Background—The −344C allele of a 2-allele (C or T) polymorphism in the promoter of the gene encoding aldosterone synthase (CYP11B2) is associated with increased left ventricular size and mass and with decreased baroreflex sensitivity, known risk factors for morbidity and mortality associated with myocardial infarction (MI). We hypothesized that this polymorphism was a risk factor for MI.

Methods and Results—We used a nested case-control design to investigate the relationships between this polymorphism and the risk of nonfatal MI in 141 cases and 270 matched controls from the Helsinki Heart Study, a coronary primary prevention trial in dyslipidemic, middle-aged men. There was a nonsignificant trend of increasing risk of MI with number of copies of the −344C allele. However, this allele was associated in a gene dosage–dependent manner with markedly increased MI risk conferred by classic risk factors. Whereas smoking conferred a relative risk of MI of 2.50 (P < 0.0001) compared with nonsmokers in the entire study population, the relative risk increased to 4.67 in −344CC homozygous smokers (relative to nonsmokers with the same genotype, P = 0.003) and decreased to 1.09 in −344TT homozygotes relative to nonsmokers with this genotype. Similar joint effects were noted with genotype and decreased HDL cholesterol level as combined risk factors.

Conclusions—Smoking and dyslipidemia are more potent risk factors for nonfatal MI in males who have the −344C allele of CYP11B2. (Circulation. 1999;100:2213-2218.)

Key Words: aldosterone • genetics • genes • risk factors • myocardial infarction
other polymorphisms in CYP11B2 might affect gene expression and thus have effects on cardiovascular function.

One potentially interesting polymorphism in CYP11B2 is located in the 5′ flanking region of the gene, 344 nucleotides upstream from the start of translation within a binding site for the transcription factor steroidogenic factor-1 (SF-1); this position may be either a C or T nucleotide (−344C and −344T alleles). These alleles are present at approximately equal frequencies in white populations. Whereas these alleles have inconsistent associations with aldosterone secretion and blood pressure, the −344C allele is strongly associated with increased left ventricular size and mass in young Finnish adults and with decreased baroreflex sensitivity in the same population, as well as in older individuals.

These associations with well-established predictors of morbidity and mortality from MI prompted us to determine whether the −344C allele was itself a risk factor for MI and to study its joint effects with classic risk factors for MI.

Methods

Subjects
The participants for this case-control study were selected from the trial cohort of the Helsinki Heart Study, a double-blind, randomized, coronary primary-prevention trial with gemfibrozil in dyslipidemic middle-aged (40 to 57 years) men. The study design, end-point definitions, and results have been published previously. During the 8.5 years of the study, 241 of 4081 subjects suffered a cardiac end point, either a nonfatal MI (189) or coronary death (52). At the final posttrial follow-up visit in 1990, a blood sample for DNA analysis was drawn from each living participant (<3500). One hundred forty-one subjects with cardiac end points gave blood samples; 25 others had died of other causes, and 23 were no longer participating in the study. Thus, the CHD cases in this study population consist of subjects with nonfatal MI. Control subjects who remained free of cardiac events during the study were selected for each CHD case and matched for geographic region and drug treatment (gemfibrozil or placebo). The ages of the cases (mean 48.2 years at entry, range 40 to 56 years) and controls (mean 47.5, range 40 to 57 years) were similar. Thirteen percent of the controls and 14% of the cases were using antihypertensive agents; such treatment was not taken into account in the analyses. Twelve cases had a single matched control, and 129 cases had 2 matched controls. All other risk factor data are from the baseline visit.

Molecular Analysis of the Aldosterone Synthase (CYP11B2) Gene
DNA samples were genotyped for the −344C/T polymorphism by polymerase chain reaction amplification followed by digestion with restriction endonuclease HaeIII as described previously.

Statistical Analysis
To describe the overall effects of classic risk factors in this study cohort and to illustrate their joint effects with CYP11B2 genotypes, the mean levels (or percentages) among cases and controls are presented both for the entire pooled data and by genotype groups (Table 1). For this purpose, the individual case-control matching had to be discarded. However, the matching was maintained when the relative risks were estimated in terms of ORs by use of conditional logistic regression analyses. For categorical variables such as the genotype, a dummy variable technique was used to allow the simultaneous presence of all genotypes in the models. Specifically, we studied the joint effects of genotype and each classic risk factor by estimating the ORs in all 6 combinations of genotype and the risk factor (eg, −344TT, −344CT, and −344CC, with and without smoking) of the 6 combinations (eg, −344TT nonsmokers) as the reference group.

For analysis of HDL cholesterol (HDL-C) as a risk factor, HDL-C levels were dichotomized with the lowest tertile for the trial population, 1.08 mmol/L (42 mg/dL), used as a limit value. To dichotomize systolic blood pressure as a risk factor, 150 mm Hg (the highest tertile for the trial population) was used as a limit value. Current smokers were coded as smokers, never and former smokers as nonsmokers. In addition to the ORs, the corresponding 95% CIs and probability value provided by Wald’s test were reported. To control for possible confounding, we also estimated the ORs by incorporating covariates in the model. All statistical analyses were performed with the SAS program (SAS Institute).

Results
The distribution of CYP11B2 genotypes of controls and MI cases is presented in Table 1. The frequencies of the T and C alleles were 0.53 and 0.47 in controls and 0.48 and 0.52 in cases, respectively. The genotype distributions in both groups were in Hardy-Weinberg equilibrium. To give an overview of the data, the mean levels (or percentages) of classic risk for MI with different CYP11B2 genotypes among cases and controls are also presented in Table 1. These findings were further analyzed by estimation of the corresponding ORs and their levels of significance (Tables 2 through 5). There was a higher proportion of smokers among cases with the −344CC and to a lesser extent the −344CT genotypes than among controls with the same genotypes, but not in the −344TT group. Similarly, HDL-C levels were lower among MI cases only in persons with the −344C allele. Systolic blood pressure was higher in cases with the −344CT genotype than in controls, but there were no differences in blood pressure between cases and controls with the other genotypes. The pattern was similar with diastolic blood pressure (data not shown). The MI cases had lower HDL-C and higher systolic blood pressure than controls (Table 1), but the difference in mean LDL cholesterol (LDL-C) was small, presumably because all individuals enrolled in the study were selected to have high LDL-C. The MI cases were more likely than controls to be smokers. In conditional logistic regression analyses (Table 2), smoking, low HDL-C (<1.08 mmol/L [42 mg/dL]), and high systolic blood pressure (>150 mm Hg)
significantly increased MI risk in both univariate and multi-variate models. There was a trend of increasing MI risk with heterozygosity or homozygosity for the \(2344C\) allele, although this trend was not statistically significant.

Because of the differences related to genotype, we estimated the ORs with corresponding levels of significance for joint effects of CYP11B2 genotypes and other risk factors on MI risk. Intriguingly, the \(2344C\) allele functioned in a gene dosage–dependent manner to increase the risk of MI in smokers; smoking was not a significant risk factor for MI in subjects homozygous for the \(2344T\) allele, whereas it increased MI risk almost 5-fold in individuals who were homozygous for the \(2344C\) allele (Table 3).

An analogous pattern was found for the joint effect of CYP11B2 genotype and HDL-C; low HDL-C was a significant MI risk factor only in the presence of the \(2344T\) allele (Table 4). Very similar results were obtained (not shown) with different cutoff levels for dichotomization, such as the median (1.18 mmol/L) and lowest quartile (1.04 mmol/L) instead of the lowest tertile (1.08 mmol/L). In contrast, there was no clear gene dose–dependent relationship between genotype and the MI risk conferred by high systolic blood pressure (Table 5).

**Discussion**

**CYP11B2 Genotype as a Risk Factor for MI**

Taken together with the present study, the associations of the \(2344C\) allele with increased left ventricular size\(^13\) and decreased baroreflex sensitivity\(^17\) suggest that this allele is associated with physiological changes to the cardiovascular and/or autonomic nervous systems that sensitize the heart to other stressors, such as smoking, thus increasing the risk of MI when such stressors are present.

Despite the rather small sample size, it seems highly unlikely that these findings are chance or trivial associations, because Finnish populations are highly homogenous ethnically, which reduces the chance of unsuspected admixture of different populations in the sample\(^20\), and because the population-based design of this study further minimizes the possibility of unintentional selection bias. Moreover, there is little variation in allele frequencies among white American and European populations.\(^12,13,15,16\) It is also unlikely that these associations are due to an effect of CYP11B2 on blood pressure, because the \(2344C\) allele does not increase blood pressure.

---

**TABLE 2. Relative Risks of MI Associated With Different Risk Factors When Analyzed Singly in Univariate Models or Simultaneously in a Multivariate Model**

<table>
<thead>
<tr>
<th></th>
<th>Univariate Models</th>
<th></th>
<th></th>
<th></th>
<th>Multivariate Model</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>(-344 CT) genotype*</td>
<td>1.41</td>
<td>0.86–2.30</td>
<td>0.17</td>
<td>1.29</td>
<td>0.76–2.20</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>(-344 CC) genotype*</td>
<td>1.57</td>
<td>0.87–2.86</td>
<td>0.14</td>
<td>1.43</td>
<td>0.74–2.75</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>2.50</td>
<td>1.62–3.87</td>
<td>0.0001</td>
<td>2.46</td>
<td>1.53–3.94</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>HDL-C (\leq1.08) mmol/L</td>
<td>1.94</td>
<td>1.24–3.02</td>
<td>0.004</td>
<td>2.00</td>
<td>1.24–3.25</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>SBP (\geq150) mm Hg</td>
<td>2.02</td>
<td>1.32–3.11</td>
<td>0.001</td>
<td>2.12</td>
<td>1.32–3.41</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.04</td>
<td>0.99–1.08</td>
<td>0.13</td>
<td>1.05</td>
<td>1.00–1.10</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure.

*Relative to \(-344TT\) genotype.

---

**TABLE 3. Joint Effects of CYP11B2 Genotype and Smoking on Risk of MI**

<table>
<thead>
<tr>
<th></th>
<th>Non-smokers</th>
<th></th>
<th></th>
<th></th>
<th>Smokers</th>
<th></th>
<th></th>
<th></th>
<th>Smokers vs Non-smokers</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-344TT)</td>
<td>1</td>
<td></td>
<td></td>
<td>1.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.06</td>
<td>0.43–2.58</td>
<td></td>
</tr>
<tr>
<td>(-344CT)</td>
<td>0.86</td>
<td>0.44–1.70</td>
<td>NS</td>
<td>2.38</td>
<td>1.21–4.68</td>
<td>0.01</td>
<td>2.75</td>
<td>1.49–5.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-344CC)</td>
<td>0.77</td>
<td>0.32–1.84</td>
<td>NS</td>
<td>3.51</td>
<td>1.51–8.16</td>
<td>0.004</td>
<td>4.54</td>
<td>1.81–11.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-344TT)</td>
<td>1</td>
<td></td>
<td></td>
<td>1.09</td>
<td>0.44–2.68</td>
<td>NS</td>
<td>1.09</td>
<td>0.44–2.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-344CT)</td>
<td>0.87</td>
<td>0.44–1.71</td>
<td>NS</td>
<td>2.51</td>
<td>1.26–4.99</td>
<td>0.009</td>
<td>2.89</td>
<td>1.56–5.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-344CC)</td>
<td>0.78</td>
<td>0.33–1.88</td>
<td>NS</td>
<td>3.66</td>
<td>1.56–8.55</td>
<td>0.003</td>
<td>4.67</td>
<td>1.86–11.73</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The columns labeled “Non-smokers” and “Smokers” represent the results of a single conditional logistic regression analysis with all 6 combinations of genotype and smoking status included in the model and with OR expressed relative to the risk in non-smokers with the \(-344TT\) genotype. The column labeled “Smokers vs Non-smokers” summarizes the risk to smokers with each genotype when the same analysis is recalculated using non-smokers with the respective genotype as the reference group.
pressure in a dose-dependent manner in this or other populations.\textsuperscript{13,15,16}

Aldosterone is known to have effects on the cardiovascular system that are independent of blood pressure. In rats fed a high-salt diet, it causes myocardial fibrosis and cardiac hypertrophy at doses that do not affect blood pressure.\textsuperscript{21,22} In the isolated perfused rat heart, it decreases coronary blood flow.\textsuperscript{23} In dogs, it decreases baroreflex sensitivity.\textsuperscript{24} Humans with primary aldosteronism are more prone to left ventricular hypertrophy than individuals with essential hypertension of equivalent severity.\textsuperscript{25} These effects may all be mediated by mineralocorticoid receptors in the heart\textsuperscript{26} or in the vascular endothelium. Thus, the simplest explanation for the associations of the $\text{CYP11B2}\text{C}_{344}$ allele with cardiovascular disease would be that this allele increases expression of \text{CYP11B2} and thereby increases aldosterone secretion. However, as previously discussed, this allele is only inconsistently associated with increased aldosterone secretion.\textsuperscript{14,15}

Several other explanations are consistent with the available data. \text{CYP11B2} expression has been reported in human\textsuperscript{27} and rodent\textsuperscript{28} vascular endothelium and in rodent heart,\textsuperscript{29} which suggests that aldosterone is synthesized in these tissues. If the $\text{CYP11B2}\text{C}_{344}$ allele increased expression of \text{CYP11B2} in these tissues, it might increase local concentrations of aldosterone and thus have cardiovascular effects without significantly increasing circulating aldosterone levels.

Alternatively, an allele of another polymorphic locus in or near \text{CYP11B2} may be responsible for the observed effects if that allele is associated with $\text{CYP11B2}\text{C}_{344}$. Linkage disequilibrium (associations between particular alleles of linked polymorphic loci) in this region extends at least as far as the distal (3') end of the adjacent steroid 11\textbeta hydroxylase gene (\text{CYP11B1}).\textsuperscript{10,30} Indeed, 11-deoxycortisol responses to exogenous adrenocorticotrophic hormone stimulation are higher in males with $\text{CYP11B2}\text{C}_{344}\text{C}$ than with $\text{CYP11B2}\text{C}_{344}\text{T}$ genotypes.\textsuperscript{14} This might reflect decreased \text{CYP11B1} activity associated with the

### Table 4. Joint Effects of \text{CYP11B2} Genotype and Low HDL-C on Risk of MI

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normal HDL-C</th>
<th></th>
<th></th>
<th>Low HDL-C</th>
<th></th>
<th></th>
<th>Low vs Normal HDL-C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>P</td>
<td></td>
<td>OR 95% CI</td>
<td></td>
<td></td>
<td>OR 95% CI</td>
<td></td>
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<tr>
<td>No adjustments</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>$\text{CYP11B2}\text{C}_{344}\text{T}$</td>
<td>1.27 0.51–3.16</td>
<td>NS</td>
<td>1.27 0.51–3.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CYP11B2}\text{C}_{344}\text{C}$</td>
<td>2.62 1.25–5.50</td>
<td>0.01</td>
<td>2.18 1.19–4.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adjusted for age and smoking</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CYP11B2}\text{C}_{344}\text{T}$</td>
<td>1.27 0.51–3.16</td>
<td>NS</td>
<td>1.27 0.51–3.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CYP11B2}\text{C}_{344}\text{C}$</td>
<td>3.20 1.30–7.88</td>
<td>0.01</td>
<td>2.52 1.00–6.36</td>
<td></td>
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</tbody>
</table>

The columns labeled “Normal HDL-C” and “Low HDL-C” represent the results of a single conditional logistic regression analysis with all 6 combinations of genotype and HDL-C levels included in the model and with OR expressed relative to the risk in individuals with normal HDL-C and the $\text{CYP11B2}\text{C}_{344}$ genotype. The column labeled “Low vs Normal HDL-C” summarizes the risk to individuals with low HDL-C and each genotype when the same analysis is recalculated using individuals with normal HDL-C and the respective genotype as the reference group.

*Low HDL-C signifies HDL-C <1.08 mmol/L. Similar results are obtained when other cutoff levels for dichotomization are used, including 1.18 and 1.04 mmol/L.

### Table 5. Joint Effects of \text{CYP11B2} Genotype and High Systolic Blood Pressure on Risk of MI

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normal SBP</th>
<th></th>
<th></th>
<th>High SBP</th>
<th></th>
<th></th>
<th>High vs Normal SBP</th>
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<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>P</td>
<td></td>
<td>OR 95% CI</td>
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<td>OR 95% CI</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CYP11B2}\text{C}_{344}\text{T}$</td>
<td>2.01 0.84–4.79</td>
<td>NS</td>
<td>2.01 0.84–4.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CYP11B2}\text{C}_{344}\text{C}$</td>
<td>2.77 1.42–5.40</td>
<td>0.003</td>
<td>2.22 1.24–3.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adjusted for age and smoking</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CYP11B2}\text{C}_{344}\text{T}$</td>
<td>2.16 0.88–5.33</td>
<td>0.09</td>
<td>2.16 0.88–5.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CYP11B2}\text{C}_{344}\text{C}$</td>
<td>2.85 1.42–5.71</td>
<td>0.003</td>
<td>2.29 1.24–4.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The columns labeled “Normal SBP” and “Low SBP” represent the results of a single conditional logistic regression analysis with all 6 combinations of genotype and systolic blood pressure (SBP) included in the model, with OR expressed relative to the risk in individuals with normal blood pressure and the $\text{CYP11B2}\text{C}_{344}$ genotype. The column labeled “High vs Normal SBP” summarizes the risk to individuals with high SBP and each genotype when the same analysis is recalculated using individuals with normal SBP and the respective genotype as the reference group.

*High SBP signifies SBP >150 mm Hg. Similar results are obtained when SBP >160 mm Hg is used as a cutoff level.
Joint Effects of CYP11B2 Genotype and Smoking

Smoking is a very-well-established risk factor for CHD and for progression of atherosclerosis. The mechanisms by which CHD risk is increased are not completely understood but probably include effects on myocardial oxygen supply and consumption, platelet activation, decreased HDL-C levels, and generation of free radicals. Catecholamine release induced by nicotine might act synergistically with aldosterone to adversely affect the heart, perhaps because both agents increase cardiac output but decrease coronary blood flow. However, currently available data do not permit definitive identification of the mechanism(s) underlying our observations.

Study Limitations

This study provides evidence that a polymorphism in CYP11B2 increases the risk of nonfatal MI in smokers. Because many subjects died before DNA could be obtained from them, no firm conclusions can be drawn regarding risk of fatal MI. Only middle-aged, dyslipidemic Finnish males participated, and so the conclusions of this study cannot yet be extrapolated to other nationalities, to lower-risk men, to other age groups, or to women. However, it is likely that a similar association will be found in women, because the effects of CYP11B2 genotype on left ventricular size and baroreflex sensitivity persist better in middle-aged women than in men (Reference 17 and A. Ylitalo et al., unpublished data, 1999).

Conclusions

In conclusion, smoking and dyslipidemia are more potent risk factors for nonfatal MI in males who have the CYP11B2. Knowledge of how the mineralocorticoid system and smoking interact to increase the risk of acute MI may provide novel insights into the genesis of thrombotic coronary occlusion. It will be important to determine the mechanisms underlying our observations because of the implications for the pharmacological prophylaxis of CHD. For example, if our observations were due to increased local or systemic concentrations of aldosterone, then drugs that block the mineralocorticoid receptor might in theory reduce the risk of acute coronary events in high-risk individuals. Animal experiments to evaluate possible synergistic effects of smoking and aldosterone on the myocardium and coronary arteries might be one way to address this possibility.

Acknowledgments

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Joint Effects of an Aldosterone Synthase (CYP11B2) Gene Polymorphism and Classic Risk Factors on Risk of Myocardial Infarction
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