The V433M Variant of the CYP4F2 Is Associated With Ischemic Stroke in Male Swedes Beyond Its Effect on Blood Pressure

Cristiano Fava, Martina Montagnana, Peter Almgren, Lena Rosberg, Giuseppe Lippi, Bo Hedblad, Gunnar Engström, Göran Berglund, Pietro Minuz, Olle Melander

Abstract—Cytochrome (CYP) 4A11 and CYP4F2 are responsible for renal production of 20-hydroxyeicosatetraenoic acid, a vasoconstrictor and natriuretic substance. The CYP4A11 F434S and CYP4F2 V433M polymorphisms reduce 20-hydroxyeicosatetraenoic acid production in vitro. The aim of the present study was to evaluate the effect of these polymorphisms on blood pressure (BP) levels, hypertension prevalence, and risk of incident cardiovascular events in middle-aged Swedes. The polymorphisms were genotyped in the cardiovascular cohort of the Malmö Diet and Cancer Study. The incidence of cardiovascular events (coronary events, n=276; ischemic stroke, n=199) was monitored over 10 years of follow-up. The analysis of BP levels was performed twice: either excluding or including subjects under antihypertensive treatment. In the whole population, CYP4A11 S434S homozygotes had higher systolic BP, both crude and adjusted for the number of antihypertensive drugs, and higher prevalence of hypertension with respect to F434 carriers. Male, but not female, CYP4F2 M433 carriers had significantly higher crude and adjusted systolic and diastolic BP levels and a trend toward higher hypertension prevalence (P=0.06) with respect to V433M homozygotes. After adjustment for major cardiovascular risk factors, the hazard ratio for incident ischemic stroke in male CYP4F2 M433 carriers was significantly higher with respect to V433M homozygotes (hazard ratio: 1.69; 95% CI: 1.10 to 2.60) even when baseline BP levels and hypertension prevalence were included in the Cox proportional hazard model. A common CYP4F2 V433M polymorphism might increase the risk of incident ischemic stroke in male subjects only partially through its elevating effect on BP. Additional studies are needed to confirm these data. (Hypertension. 2008;52:373-380.)

Key Words: cytochrome P450 ■ 20-HETE ■ hypertension ■ stroke ■ genetics

During the last 3 decades, in different animal models, metabolites of arachidonic acid derived via cytochrome (CYP) P450 have been shown to play a pivotal role in the control of systemic and renal vascular tone and salt excretion. In particular, 20-hydroxyeicosatetraenoic acid (20-HETE) is involved in modulation of ion transport in the proximal tubules and thick ascending limb of the Henle’s loop, promoting natriuresis. In the human kidney, 20-HETE is generated through 2 isoforms, CYP4A11 and CYP4F2. Interestingly, mice with targeted disruption of CYP4A14, a murine homologue of CYP4A11, have a severe form of hypertension that has been shown to be testosterone dependent, suggesting a complex interplay between CYPs, sex hormones, and blood pressure (BP). A few studies in humans have tried to unravel the relation between 20-HETE excretion and salt sensitivity, and, more recently, it was found that patients with renovascular and essential hypertension had a diminished excretion of 20-HETE with respect to normotensive control subjects. Functional studies have shown that 2 nonsynonymous single nucleotide polymorphisms (SNPs) resulting in a phenylalanine-to-serine substitution at amino acid 434 (F434S) for the CYP4A11 and in a methionine-to-valine substitution at amino acid 433 (V433M) of the CYP4F2 lead to proteins with a significantly reduced arachidonic acid metabolizing activity. Moreover, the CYP4A11 F434S polymorphism has been associated with hypertension prevalence and/or with higher systolic BP in different samples. For the CYP4F2 V433M polymorphism, only 1 study exists to evaluate its effect on BP, whereas the potential role of both polymorphisms on cardiovascular events has never been evaluated. The aim of the present study was to test the association of the CYP4A11 F434S and of the CYP4F2 V433M polymorphisms with BP levels, hypertension prevalence, and incidence of cardiovascular events in a large, urban-based population of middle-aged Swedes.

Materials and Methods
All of the study participants had given written informed consent. The ethics committee of the medical faculty of Lund University approved...
the study. The procedures were in accordance with the institutional guidelines. The study population is derived from the Malmo Diet and Cancer Study (MDC; please see the data supplement available online http://hyper.ahajournals.org). BP and other cardiovascular risk factors were measured in a random subsample referred to as the MDC cardiovascular arm (MDC-CVA; n=6103). Successfully extracted genomic DNA was available from 6053 MDC-CVA participants. The study of BP as a continuous variable in MDC-CVA was performed both excluding people with antihypertensive medication (AHT; n=990 for the CYP4A11 and n=962 for the CYP4F2) and in the entire cohort (n=5975 successfully genotyped subjects). To overcome the possibility that a biased selection might result from selecting only individuals who were free of antihypertensive treatments, we conducted an analysis adjusting the systolic BP and diastolic BP of hypertensive individuals who were taking antihypertensive drugs at the time of investigation by 2 methods recently reviewed by Harrap et al (please see the data supplement). Phenotyping
BP was measured twice after 10 minutes of rest in the supine position by specially trained nurses on the right brachial artery using a mercury sphygmomanometer. The systolic BP was defined by phase I and the diastolic BP defined by phase V Korotkoff sounds. “Hypertension” in the MDC-CVA was defined as being on antihypertensive treatment or having systolic BP or diastolic BP \( \geq 140/90 \) mm Hg according to current diagnostic criteria and “normotension” as having systolic BP and diastolic BP \( <140/90 \) mm Hg. Genotype frequencies were also compared between individuals with clinically diagnosed hypertension (under treatment or answering yes to diagnosed hypertension according to questionnaire; n=1944 for the CYP4A11 and n=1884 for the CYP4F2) and individuals with normal BP (BP \( <130/85 \) mm Hg; n=1190 for the CYP4A11 and n=1140 for the CYP4F2).

Table 1. Anthropometric and Metabolic Features of the Individuals With and Without Ischemic Events

<table>
<thead>
<tr>
<th></th>
<th>MDC-CVA: All Subjects With at Least a Valid CYP Genotype (n=6002)</th>
<th>Subjects Free From Ischemic Cardiovascular Events (n=5554)</th>
<th>Coronary Events* (n=276)</th>
<th>Ischemic Strokes* (n=199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male %</td>
<td>40.7</td>
<td>63.6†</td>
<td>55.3†</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>57.2±5.9</td>
<td>60.2±5.3†</td>
<td>61.1±4.8†</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²§</td>
<td>25.8±3.9</td>
<td>26.8±4.1†</td>
<td>27.3±4.6†</td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>140.4±18.7</td>
<td>151.9±19.7†</td>
<td>152.7±19.6†</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>86.6±9.3</td>
<td>90.8±9.8†</td>
<td>92.2±10.2†</td>
<td></td>
</tr>
<tr>
<td>Obesity, %§</td>
<td>12.6</td>
<td>19.4†</td>
<td>25.3†</td>
<td></td>
</tr>
<tr>
<td>Diabetes, %</td>
<td></td>
<td></td>
<td>8</td>
<td>21.1†</td>
</tr>
<tr>
<td>Smoking habit, %¶</td>
<td>27.3</td>
<td>40.2†</td>
<td>31.5†</td>
<td></td>
</tr>
<tr>
<td>History of previous cardiovascular event, %</td>
<td>1.9</td>
<td>8.6†</td>
<td>8.9†</td>
<td></td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>62</td>
<td>84.3†</td>
<td>86†</td>
<td></td>
</tr>
<tr>
<td>Waist, cm#</td>
<td>83.65±12.75</td>
<td>90.58±13.30†</td>
<td>89.05±13.13†</td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L#</td>
<td>5.15±1.30</td>
<td>5.88±2.49†</td>
<td>5.84±2.38†</td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mmol/L#</td>
<td>6.17±1.08</td>
<td>6.26±1.04†</td>
<td>6.10±1.0</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L#</td>
<td>1.36±0.74</td>
<td>1.56±0.73†</td>
<td>1.60±0.88†</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L#</td>
<td>1.39±0.37</td>
<td>1.21±0.36†</td>
<td>1.24±0.37†</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L#</td>
<td>4.16±0.99</td>
<td>4.34±0.99†</td>
<td>4.16±0.90</td>
<td></td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein. Data are number or mean±SD. 
*A total of 27 subjects had both coronary and ischemic stroke events. 
†P<0.001 vs subjects free from ischemic cardiovascular events. 
‡P<0.01 vs subjects free from ischemic CV events. 
§A total of 7 subjects were not included because of missing values. 
¶A total of 545 subjects were not included because of missing values. 
#A total of 318 subjects were not included because of missing values.

Follow-Up and Definition of End Points
All of the subjects were followed from the baseline examination until the first cardiovascular event, death, or December 31, 2003. Mean follow-up was 10.3±2.1 years. A cardiovascular event was defined as fatal or nonfatal myocardial infarction (International Classification of Diseases, 9th Revision, code 410), death because of chronic ischemic heart disease (International Classification of Diseases, 9th Revision, code 412 or 414) or fatal or nonfatal stroke.
Classification of Diseases, 9th Revision, code 434 or 436). In 276 cases, the cardiovascular event was a coronary event (fatal: n=89; nonfatal: n=187), and in 199 cases the cardiovascular event was an ischemic stroke (fatal: n=9; nonfatal: n=190). The end points were retrieved by data linkage with the national Swedish Hospital Discharge and Cause of Death Registers and local stroke and myocardial infarction registers of Malmö.16

**Genotyping**

Genotypes of the CYP4A11 F434S polymorphism (database SNP accession No. rs4233507) and the CYP4F2 V433M polymorphism (database SNP accession No. rs2108622) were determined by endpoint fluorescent measurements (please see the extended Methods section in the data supplement).17

**Statistics**

Continuous variables are presented as the means±SDs. All of the data, except for the power analysis, were analyzed with SPSS statistical software (version 14.0; SPSS Inc). Power calculation was performed using the Power and Sample Size calculator version 2.1.31 (Vanderbilt University Medical Center).

Frequency differences and deviation from Hardy-Weinberg equilibrium were analyzed by χ² test. Significance of differences in continuous variables was tested by ANOVA followed by Turkey’s test and t test. Multiple logistic regression analyses were used in the multivariate models with hypertension status as dependent variables and genotype, age, sex, BMI, and the interaction variables (computed by multiplying the genotype with age, sex, and BMI, respectively) as independent variables.

Kaplan-Meier curves and log-rank tests compared cumulative incidence of ischemic strokes and coronary events in carriers of different genotypes. Age-, sex-, and BMI-adjusted Cox proportional hazard models were used to study the relationships between the polymorphisms and time (in years) to first cardiovascular events. The fit of the proportional hazards model was confirmed by plotting the cardiovascular incidence rates over time. Hazard ratios (HRs) and 95% CIs were calculated for variables with skewed distributions, log-normalized values were used in the analysis. All of the tests were 2 sided, and P values <0.05 were considered statistically significant.

**Results**

Information about genotyping success rate, genotype frequency, Hardy-Weinberg equilibrium, and the statistical power of the study can be found in the online Results section. Clinical characteristics of all of the participants are presented in Table 1.

Excluding subjects under chronic AHT, we found no difference either in systolic or diastolic BP in subjects carrying different genotypes (data not shown; P > 0.05 for all). When the analysis was repeated including people with AHT, CYP4A11 S434S homozygotes had higher crude and adjusted systolic BP, higher adjusted diastolic BP, and higher prevalence of hypertension respect to CYP4A11 F-carriers (F434F→F434S; Tables 2 and 3). No evidence of interaction between the CYP4A11 F434S polymorphism and age, sex, and BMI with respect to either BP level or hypertension prevalence was found (see Table S1).

In the entire cohort CYP4F2 M-carriers (M433M+V433M) had higher adjusted systolic and diastolic BP (the latter only for the stepped addition) and no difference in hypertension prevalence (P = 0.90) with respect to V433V homozygotes (Table 2). A positive interaction was detected between CYP4F2 V433M and male sex with respect to both

### Table 2. Crude and Adjusted BP According to Genotypes

<table>
<thead>
<tr>
<th>Type of BP Adjustment for AHT</th>
<th>CYP4A11 F434F (n=4589), Mean±SD</th>
<th>CYP4A11 F434S (n=1275), Mean±SD</th>
<th>CYP4A11 S434S (n=111), Mean±SD</th>
<th>CYP4A11 F-Carriers (n=5864), Mean±SD</th>
<th>P, ANOVA</th>
<th>P, t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonadjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>114.43±19.10</td>
<td>140.80±19.25*</td>
<td>145.71±19.03</td>
<td>141.29±19.13</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>87.02±9.46</td>
<td>86.70±9.51</td>
<td>88.67±9.51</td>
<td>86.95±9.47</td>
<td>0.06</td>
<td></td>
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<tr>
<td>Fixed addition</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>134.12±20.37*</td>
<td>142.30±20.40*</td>
<td>147.96±21.05</td>
<td>142.94±20.38</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>87.86±10.09</td>
<td>87.45±9.98*</td>
<td>89.79±10.46</td>
<td>87.77±10.07</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Stepped addition</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>143.05±20.43*</td>
<td>142.23±20.43*</td>
<td>147.82±20.99</td>
<td>142.88±20.43</td>
<td>0.01</td>
<td></td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>87.99±10.36</td>
<td>87.54±10.19</td>
<td>89.91±10.85</td>
<td>87.89±10.32</td>
<td>0.04</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of BP Adjustment for AHT</th>
<th>CYP4F2 V433V (n=3156)</th>
<th>CYP4F2 V433M (n=2247)</th>
<th>CYP4F2 M433M (n=389)</th>
<th>CYP4F2 M-Carriers (n=2636)</th>
<th>P, ANOVA</th>
<th>P, t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonadjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>141.02±18.94</td>
<td>141.79±19.15</td>
<td>142.88±19.62</td>
<td>141.95±19.22</td>
<td>0.06</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>86.85±9.41</td>
<td>87.24±9.42</td>
<td>86.97±9.64</td>
<td>87.20±9.45</td>
<td>0.16</td>
<td></td>
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<tr>
<td>Fixed addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>142.57±20.07</td>
<td>143.56±20.57</td>
<td>144.76±21.04</td>
<td>143.74±20.64</td>
<td>0.03</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>87.63±9.97</td>
<td>88.13±10.08</td>
<td>87.91±10.36</td>
<td>88.10±10.12</td>
<td>0.08</td>
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<tr>
<td>Stepped addition</td>
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<td></td>
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</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>142.48±20.11</td>
<td>143.53±20.65</td>
<td>144.72±21.11</td>
<td>143.70±20.72</td>
<td>0.02</td>
<td></td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>87.72±10.20</td>
<td>88.29±10.37</td>
<td>88.10±10.70</td>
<td>88.26±10.42</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 by Turkey’s posthoc test vs CYP4A11 S434S homozygotes.
Carriers of CYP4A11 mode of inheritance. The prevalence of hypertension in polymorphism in men according to an autosomal dominant recessive mode of inheritance and for the polymorphism with hypertension according to an autosomal significant association for the CYP4A11 association and individuals with BP homozygotes (Table 4).

BP level and hypertension prevalence (see Table S2). Stratifying the population according to sex, male CYP4F2 M-carriers showed a trend toward higher hypertension prevalence (P=0.06) (Table 3) and higher crude and adjusted systolic and diastolic BP with respect to male V433V homozygotes (Table 4).

A comparison between patients with a history of hypertension and individuals with BP =130/85 mm Hg confirmed the significant association for the CYP4A11 F434S polymorphism with hypertension according to an autosomal recessive mode of inheritance and for the CYP4F2 V433M polymorphism in men according to an autosomal dominant mode of inheritance. The prevalence of hypertension in CYP4A11 S434S homozygotes was 74.2% versus 61.8% in carriers of ≥1 F-allele (S434F+F434F; P=0.046) and 76.2% in male CYP4F2 M-carriers versus 67.4% in V433V homozygotes (P=0.001).

Logistic regression analysis using age, sex, and BMI as covariates showed a doubled risk of being hypertensive for CYP4A11 S434S homozygotes compared with F-carriers (odds ratio: 2.02; 95% CI: 1.07 to 3.78; P=0.029; see Table S1) and a 50% higher risk of being hypertensive for male carriers of the CYP4F2 M-carriers (odds ratio: 1.51; 95% CI: 1.14 to 1.98; P=0.003). We found no difference in the incidence of coronary events and ischemic strokes in carriers of different CYP4A11 F434S genotypes, whereas a higher incidence of ischemic strokes was evident in male CYP4F2 M-carriers versus V433V homozygotes (6.5% versus 3.8%; P=0.006 by log-rank test; see Figure).

Using cyclooxygenase regression, the independent association of the CYP4F2 polymorphism with incident ischemic strokes in male subjects was significant in all of the models tested taking into account age, BMI, history of previous cardiovascular event, diabetes and metabolic syndrome (National Cholesterol Education Program Adult Treatment Panel III criteria) prevalence at baseline, smoking habit, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, glucose, triglycerides, and waist circumference (see Table 5). When BP levels and hypertension prevalence were also included into the cyclooxygenase regression model, the association of the polymorphism with ischemic stroke remained significant.

**Discussion**

The main result of this work is that a common functional polymorphism of the CYP4F2, V433M, is associated with BP level, hypertension prevalence, and ischemic strokes in an urban population-based sample of middle-aged Swedes, but only in men. We indeed confirm a role of the CYP4A11 F434S polymorphism in systolic BP and hypertension prevalence.

A potential role of metabolites of arachidonic acids via CYP450 in BP homeostasis is suggested by a large quantity of experimental work in different rats strains and knockout mice and only recently in humans. 20-HETE is both a vasoconstrictor and a natriuretic substance so that a dual role in hypertension could be hypothesized according to the site of action. According to the renal natriuretic property, it was found recently that hypertensive subjects have a diminished excretion of 20-HETE compared with normotensive control subjects, whereas salt-sensitive subjects cannot augment their 20-HETE excretion in response to a salt load versus salt-resistant ones.

Moreover, CYP4A11 S-carriers showed higher aldosterone:renin and waist:hip ratios but...
lower furosemide-induced fractional excretions of Na\(^+\) and K\(^+\) versus F434F homozygotes, probably because of a diminished 20-HETE responses to salt loading, after adjustment for the serum insulin concentration.\(^7\)

Here, we found that the CYP4A11 F434S and the CYP4F2 V433M polymorphisms in the gene codifying for the 2 major 20-HETE metabolizing enzymes in the kidney are associated with BP levels and hypertension. We speculate that, as suggested from in vitro experiments, in our sample, a diminished production of 20-HETE at tubular level could have impaired the capacity of the kidney to excrete salt, indeed heightening BP levels and predisposing to hypertension.

On the contrary, a study in Chinese subjects found that carriers of a CYP4F2 construct haplotype, not containing the V433M polymorphism, was associated with augmented 20-HETE metabolizing activities in the kidney and hypertension. We speculate that, as suggested from in vitro experiments, in our sample, a diminished production of 20-HETE at tubular level could have impaired the capacity of the kidney to excrete salt, indeed heightening BP levels and predisposing to hypertension.

The hypertension status classification has the limitation that it is based on BP measured on a single occasion. Our data do, however, agree with the prevalence of hypertension measured on single occasions in other studies,\(^22\) and casual BP values, also in the MDC, are strongly associated with vascular risk factors, including BP and hypertension prevalence,\(^9,11,21\) whereas is not detectable in the vasculature, at least in veins.\(^25\) No study has sought this isoform in the human brain, but its presence is unlikely, because CYP4F1, the rat ortholog of CYP4F2, despite being expressed in rat kidney and liver, is absent in the brain.\(^26\) Thus, the effect of the tubular epithelia level is in agreement with a putative natriuretic and antihypertensive function of renal 20-HETE produced by this enzyme.\(^2\)

Two independent groups found an effect of the functional CYP4A11 F434S polymorphism on hypertension prevalence and BP values in 4 cohorts of patients\(^9,11,21\) but raised questions about the mode of inheritance.\(^9,11,21\) Our data indicate a recessive mode of inheritance in agreement with European studies in white subjects.\(^9,11,21\)

The effect of the CYP4F2 V433M polymorphism on stroke incidence independent from that of major cardiovascular risk factors, including BP and hypertension prevalence, is more unexpected. Animal studies could have suggested a potential beneficial effect for stroke of decreased 20-HETE levels,\(^24\) but it has to be underlined that CYP4F2 is only expressed in the human kidney and liver,\(^2\) whereas is not detectable in the vasculature, at least in veins.\(^25\) No study has sought this isofrom in the human brain, but its presence is unlikely, because CYP4F1, the rat ortholog of CYP4F2, despite being expressed in rat kidney and liver, is absent in the brain.\(^26\) Thus, the effect of the
present polymorphism in humans should be confined to the kidney. Other than the postulated antinatriuretic action of the SNP, there are no other well-defined cardiovascular properties that could explain an augmented risk for incidental ischemic stroke in our study. On the other hand, prospective data support the hypothesis that dietary salt, independent of BP levels, increases the risk of death from stroke and other cardiovascular disease, and salt sensitivity has been shown associated with cardiac hypertrophy and kidney function impairment.

![Cumulative incidence (percentage) of ischemic strokes (left) and coronary events (right) in MDC-CVA according to the CYP4F2 M433V genotype in the entire cohort (A and D), female subjects (B and E), and male subjects (C and F).](image)

**Figure.** Cumulative incidence (percentage) of ischemic strokes (left) and coronary events (right) in MDC-CVA according to the CYP4F2 M433V genotype in the entire cohort (A and D), female subjects (B and E), and male subjects (C and F).

### Table 5. HR of the CYP4F2 V433M Polymorphism in Different Cyclooxygenase Proportional Hazard Regression Models of Ischemic Stroke (n=5791)

<table>
<thead>
<tr>
<th>Models</th>
<th>Whole Population, HR (95% CI)</th>
<th>Male Subjects, HR (95% CI)</th>
<th>Female Subjects, HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP4F2 V433M*</td>
<td>1.17 (0.88 to 1.54)</td>
<td>1.64 (1.12 to 2.40)</td>
<td>0.73 (0.48 to 1.14)</td>
</tr>
<tr>
<td>CYP4F2 V433M (age and sex adjusted)*</td>
<td>1.13 (0.85 to 1.50)</td>
<td>1.61 (1.10 to 2.36)</td>
<td>0.72 (0.47 to 1.18)</td>
</tr>
<tr>
<td>CYP4F2 V433M (age, sex, BP, and hypertension adjusted)*</td>
<td>1.12 (0.84 to 1.49)</td>
<td>1.59 (1.08 to 2.33)</td>
<td>0.73 (0.47 to 1.13)</td>
</tr>
<tr>
<td>CYP4F2 V433M (age, sex, and cardiovascular risk factors adjusted; excluded BP and hypertension)†‡</td>
<td>1.16 (0.85 to 1.58)</td>
<td>1.66 (1.08 to 2.56)</td>
<td>0.73 (0.45 to 1.18)</td>
</tr>
<tr>
<td>CYP4F2 V433M (age, sex, and cardiovascular risk factors adjusted; included BP and hypertension)†‡</td>
<td>1.17 (0.86 to 1.59)</td>
<td>1.65 (1.08 to 2.53)</td>
<td>0.74 (0.46 to 1.20)</td>
</tr>
</tbody>
</table>

* CYP4F2 V433M polymorphism (V433V vs V433M+M433M).
† Cardiovascular risk factors indicates history of cardiovascular disease, diabetes, metabolic syndrome (adult treatment panel III criteria), smoking habit, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, glucose, triglycerides, and waist circumference.
‡ A total of 862 subjects were not included in the analysis because of missing values.
Thus, the independent effect of the CYP4F2 V433M polymorphism on incident ischemic stroke could be mediated by its deleterious effect beyond BP. However, because we have information only on baseline BP, we cannot exclude that subjects with the deleterious CYP4F2 M-allele polymorphism could have had a steeper worsening of BP levels or that a complex relationship among BP levels, antihypertensive treatment, and the polymorphism is the main cause of the major incidence of ischemic strokes in these subjects.

Interestingly the effect of the CYP4F2 V433M polymorphism in our study was evident only in male subjects. This issue is in agreement with a well-known testosterone-CYP interaction in animal models. In fact, both CYP4A14 null mice and normotensive rats treated with 5α-dihydrotestosterone present a form of hypertension that is gender/testosterone-mediated, with a concomitant upregulation of CYP isoforms responsible for the renal vascular synthesis of 20-HETE. These findings suggest that, in humans, the interaction of CYPs and gonadal hormones could also play a primary role in the well-established sexual dimorphism of BP, the molecular basis of which is still largely unknown. We did not find the same kind of interaction for the CYP4A11 F434S polymorphism, but the statistical power that we had was less because of the autosomal recessive mode of inheritance and the relatively low prevalence of homozygotes for the deleterious S-allele.

In conclusion, our data indicate that a common CYP4F2 V433M polymorphism of the CYP4F2 might increase the risk of incident ischemic stroke in male subjects only partially through its elevating effect on BP and confirm that the CYP4A11 F434S polymorphism is associated with hypertension and systolic BP.

Perspectives
In light of our and recent studies, research concerning the metabolic pathway of CYP-derived metabolites of arachidonic acids in hypertension should also assume greater importance in humans. A challenge for future studies will be to determine the interactions among CYPs, enzymatic pathways, and other hormonal factors, including sex hormones, clarifying their contribution to renal hemodynamics and regulation of BP.

Functional variants of genes implicated in sodium handling are attractive candidates for the regulation of BP homeostasis and the development of hypertension. Nevertheless, because only a minor effect on these variables is expected from SNPs, studies of adequate size are needed to confirm previous analyses or to test a new hypothesis. In particular, samples derived from a population-based study, analyzed under prospective conditions, such as the present one, could provide unbiased information about the relevance of different SNPs at the population level and render a possible future translation for public health of genetic studies easier regarding complex traits.

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Disclosures
None.

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Disclosures
None.

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