinations approximately every 4 years
starting in 1971. DNA samples were obtained for 1920 individuals
from June 1987 to February 1991.21 Blood pressure measurements
were the mean of 2 physician-obtained readings taken
apart in the left arm of the seated subject with a mercury column
sphygmomanometer, in which appearance of Korotkoff sounds
(phase I) denoted systolic blood pressure and disappearance (phase
V) determined diastolic blood pressure.8 Hypertension was defined
as systolic blood pressure >140 mm Hg or diastolic blood pressure
>90 mm Hg or current use of antihypertensive medication.8 Diabe-
tes (type 2) was defined as use of hypoglycemic medication or
fasting plasma glucose levels >7 mmol/L (>125 mg/dL) at any of
6 examinations. Participants considered in the present study
(n=1880) are a subset of unrelated individuals who provided blood
samples for DNA extraction at the sixth examination cycle
(n=2933). Selection criteria were established to provide DNA
samples of biologically unrelated subjects with approximately
equal numbers of men and women who have characteristics similar
to the remainder of the participants. Included in this report were
1538 (82%) of the 1880 subjects genotyped for T8590C. All
subjects gave written consent, and the protocol was approved by the
Boston University Medical Center Institutional Review
Board. Further details of the Framingham Heart Study are
available online (http://www.nhlbi.nih.gov/about/framingham).

Polymorphism Discovery and Genotyping
In addition to single base differences found throughout the gene
sequences, CYP4A11 and CYP4A22 are distinguished by the pres-
cence in CYP4A11 of short nucleotide insertions (≤10 bp) in introns
1, 3, 4, and 11 and deletions (≤18 bp) in introns 4, 6, and 11.22
Polymerase chain reaction (PCR) amplification was performed with
intron-specific primers designed to maximize the difference between
the 2 genes and to generate overlapping CYP4A11 specific amplicons
covering all 12 exons and intervening introns.22 The selectivity of
this approach was corroborated by both sequence analysis and
restriction enzyme digestion with sites unique to the CYP4A11
gene.22 Primer sequences, reaction conditions, DNA extraction, and
genotyping methods can be found at www.bioventures.com. To
define CYP4A11 variants, both forward and reverse sequencing of
PCR products were performed in a screening subset (n=34) of
predominantly hypertensive subjects divided equally by gender and
ethnicity. Variants were numbered by nucleotide position in refer-
ence to the translation start site.

Biochemical Characterization
By reverse transcription–PCR of a mixture of total kidney RNAs
isolated from 15 nonsexed individuals (Clontech, Palo Alto, Calif.),
we obtained a single cDNA encoding CYP4A22 (GenBank acces-
sion: AY280371). CYP4A11 cDNA was expressed and protein
purified as described except the PD-10 column was replaced by
hydroxyapatite and 1% Emulgen 913 was replaced by 0.02% sodium
cholate.19 Membrane fractions were isolated by differential centrif-
gation.23 Partially purified proteins were dialyzed against Tris-Cl
buffer (pH 7.5) and kept at −80°C until use. Enzyme incubations were
performed at 35°C in 0.05 mol/L Tris-Cl buffer (pH 7.5) containing
10 mmol/L MgCl2, 1 mmol/L GSH, 1 mmol/L NADPH, 50 μg/mL
dilauroylphosphatidylcholine, 8 mmol/L isocitric acid, 0.5 IU/mL
isocitric dehydrogenase, and 70 to 100 μmol/mL [1-14C]labeled lauric
or AA (20 μCi/mmol).24 Purified recombinant P450os was mixed with
a 10-molar excess of purified liver P450 reductase and an equimolar
amount of purified cytochrome b₅ and incubated for 15 minutes at
room temperature before the addition of reaction components.23
Reaction products were resolved and quantified.19

Statistical Methods
To determine whether the CYP4A11 polymorphisms were in Hardy-
Weinberg equilibrium, actual and predicted genotype counts were
compared by a χ² test statistic with 2 degrees of freedom. Logistic
regression models were used to compute the ORs for the dichoto-
mous trait of hypertension (the presence or absence of hypertension)
with the genotype indicator variable(s) as the primary independent
variable. Three analytical models were used to consider the crude
relationship between these variables and adjustment for age, gender,
and body mass index (BMI). Analysis was performed for men and
women separately given that we previously observed a significant
effect of gender on hypertension in mice with targeted disruption of
Cyp4a11, a murine homologue of CYP4A11. The analysis of the
effect of diabetes on the CYP4A11 variant in the Framingham cohort
was prespecified on the basis of animal24 and human25 data that
suggest that insulin downregulates CYP4A4 expression, potentially
impacting the effect of the variant. Diabetes prevalence information
was not available for the Tennessee cohort. Descriptive data are
presented as mean±SD for continuous variables and as proportions
for categorical variables. ORs and 95% CIs are presented from
logistic regression analyses. A 2-tailed probability value of ≤0.05
was considered significant. Analyses were done with SPSS for
Windows (version 11.0, SPSS; Tennessee data) and SAS (release
8.2, SAS Institute Inc; Framingham data).

Results

CYP4A11 Is the Functional CYP4A 20-HETE
Synthase in Humans
CYP4A11 has been cloned, expressed, and characterized as a
renal 20-HETE synthase.19,20 However, the enzymatic activity
of the other human CYP4A gene product, CYP4A22, and
its role in renal fatty acid metabolism are unknown, and its
mRNA is detected in the kidney only after reverse transcription–PCR
amplification.22 Western blot analysis of the mem-
brane preparations from cells expressing CYPs 4A11 and
4A22 cDNAs showed the presence of CYP4A11 with ex-
CYP4A11 catalyzed the formation of 11- and 12-hydroxydocosanoic acids (not shown), which confirms that CYP4A11 lacks hydroxylase activity. Sequence comparisons showed that the glycine at position 130, conserved among all CYP4A isoforms, is replaced by serine in CYP4A22. Site-specific mutation of the CYP4A11 glycine residue 130 to serine resulted in a protein that lacked a functional heme group and was catalytically inactive (not shown).

**CYP4A11 Polymorphism Discovery**

Screening of CYP4A11 revealed 9 variants with allelic frequencies >1% (Table 1) and in Hardy-Weinberg equilibrium within ethnic groups (not shown). Additional variants with allele frequencies <1% and ethnic comparisons of variants are posted at www.bioventures.com. Of the 2 variants in the open reading frame, the cysteine-for-thymidine transition at nucleotide 8590 (exon 11) resulted in a nonsynonymous phenylalanine (F)-to-serine (S) substitution at residue 434 of CYP4A11, which was chosen for functional characterization.

**Functional Effects of the CYP4A11 Gene Polymorphism**

To determine the functional consequences of an F-to-S replacement at amino acid 434, we generated the corresponding cDNA by site-specific mutation, expressed it in Escherichia coli, partially purified it, and compared the fatty acid hydroxylating activities of the wild-type (F434) and mutated (S434) proteins. As shown in Table 2, compared with F434 (wild type), the S434 replacement reduced by more than half the 20-HETE synthase activity of CYP4A11, with only a small effect on its apparent $K_m$ values and thus, its affinity for AA (Table 2). Similarly, the apparent $V_m$ of the lauric acid conversion to 12-hydroxylaurate was reduced by more than half, but for this fatty acid, there was also a significant reduction in apparent $K_m$ values (Table 2). These results suggest that the T8590C variant, which corresponds to the F434S protein polymorphism, affects the catalytic activity of the 20-HETE synthase through a loss-of-function mechanism.

**The CYP4A11 8590 C Allele is Associated With Hypertension**

**Tennessee Cohort**

To determine whether an association exists between the CYP4A11 variant and hypertension, each of 512 subjects were genotyped at the T8590C (also referred to as F434S) locus (blacks: hypertensive n=60, normotensive, n=60; and whites: hypertensive n=197, normotensive n=195). As displayed in Table 3, age was similar by blood pressure status and between gender in each ethnic group. BMI and mean arterial pressure were greater in hypertensive than in normotensive subjects but were not different between genders. All subjects with hypertension were receiving antihypertensive medications. Compared with the reference 8590TT genotype, there was no association of the 8590C allele with hypertension in blacks (Table 4). In whites, the 8590C allele was associated with an increased prevalence of hypertension after adjustment for age, gender, and BMI in both men and women (OR 2.31, 95% CI 1.41 to 3.78). No significant interaction between gender and genotype was detected, nor were there...
significant differences in age and BMI across genotype groups.

**Framingham Offspring Cohort**

To test the hypothesis raised in the initial study findings in an independent cohort and to further characterize the association of the CYP4A11 8590C (434S) variant with hypertension, we characterized this variant in the large population-based Framingham cohort (n=1538). Table 5 shows baseline characteristics of male and female subjects.21 Approximately 50% of the men and 40% of the women were hypertensive, and 60% to 75% of them were taking antihypertensive medications. Table 6 shows that the OR of having hypertension attributable to the 8590C allele was 1.21 (95% CI 0.93 to 1.59) after adjustment for age, BMI, and gender was 1.23 (95% CI 0.94 to 1.59) with a model that additionally adjusted for cigarette and alcohol use. After exclusion of diabetics, the OR tended to be greater for cigarette and alcohol use. After exclusion of diabetics, the OR was 1.56 (95% CI 0.94 to 1.59) after adjustment for age, BMI, and gender and was 1.23 (95% CI 0.92 to 1.66/P=0.06) with a model that additionally adjusted for cigarette and alcohol use. After exclusion of diabetics, the ORs were 1.31 (95% CI 0.99 to 1.74) and 1.33 (95% CI 1.01 to 1.77), respectively. ORs tended to be greater in men than in women, but no significant interaction between gender and CYP4A11 genotypes was detected.

**Discussion**

In the present study, we have provided evidence that although CYP4A11 functions as a 20-HETE synthase, CYP4A22 does not contribute to renal 20-HETE biosynthesis. Furthermore, to determine the relevance of genetic variation in CYP4A11 to hypertension, we identified a coding polymorphism (T8590C), biochemically characterized 8590C as a loss-of-function variant, and demonstrated its association with hypertension in whites. Initial findings of associations between biologically plausible functional variants are often not replicated in follow-up studies, and even when associations are replicated, they are often less strong than in the initial validation cohort.27 For this reason, we conducted a study of replication in a population-based cohort of whites. Our results suggest the existence of a modest association finding in the Framingham cohort. Clearly, our findings strongly support further study of the relationship of variants in this gene with hypertension in other populations.

Both animal14,16 and human data support CYP4A11 as a candidate gene in hypertension. Numerous studies in the spontaneously hypertensive and Dahl salt-sensitive rats have shown an association between CYP4A 20-HETE synthase expression levels and/or activity and (1) altered nephron-specific transport of sodium chloride and (2) increased blood pressure.14,16 These studies support the contention that 20-HETE exerts prohypertensive and antihypertensive actions that are critically dependent on the site of biosynthesis and action along the nephron.14,17 In the renal tubule, 20-HETE blocks sodium transport and functions primarily as a natriuretic, antihypertensive substance.16,17 In contrast, 20-HETE acting in the renal vasculature has vasoconstricting, prohypertensive properties.16 Analyses of CYP4A expression and of 20-HETE synthase activity demonstrated abundant CYP4A expression and enzyme activity in the proximal and distal tubule, 2 segments of the rat nephron identified as

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**TABLE 1. Selected CYP4A11 Polymorphisms**

<table>
<thead>
<tr>
<th>No.</th>
<th>Common → Minor Variant</th>
<th>Position</th>
<th>Region</th>
<th>Flanking Region (Minor Allele Capitalized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A→G</td>
<td>−845</td>
<td>Promoter</td>
<td>gtgtaattac G tactatggta</td>
</tr>
<tr>
<td>2</td>
<td>T→A</td>
<td>−34</td>
<td>Exon 1</td>
<td>cagagagag G aagggccacct</td>
</tr>
<tr>
<td>3</td>
<td>A→G</td>
<td>4265</td>
<td>Intron 3</td>
<td>tccctctctt G acaagaccc</td>
</tr>
<tr>
<td>4</td>
<td>A→G</td>
<td>4271</td>
<td>Intron 3</td>
<td>ttctaaacag G cccctacccce</td>
</tr>
<tr>
<td>5</td>
<td>A→T</td>
<td>5670</td>
<td>Intron 4</td>
<td>gatacttgca T gctacaggaa</td>
</tr>
<tr>
<td>6</td>
<td>C→T</td>
<td>7026</td>
<td>Intron 7</td>
<td>aaggccgtga C tttacaccgc</td>
</tr>
<tr>
<td>7</td>
<td>C→T</td>
<td>7119</td>
<td>Exon 8</td>
<td>tgtagagccca T gcacaccag</td>
</tr>
<tr>
<td>8</td>
<td>T→G</td>
<td>8590</td>
<td>Exon 11</td>
<td>tttgacccct C cgttttgcA</td>
</tr>
<tr>
<td>9</td>
<td>A→T</td>
<td>11367†</td>
<td>Exon 12</td>
<td>ctgtctgcce T tatctgttt</td>
</tr>
</tbody>
</table>

Nine single-nucleotide polymorphisms in CYP4A11 are shown with variant name, position, and 10-base flanking regions. Positions of each variant are numbered relative to the translational start site.

*Results in nonsynonymous amino acid change of phenylalanine to serine (codon 434).
†Noncoding portion of exon.

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**TABLE 2. Kinetic Properties of the Fatty Acid Hydroxylase Activities of Wild Type and the Serine434 Variant CYP4A11**

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$, μmol/L</td>
<td></td>
<td>$V_m$, min$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lauric Acid</td>
<td>AA</td>
<td>Lauric Acid</td>
<td>AA</td>
<td></td>
</tr>
<tr>
<td>Wild type (phenylalanine 434)</td>
<td>11±1.0</td>
<td>37±3.0</td>
<td>20±2.0</td>
<td>2.2±0.06</td>
<td></td>
</tr>
<tr>
<td>Variant (serine 434)</td>
<td>5±0.3*</td>
<td>25±2.0</td>
<td>7±1.0†</td>
<td>0.9±0.06‡</td>
<td></td>
</tr>
</tbody>
</table>

Values represent average of 8 or 3 different experiments for AA and lauric acid, respectively, ±SEM.

Significance vs wild type: *P=1.66×10$^{-4}$; †P=4.8×10$^{-6}$; ‡P=1.5×10$^{-4}$. 
targets for the natriuretic, antihypertensive actions of 20-HETE.14-17 The relevance of these observations to the pathophysiology of human hypertension has been highlighted by measurements of urinary sodium and 20-HETE excretion that show the ability of dietary salt to regulate 20-HETE excretion28 and impairment of 20-HETE-dependent natriuretic mechanisms in salt-sensitive compared with salt-resistant hypertensive patients.29 Moreover, recent studies of the natriuretic response to furosemide in humans suggested that 20-HETE modulates its natriuretic effects and that altered triuretic response to furosemide in humans suggested that altered triuretic response to furosemide in humans suggested increased 20-HETE synthase activity. In contrast, such an overt effect of gender was not detected in the present study, and although CYP4A11 accounts for significant renal 20-HETE synthase in humans, the role that androgens play in regulating its renal expression is yet to be defined. Nevertheless, the possibility exists that additional, non-CYP4A-dependent ω-hydroxylases may contribute to 20-HETE biosynthesis in the human kidney.17

The use of the Framingham cohort allowed us to address the effects of gender and phenotypic context. Significant gender differences have long been recognized in both the prevalence of hypertension and target-organ damage attributable to increased blood pressure, the mechanisms of which are largely unknown.20 The rationale to investigate the effect of gender was prompted by the finding that hypertension in Cyp4a14 (−/−) mice, a human homologue of CYP4A11, is sexually dimorphic and androgen sensitive.18 Although Cyp4a14 does not metabolize arachidonic acid, Cyp4a14 (−/−) mice had an upregulation of Cyp4a12 that resulted in increased 20-HETE synthase activity. In contrast, such an overt effect of gender was not detected in the present study, and although CYP4A11 accounts for significant renal 20-HETE synthase in humans, the role that androgens play in regulating its renal expression is yet to be defined. Nevertheless, the possibility exists that additional, non-CYP4A-dependent ω-hydroxylases may contribute to 20-HETE biosynthesis in the human kidney.17

The well-characterized nature of the Framingham cohort allowed us to analyze the effect of the CYP4A11 variant without a potential confounding influence of diabetes. Both animal24 and human studies25 have suggested that diabetes induces and insulin suppresses CYP4A expression. We hypothesized that the effects of a loss-of-function CYP4A variant may be muted in the context of altered glucose/insulin

### TABLE 3. Tennessee Cohort: Baseline Characteristics of Black and White Subjects by Gender and Presence or Absence of Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blacks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>34.5±8.7</td>
<td>38.2±7.2</td>
<td>37.1±8.8</td>
<td>38.9±7.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.8±4.6</td>
<td>33.0±6.1*</td>
<td>26.4±3.8</td>
<td>31.6±8.6*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>90.2±7.4</td>
<td>108.9±11.7*</td>
<td>86.1±9.1</td>
<td>110.2±16.1*</td>
</tr>
<tr>
<td><strong>Whites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>39.7±12.4</td>
<td>42.1±10.7</td>
<td>42.0±11.0</td>
<td>44.0±9.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6±4.1</td>
<td>29.3±6.0*</td>
<td>23.5±3.8</td>
<td>29.3±7.0*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>89.7±7.9</td>
<td>105.0±17.2*</td>
<td>86.2±8.3</td>
<td>105.3±14.4*</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure.

Data are mean±SD.

*P< 0.001 vs normotensive subjects within ethnic group.

### TABLE 4. Tennessee Cohort: Genotype and Allele Frequencies of T8590C Variant by Ethnicity, Gender, and Presence or Absence of Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blacks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8590 C allele frequency</td>
<td>0.26</td>
<td>0.32</td>
<td>0.29</td>
<td>0.33</td>
</tr>
<tr>
<td>Adjusted OR (95% CI)*</td>
<td>0.95 (0.29–3.11)</td>
<td>1.32 (0.59–2.94)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Whites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8590 CC, n (%)</td>
<td>2 (2.0)</td>
<td>2 (2.0)</td>
<td>2 (2.0)</td>
<td>3 (3.1)</td>
</tr>
<tr>
<td>8590 CT, n (%)</td>
<td>18 (18.2)</td>
<td>31 (31.3)</td>
<td>23 (23.5)</td>
<td>33 (34.4)</td>
</tr>
<tr>
<td>8590 TT, n (%)</td>
<td>79 (79.8)</td>
<td>66 (66.7)</td>
<td>73 (74.5)</td>
<td>60 (62.5)</td>
</tr>
<tr>
<td>8590 C allele frequency</td>
<td>0.11</td>
<td>0.18</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>Adjusted OR (95% CI)*</td>
<td>2.15 (1.08–4.26)</td>
<td>2.56 (1.24–5.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adjusted OR (95% CI), combined men and women</strong></td>
<td>2.31 (1.41–3.78)†</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ORs (95% CIs)** were computed for 8590 CC+CT vs TT (reference) genotype by logistic regression and adjusted for *age, BMI, and gender where appropriate.

†P=0.001; 8590C variant: blacks vs whites, all \( \chi^2 = 28.6; 2df, P<0.001. \)
regulation (eg, diabetes). That contention is supported by the Framingham data, which show a significant association of the CYP4A11 variant with hypertension in the subpopulation without diabetes. These data serve to focus future validation studies and demonstrate the utility of a large, well-characterized genetic resource in the characterization of polygenic effects underlying hypertension. The apparent differences in the relationship of the variant and hypertension between the Tennessee and Framingham cohorts are not known but may relate to population-specific factors. The prevalence of diabetes in the Tennessee cohort was not known, and hypertensive subjects tended to be younger, heavier, and to have higher mean blood pressures than those in the Framingham cohort.

The lack of a significant association of the CYP4A11 variant and hypertension in blacks is somewhat surprising, despite an increased allelic frequency of the CYP4A11 variant in blacks compared with whites, given the potential link of 20-HETE to salt-sensitive hypertension28,29 and the reported greater prevalence of salt-sensitive hypertension in blacks.31 This fact could be due to a limited sample size of blacks or possibly to population stratification, a limitation inherent to the population case-control study design.32 Given the data in the present study that establish a significant loss of CYP4A11 metabolizing activity with the 8590C variant, there exists the possibility that other genetic or physiological factors modify the activity of the CYP4A system in this ethnic group. Further studies are required to confirm the lack of association of the CYP4A11 variant in blacks and to address CYP4A regulation.

In summary, we have identified a coding variant of the human CYP4A11 gene that results in a CYP4A11 protein

TABLE 5. Framingham Offspring Cohort: Baseline Characteristics of Subjects by Gender and Presence or Absence of Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotensive (n = 402)</td>
<td>Hypertensive (n = 367)</td>
</tr>
<tr>
<td>Age, y</td>
<td>56.7 ± 9.2</td>
<td>63.3 ± 8.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.3 ± 4.5</td>
<td>29.0 ± 4.1</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>90.0 ± 7.5</td>
<td>100.5 ± 11.0*</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure. Data are mean ± SD.

*P < 0.001 vs normotensive subjects.

TABLE 6. Framingham Offspring Cohort: Genotype and Allele Frequencies of T8590C Variant by Gender and Presence or Absence of Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotensive (n = 402)</td>
<td>Hypertensive (n = 367)</td>
</tr>
<tr>
<td>Genotype/variant</td>
<td>Genotype/variant</td>
<td>Genotype/variant</td>
</tr>
<tr>
<td>8590 CC, n (%)</td>
<td>3 (0.8)</td>
<td>7 (1.9)</td>
</tr>
<tr>
<td>8590 CT, n (%)</td>
<td>83 (20.6)</td>
<td>94 (25.6)</td>
</tr>
<tr>
<td>8590 TT, n (%)</td>
<td>316 (78.6)</td>
<td>266 (72.5)</td>
</tr>
<tr>
<td>8590 C allele frequency</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>Adjusted*</td>
<td>1.29 (0.90–1.84)</td>
</tr>
<tr>
<td></td>
<td>Adjusted†</td>
<td>1.30 (0.90–1.87)</td>
</tr>
<tr>
<td>Combined men and women, OR (95% CI)</td>
<td>Adjusted*</td>
<td>1.21 (0.93–1.56)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>Adjusted*</td>
<td>1.40 (0.94–2.09)</td>
</tr>
<tr>
<td></td>
<td>Adjusted†</td>
<td>1.43 (0.95–2.14)</td>
</tr>
<tr>
<td>Nondiabetics n</td>
<td>354</td>
<td>292</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>Adjusted*</td>
<td>1.31 (0.99–1.74)</td>
</tr>
<tr>
<td></td>
<td>Adjusted†</td>
<td>1.33 (1.01–1.77)</td>
</tr>
</tbody>
</table>

ORs (95% CIs) were computed for 8590 CC + CT vs TT (reference) genotype by logistic regression and adjusted for age, BMI, and gender, where appropriate, and for age, BMI, cigarettes, alcohol use, and menopause and estrogen usage in women and for gender, where appropriate.
with reduced 20-HETE synthase activity and that has a greater prevalence in hypertensive compared with normotensive whites. These results, in conjunction with published studies showing abundant renal CYP4A11 expression\(^2\) and antihypertensive, natriuretic effects of 20-HETE,\(^6,17\) provide compelling evidence that a genetically controlled alteration in CYP4A11 expression or activity plays a role as a determinant of polygenic blood pressure control in humans.

Acknowledgments

This work was supported by DK38226 (Dr Capdevila), DK28350 (Dr Waterman), HL04221, RR00095 (Dr Gainer), HL65193, HL67308, HL60906 (Dr Brown), and N01-HC-25195 (Dr O’Donnell). cDNAs were sequenced using facilities supported by the National Cancer Institute (CA68485).

References

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James V. Gainer, Aouatef Bellamine, Elliott P. Dawson, Kristie E. Womble, Sarah W. Grant, Yarong Wang, L. Adrienne Cupples, Chao-Yu Guo, Serkalem Demissie, Christopher J. O'Donnell, Nancy J. Brown, Michael R. Waterman and Jorge H. Capdevila

Circulation. published online December 20, 2004;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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