Factor VII Gene Polymorphism, Factor VII Levels, and Prevalent Cardiovascular Disease
The Framingham Heart Study

Abstract—Elevated factor VII levels have been associated with increased cardiovascular risk in some studies. The arginine/glutamine (Arg/Gln) polymorphism of the factor VII gene has been previously shown to modify factor VII levels. However, the presence of a gene/environment interaction on factor VII levels or a link with cardiovascular disease (CVD) remains uncertain. We studied subjects from the Framingham Heart Study to determine (1) the extent to which this genetic polymorphism affects factor VII levels; (2) whether interactions exist between this polymorphism and environmental factors on factor VII levels; and (3) the association between the polymorphism and CVD. Genotype data and factor VII antigen levels were available in 1816 subjects. Factor VII levels differed significantly among genotypes in an additive fashion: Gln homozygous, 82.7 ± 2.5%; heterozygous, 92.2 ± 0.7%; and Arg homozygous, 100.5 ± 0.4% (P < 0.0001). The polymorphism was the strongest, single predictor of factor VII levels, explaining 7.7% of the total variance of factor VII levels, whereas other traditional risk factors combined explained an additional 11.5% of the variance. There was an interaction (P = 0.02) between the genotype and total cholesterol on factor VII levels, such that the correlation coefficient and slope (factor VII level/total cholesterol) were greatest in Gln/Gln subjects. Among 3204 subjects characterized for genotype and CVD, there was no significant relationship between the genotype and CVD (P = 0.12). In the Framingham Heart Study, the Arg/Gln polymorphism was significantly associated with factor VII antigen levels. The strength of the association suggests that genetic variation plays an important role in determining factor VII levels. However, despite being associated with factor VII levels, the Arg/Gln polymorphism was not associated with prevalent CVD. (Arterioscler Thromb Vasc Biol. 2000;20:593-600.)

Key Words: factor VII • genetics • polymorphisms • cardiovascular disease • risk factors

The importance of the hemostatic system in coronary heart disease is well recognized. Thrombosis on top of an eroded or ruptured atherosclerotic plaque is the most common pathological finding in acute coronary syndromes. In addition, there is accumulating evidence that the hemostatic system plays a part in plaque formation and plaque growth. In the Northwick Park Heart Study, high plasma factor VII coagulant activity was associated with an increased risk of a subsequent fatal coronary event among middle-aged men. This association was independent of plasma cholesterol and fibrinogen levels. This finding has been supported by some but not all studies. Recently, an association has been observed between factor VII levels and the arginine/glutamine (Arg/Gln) polymorphism of the factor VII gene, with individuals who carry a Gln allele displaying lower factor VII levels than those who are Arg-homozygous. Humphries and colleagues subsequently reported an interaction between genotype and plasma triglyceride levels on factor VII levels such that the correlation between factor VII levels and triglyceride concentration was more pronounced in subjects who were Arg-homozygous than among those with a Gln allele. In addition, Meilahn et al reported that women with the Gln allele did not exhibit the elevation in factor VII level with menopause or use of hormone replacement therapy that was observed in the Arg-homozygous women, suggesting that the genotype may modify hormone-induced changes in factor VII levels. Although the Arg/Gln polymorphism has consistently modified factor VII levels, gene-environment interactions have not always been seen. It also remains uncer-
tain whether this polymorphism influences the risk for cardiovascular disease (CVD). Although Iacoviello et al. found that this polymorphism was associated with myocardial infarction, others have failed to find such an association.19,21 Because the identification of genetic predictors of CVD has implications for risk stratification and disease prevention, we evaluated subjects from the Framingham Heart Study to determine (1) the extent to which the Arg/Gln polymorphism influences factor VII levels; (2) the presence of any interaction between this polymorphism and environmental factors on factor VII antigen levels; and (3) the association between the polymorphism and prevalent CVD.

Methods

Subjects

Details of the design and methodology of the Framingham Heart Study have been presented previously.24,25 Starting in 1948, 5029 subjects between the ages of 28 and 62 years were enrolled into the original cohort study, and they have been examined biennially. Children and spouses of the cohort members, totaling 5124 subjects, were enrolled beginning in 1971 and have undergone subsequent follow-up examinations (the Framingham Offspring Study).24,25 We studied these 2 overlapping Framingham subject groups to analyze specific questions. First, to determine the relation between factor VII antigen levels and the Arg/Gln polymorphism, and any gene-environment interaction on factor VII levels, we studied the offspring cohort individuals (n=1816), because only this group had factor VII antigen data. Second, to evaluate the relation between the Arg/Gln polymorphism and CVD, we were able to study both the original cohort and the offspring cohort, since genetic data and CVD data were available from both cohorts. Of the 5041 samples collected for genetic analysis between June 1987 and February 1991, 3291 were members of families in which DNA specimens had been obtained for a parent-child pair or a sibling pair. These 3291 DNA samples were genotyped for this analysis while the rest (singleton) samples were not available for genotyping. Of these 3291 samples, 83 (2.5%) could not be successfully genotyped. Therefore, 3204 subjects were used for the CVD analyses. We compared the characteristics of subjects included in the study (3024) with the 2285 subjects who were not genotyped for this polymorphism. The 2 groups of subjects had very similar clinical profiles (data not shown).

The prevalence of CVD was evaluated at the time of the DNA blood draw during the 19th, 20th, or 21st examination cycle for the original cohort and during the fourth or fifth cycle for the offspring study. Prevalent CVD was defined as the presence of a diagnosis of coronary heart disease (stable angina, unstable angina, and myocardial infarction), cerebrovascular disease (stroke and transient ischemic attack), intermittent claudication, or congestive heart failure. At the time of blood draw for DNA analysis, 516 of 3204 subjects had prevalent CVD. For a secondary analysis of CVD, we defined the 278 individuals with a history of unstable angina, myocardial infarction, or ischemic stroke as a thrombotic CVD group while the remaining 238 individuals were considered a nonthrombotic CVD group.

For the factor VII antigen levels analysis, study subjects were members of the Framingham Offspring Study, with blood samples obtained during the fifth examination cycle. Of 3204 who were genotyped, 1980 were Offspring Study members. We excluded 164, including 28 owing to anticoagulant use and 136 without factor VII antigen levels measured. A total of 1816 subjects met the inclusion criteria.

Genotyping

To detect the substitution of guanine to adenine in codon 353 in the eighth exon of the factor VII gene, which is responsible for the Arg/Gln polymorphism, we used a polymerase chain reaction (PCR)–based restriction fragment length polymorphism analysis. Genomic DNA was isolated from whole blood. Genomic DNA, 10 to 20 ng (5 μL), was incubated at 96°C for 3 minutes, followed by addition of 10 μL of reagents to yield final reagent concentrations of 333 nmol/L for sense and antisense primers; 167 μmol/L each of dATP, dTTP, dCTP, and dGTP; 2.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.4 at 25°C); 0.1% Triton X-100; 0.02 mM L-cresol red; 83 mM/L sucrose; and 0.15 U of Taq polymerase. The sequences of the sense primer and antisense primer were 5′-cagctgagctgctgtgccgctac-3′ and 5′-gtctgctgtagctgccagct-3′, respectively. DNA was amplified by 39 cycles of denaturing at 96°C for 20 seconds, annealing at 56°C for 40 seconds, and extension at 72°C for 30 seconds. Restriction buffer (10 mM) was added to give final concentrations of 10 mM L-Tris-HCl, 5.5 mM L-MgCl2, 12.5 mM L-NaCl, 30 mM L-KCl, 0.4 mM L-DTT, and 0.1% Triton X-100. The 206-bp amplification product was incubated at 37°C overnight with 10 U of the restriction endonuclease MspI, which cleaves the Gln allele into fragments of 22 and 184 bp, whereas the Arg allele yields 3 restriction products of 22, 67, and 117 bp. The MspI-digested amplification product (8 μL) was loaded onto 2% agarose gel slabs containing 40 mM/L Tris acetate and 2 mM/L EDTA. Samples were size-fractionated at 6 V/cm for 30 minutes. Bands were visualized after being stained with ethidium bromide by 300-nm UV transillumination. PCR results were scored without knowledge of the factor VII antigen level results. When there was any ambiguity, genotyping was repeated. Ninety-seven percent of subjects were successfully genotyped.

Factor VII Antigen Levels

Blood samples were obtained in the morning to avoid circadian changes. Blood was drawn into tubes containing 3.8% sodium citrate (9:1, vol/vol). Plasma was separated by centrifugation for 20 minutes at 2000g and stored at −80°C for later analysis. Factor VII antigen levels were determined by ELISA (Diagnostica Stago). Values were expressed as percentage of the standard, which is 100% by definition. The coefficient of variation of the assay was 3.0% in our laboratory.

Statistical Analysis

Clinical and demographic characteristics were compared among groups of subjects. Differences in means were tested by ANOVA or ANCOVA and differences in proportions by the x2 test. The x2 test was also used to assess the genotype frequencies for Hardy-Weinberg equilibrium. For analysis of factor VII levels, multiple regression models were used to determine incremental contributions to explained variance (R2) and to adjust for age, sex, body mass index (BMI), triglyceride, total and HDL cholesterol, systolic blood pressure, alcohol consumption, smoking status, diabetes, CVD status, menopausal status, and use of estrogen replacement therapy.26,27 Separate models were evaluated for recessive, dominant, and additive genetic effects. Generalized estimating equation algorithms were used to correct for intrafamily correlation in analyses of factor VII antigen levels and CVD prevalence.28 Gene-environment interactions were evaluated. P<0.05 was regarded as statistically significant.

Results

Subject Characteristics

Factor VII Antigen Levels and Genotype Analysis

Of the 1816 individuals who had both factor VII antigen and genotype data, 931 (51%) were females. Among the females, 596 (64%) were postmenopausal and 122 (13%) were receiving estrogen replacement therapy. The allele frequencies of Arg and Gln were 0.86 and 0.14, respectively, and are in accord with Hardy-Weinberg equilibrium (P=0.96). As shown in Table 1, there were no significant differences among genotype groups regarding age, sex, BMI, triglyceride, total and HDL cholesterol, systolic blood pressure, alcohol consumption, smoking, diabetes, CVD status, hypertension, or, in women, menopausal status or estrogen replacement therapy.
Prevalent CVD Analysis

Compared with the non-CVD group, subjects with either thrombotic or nonthrombotic CVD (see Table 2) were older and had a higher prevalence of hypertension and diabetes mellitus as well as higher triglyceride levels and lower HDL cholesterol levels (all $P<0.0001$). They also consumed significantly less alcohol than did the non-CVD group ($P<0.0001$). Compared with the non-CVD group, those with thrombotic CVD were more likely to be male, and those with nonthrombotic CVD had higher total cholesterol (both $P<0.0001$). Among the women, those with thrombotic and nonthrombotic CVD were more likely to be postmenopausal than those without CVD ($P<0.0001$). There were no significant differences among groups regarding current smoking status, BMI, or estrogen replacement therapy.

Arg/Gln Polymorphism and Factor VII Levels

Factor VII levels differed significantly among genotypes, with the Gln allele being associated with lower factor VII antigen levels. Mean factor VII antigen levels were

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Arg/Arg</th>
<th>Arg/Gln</th>
<th>Gln/Gln</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1339</td>
<td>441</td>
<td>36</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>54±0.3</td>
<td>54±0.5</td>
<td>53±1.7</td>
<td>0.46</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>47</td>
<td>49</td>
<td>58</td>
<td>0.41</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.7±0.1</td>
<td>27.2±0.2</td>
<td>27.6±0.8</td>
<td>0.23</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.65±0.03</td>
<td>1.73±0.06</td>
<td>1.57±0.20</td>
<td>0.46</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.28±0.03</td>
<td>5.30±0.05</td>
<td>5.40±0.16</td>
<td>0.59</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.29±0.01</td>
<td>1.27±0.02</td>
<td>1.29±0.06</td>
<td>0.74</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>126±0.5</td>
<td>126±0.9</td>
<td>125±3.1</td>
<td>0.91</td>
</tr>
<tr>
<td>Alcohol, oz/wk</td>
<td>2.7±0.1</td>
<td>2.7±0.2</td>
<td>3.1±0.7</td>
<td>0.86</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>6.5</td>
<td>6.3</td>
<td>5.6</td>
<td>0.97</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>8.8</td>
<td>9.3</td>
<td>8.3</td>
<td>0.95</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>33</td>
<td>35</td>
<td>28</td>
<td>0.67</td>
</tr>
<tr>
<td>Menopause, % of women</td>
<td>63</td>
<td>67</td>
<td>60</td>
<td>0.57</td>
</tr>
<tr>
<td>Estrogen therapy, % of women</td>
<td>13</td>
<td>15</td>
<td>7</td>
<td>0.57</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure. Data are expressed as mean±SEM or percentages.

Arg/Gln Polymorphism and Factor VII Levels

Factor VII levels differed significantly among genotypes, with the Gln allele being associated with lower factor VII antigen levels. Mean factor VII antigen levels were

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Thrombotic CVD</th>
<th>Nonthrombotic CVD</th>
<th>Non-CVD</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>278</td>
<td>238</td>
<td>2688</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>70.9±0.8</td>
<td>69.9±0.9</td>
<td>56.1±0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>63</td>
<td>42</td>
<td>45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>82</td>
<td>75</td>
<td>42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>22</td>
<td>15</td>
<td>5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.39±0.07</td>
<td>5.69±0.07</td>
<td>5.37±0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.08±0.02</td>
<td>1.18±0.03</td>
<td>1.29±0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.92±0.12</td>
<td>1.66±0.12</td>
<td>1.38±0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Menopause, % of women</td>
<td>99</td>
<td>92</td>
<td>67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Alcohol, oz/wk</td>
<td>2.2±0.3</td>
<td>2.0±0.3</td>
<td>2.8±0.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>20</td>
<td>19</td>
<td>22</td>
<td>0.56</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.5±0.3</td>
<td>26.5±0.3</td>
<td>26.5±0.1</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Estrogen therapy, % of women</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td>0.54</td>
</tr>
<tr>
<td>Factor VII levels, %†</td>
<td>93.6±1.9%</td>
<td>99.5±1.7%</td>
<td>98.0±0.4%</td>
<td>0.051</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM or percentages.

* Differences among 3 groups by ANOVA or $\chi^2$.
† Because factor VII levels were available only in the Offspring subjects, the respective numbers were 65, 79, and 1697 for the 3 groups. The levels here were adjusted for variables listed in Statistical Analysis.
The magnitude of difference in factor VII antigen associated with difference in cholesterol was greater in subjects with the Gln allele than among subjects with the Arg/Arg genotype. The interaction between genotype and total cholesterol on factor VII levels was $P=0.02$. The ranges of low-, medium-, or high-tertile cholesterol were 2.35 to 4.85, 4.86 to 5.62, and 5.63 to 9.78 mmol/L.

100.5±0.4% for Arg/Arg (n=1339), 92.2±0.7% for Arg/Gln (n=441), and 82.7±2.5% for Gln/Gln (n=36) ($P<0.0001$). These differences remained significant after adjustment for potential confounders ($P<0.0001$). Tests for mode of inheritance indicated that the additive model was the best fit, whereas dominant or recessive models fit less well. Accounting for residual sibling-sibling correlation ($r=0.11$) produced virtually identical estimates and tests of genotype coefficients as did the initial analyses.

Components of Variance in Factor VII Levels
The Arg/Gln polymorphism explained 7.7% of the total variance of factor VII ($P<0.0001$) and was the single, most important determinant of factor VII levels in the population. Environmental factors accounted for an additional 11.5%. These included triglycerides, 5.1% ($P<0.0001$); sex, 2.4% ($P<0.0001$); BMI, 1.3% ($P<0.0001$); total cholesterol, 1.1% ($P<0.0001$); estrogen replacement therapy, 0.7% ($P=0.0001$); alcohol consumption, 0.3% ($P=0.01$); HDL cholesterol, 0.3% ($P=0.01$); and systolic blood pressure, 0.3% ($P=0.01$). Women had higher factor VII levels than did men. All variables listed above were positively related to factor VII levels, except alcohol consumption, which showed a negative association with factor VII levels. In the multivariable analysis, several variables were not significantly associated with factor VII levels ($P>0.15$), including age, diabetes, CVD status, hypertension treatment, diastolic blood pressure, smoking status, and menopausal status.

Gene-Environment Interaction on Factor VII Antigen Levels
Cholesterol level was positively associated with factor VII levels in all genotypes (see Figures 1 and 2). The Pearson correlation coefficients were 0.17 ($P<0.0001$) in the Arg/Arg genotype, 0.21 ($P<0.0001$) in the Arg/Gln genotype, and 0.47 ($P=0.0044$) in the Gln/Gln genotype. Similar partial correlation coefficients were obtained after adjustment for other variables in the multiple regression model. The slopes of factor VII levels versus total cholesterol (expressed as the percent difference in factor VII antigen per 1-mg/dL difference of cholesterol) differed among genotypes: 0.031±0.011 (slope±SE) among Arg homozygotes, 0.050±0.018 among heterozygotes, and 0.163±0.048 among Gln homozygotes ($P=0.02$). Thus, the percent difference in factor VII associated with the difference in total cholesterol was directly proportional to the number of Gln alleles present (Figure 1). Although the association between factor VII levels and factor VII genotype was less prominent among those with high cholesterol, there were still highly significant relationships for this tertile ($P<0.0001$).

Triglyceride levels were positively associated with factor VII levels, with a correlation between factor VII antigen and triglyceride levels of $r=0.21$ ($P<0.0001$) in the Arg/Arg genotype, $r=0.30$ ($P<0.0001$) in the Arg/Gln genotype, and $r=0.28$ ($P=0.09$) in the Gln/Gln genotype. Similar partial correlation coefficients were obtained after adjustment for other variables in the multiple regression model. There was a trend toward differences in slopes of factor VII level versus triglyceride (percent per mg/dL) among genotypes proportional to the number of copies of Gln alleles present: 0.027±0.004 (slope±SE) for Arg homozygotes, 0.044±0.008 for heterozygotes, and 0.055±0.027 for Gln homozygotes ($P=0.07$, Figure 2). Although the association between factor VII levels and factor VII genotype was less prominent among the high-triglyceride individuals, there were still highly significant relationships for this tertile ($P<0.0001$).

There was no evidence of significant gene-environment interactions with menopausal status or hormone replacement therapy in women or with the other variables studied ($P>0.40$) for all.
TABLE 3. The Arg/Gln Polymorphism and Prevalent CVD in 3204 Subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Thrombotic CVD (n=278)</th>
<th>Nonthrombotic CVD (n=238)</th>
<th>No CVD (n=2688)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>77.7%</td>
<td>71.0%</td>
<td>73.9%</td>
<td>0.12</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>19.1%</td>
<td>27.7%</td>
<td>23.9%</td>
<td></td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>3.2%</td>
<td>1.3%</td>
<td>2.2%</td>
<td></td>
</tr>
</tbody>
</table>

*There were no significant differences in genotype frequencies among ischemic CVD, nonischemic CVD, and non-CVD groups, unadjusted P=0.12. Similar results were obtained for men and women.

Factor VII Antigen Levels and Prevalent CVD
There was a nonsignificant trend for factor VII antigen levels to be lower among subjects with thrombotic CVD compared with those with either nonthrombotic CVD or no CVD (see Table 2). The adjusted mean levels were 93.6±1.9% (n=65), 99.5±1.7% (n=79), and 98.0±0.4% (n=1697), respectively (P=0.051).

Factor VII Polymorphism and Prevalent CVD
Despite differences in factor VII antigen levels among genotypes, there were no significant differences in genotype distribution among those with or without CVD or when they further classified as thrombotic CVD, nonthrombotic CVD, and non-CVD groups (P=0.12, Table 3). The odds ratios (ORs) for total CVD were 0.93, 0.89, and 1.11, respectively (P=0.51, 0.39, and 0.78), when a respective additive, dominant, and recessive effect of the Gln allele was assumed. By using logistic models to adjust for age, sex, BMI, current smoking status, and the presence of diabetes (model 1) or further adjustment for total and HDL cholesterol (model 2), similar nonsignificant results were obtained (Table 4). The ORs for thrombotic CVD compared with non-CVD were also not significant when general logistic models were used and after adjustments were made for other variables (Table 4).

Linear and logistic models were rerun with generalized estimating equations to account for correlations between siblings, and the initial findings were confirmed. When analyses for the genotype and CVD relation were made by including the 1816 subjects in whom both genotype and factor VII level data were available, similar results were obtained (data not shown).

Discussion
In the Framingham Heart Study sample, the Arg/Gln polymorphism of factor VII was significantly associated with factor VII levels such that the presence of 1 or 2 Gln alleles was associated with incrementally lower factor VII levels. The polymorphism was the single, most important determinant of factor VII levels in the population. A genotype–total cholesterol interaction was observed such that the differences in factor VII associated with differences in total cholesterol were greater in those with the Gln allele compared with the Arg/Arg genotype subjects. However, neither this genetic marker nor factor VII antigen levels were significantly associated with prevalent CVD.

The Arg/Gln Polymorphism, Traditional Risk Factors, and Factor VII Levels
Factor VII is a vitamin K–dependent coagulation factor synthesized in the liver. In the presence of tissue factor and calcium, factor VII converts factor X to factor Xa and initiates reaction of the common coagulation pathway. Green and colleagues first found that the Arg/Gln polymorphism influenced factor VII levels such that subjects with the Gln allele had 20% lower levels than did Arg homozygotes. The association has been consistently replicated among different ethnic groups, regardless of whether factor VII coagulation activity or antigen levels were measured.

Indeed, the polymorphism has been shown in several studies to be the single, strongest predictor of factor VII levels, accounting for 10% to 30% of the variance. In the Framingham sample, we also found that the Arg/Gln polymorphism was the primary determinant of factor VII antigen levels, explaining 7.7% of the total variance.

Factor VII is also influenced by other traditional risk factors. Age, obesity, dietary fat intake, lipids, menopause, and oral contraceptive use in women have all been described to influence factor VII levels. In our study, triglyceride levels, female sex, BMI, total cholesterol, estrogen replacement therapy, HDL cholesterol,

TABLE 4. ORs for Prevalent CVD According to Arg/Gln Genotype

<table>
<thead>
<tr>
<th>Models</th>
<th>Total CVD vs No CVD (n=516 vs 2688)</th>
<th>Thrombotic CVD vs No CVD (n=278 vs 2688)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Model 1*</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>0.97 (0.76–1.25)</td>
<td>0.82</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>0.94 (0.47–1.88)</td>
<td>0.86</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td></td>
<td>1.30 (0.59–2.88)</td>
</tr>
<tr>
<td>2 df test ‡</td>
<td>0.96</td>
<td>0.29</td>
</tr>
<tr>
<td>Model 2†</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>0.87 (0.66–1.15)</td>
<td>0.32</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>1.07 (0.53–2.17)</td>
<td>0.84</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td></td>
<td>1.47 (0.65–3.30)</td>
</tr>
<tr>
<td>2 df test ‡</td>
<td>0.59</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*Model 1 included age, sex, BMI, presence of diabetes, and current smoking status.
†Model 2 also included total and HDL cholesterol, along with the other variables in model 1.
‡The 2 df test results are from a Wald test of the null hypothesis that CVD is unrelated to genotype.
and systolic blood pressure were positively associated with factor VII antigen levels, while alcohol consumption was negatively related to factor VII levels. These traditional risk factors explained an additional 11.5% of the total factor VII variance.

The mechanism of the association between the polymorphism and factor VII levels is not well understood. One recent observation that the Arg/Gln polymorphism is in strong linkage disequilibrium with a decamer insertion polymorphism in the 5’ regulatory region at −323 bp provides a plausible explanation, particularly in view of the in vitro expression evidence that the decamer insert polymorphism reduced promoter activity by 33% compared with the wild-type allele.37

The other contributing factors for the rest of the variance remain unknown. These unknown factors may be genetic, eg, other polymorphisms in the factor VII gene, other regulatory genes, or undefined environmental and dietary factors. With linkage analysis and the use of microsatellite markers, the Framingham Heart Study is performing a genome scan to find other loci that may modulate factor VII levels.

The Interaction Between the Polymorphism and Environmental Factors

Although our findings are consistent with previous investigations regarding the correlation between genotype and factor VII plasma levels, our results on a genotype-specific interaction differ from previously published data. Whereas Humphries et al.12,13 found a correlation between triglyceride and factor VII in Arg/Arg-homozygous subjects only, we found a similar or even stronger correlation among those with the Gln allele. In addition, we found a genotype interaction with total cholesterol, such that the correlation between factor VII levels and total cholesterol was significantly greater in subjects with the Gln allele than that in the Arg/Arg-homozygous individuals. Although the association between factor VII levels and factor VII genotype was less prominent among those with high cholesterol, there were still highly significant relationships for this tertile. The reduced magnitude of association of genotype and factor VII level among those with a high cholesterol value might be due in part to a greater environmental influence. There are several possible explanations for the differences between our findings and the earlier observations. First, previous studies had a smaller sample size.12,13,19 Because of the low frequency of the Gln allele, there were only 10, 24, and 63 in those studies.12,13,19 Second, different ethnic backgrounds might influence the association.12,13,20 The Framingham Offspring Study sample is overwhelmingly white. Third, statistical methods differed. In contrast to our work, prior studies did not employ formal statistical comparison of the slopes of the correlation.13,19

The Arg/Gln Polymorphism and Prevalent CVD

On the basis of the association of the Arg allele with increased factor VII levels11,12 and the association of high factor VII coagulant activity with an increased risk of coronary heart disease,6 one could speculate that the Arg allele is a genetic risk factor for CVD.21 Given the availability of effective anticoagulant therapy, the Arg/Gln polymorphism genotype might provide important information regarding risk stratification and drug intervention. However, whereas Iacoviello et al.23 found that this polymorphism was a risk factor for myocardial infarction, we found no significant association between the polymorphism and prevalent CVD, regardless of whether the prevalence of overall CVD or of thrombotic CVD (ie, history of unstable angina, myocardial infarction, or ischemic stroke) was used as the phenotype. Several explanations are possible for the difference between our findings and the positive association from Iacoviello et al. First, that study had a smaller sample size in which they compared 165 cases with 225 controls.23 Because genetic association studies are very sensitive to the selection of representative, genetically compatible controls, a small sample size will increase the chance of a false-positive finding by selection bias. Indeed, reported allele frequencies of the Arg/Gln polymorphism have varied considerably among studies and even among subgroups in the same study.13,21 Second, the Arg/Gln polymorphism may be in linkage disequilibrium with a putative pathogenic mutation elsewhere in the factor VII gene or with another as-yet-unidentified gene that is close to the factor VII gene. The presence/preservation of linkage disequilibrium between a marker (ie, the Arg/Gln polymorphism) and the actual causative gene mutation is highly dependent on population structure and history. Thus, linkage disequilibrium that is maintained in a genetically relatively isolated sample may be lost in a more diverse population such as represented by the Framingham Heart Study. The correct interpretation of our data would be that in a general North American white population, the factor VII Arg/Gln polymorphism is not associated with significant differences in CVD prevalence.

As with any study that fails to reject the null hypothesis, assessment of statistical power is important. Our data had the power to detect a modest increase in OR of the Arg/Gln polymorphism associated with CVD. For example, if one assumes an additive effect of the Arg allele on CVD (a reasonable assumption, as the effect of the Arg allele on plasma factor VII antigen levels fits an additive model), our study provides an 80% power to detect an OR of 1.34 for CVD or of 1.54 for thrombotic CVD.

Factor VII Antigen and CVD

There was no significant association between factor VII antigen level and prevalent CVD. Indeed, the level tended to be lower in subjects with thrombotic CVD compared with nonthrombotic CVD or no CVD. A similar finding has been shown by Cortellaro et al.36 This could be either a chance finding owing to a small sample size in the thrombotic CVD group (n=65) or possibly due to “consumption” of factor VII39 caused by activation of the coagulation system associated with ongoing thrombosis in the thrombotic CVD group.40,41 Although earlier studies found that factor VII coagulation activity was associated with CVD, the relation between factor VII antigen levels and CVD is not well defined.5–8,23
Study Limitations

First, analyses for the Arg/Gln polymorphism and clinical CVD were based on prevalent, not incident, events. Survivorship bias in this cross-sectional study design is a possibility, because only subjects with nonfatal CVD were included, especially in light of the association of factor VII levels with fatal CVD death. However, we did not see significant variation in the Gln/Gln frequency across age groups (P = 0.75, data not shown), thereby lessening the likelihood of substantial survivorship bias. We plan to follow the study sample prospectively. Second, we measured factor VII antigen levels, not factor VII activity. However, a strong, positive correlation has been shown between factor VII antigen levels and factor VII activity, with correlation coefficients of 0.71 to 0.89. Thus, it is unlikely that measuring factor VII antigen levels instead of factor VII activity has materially influenced our results. Finally, we did not measure makers of activation of the coagulation system. However, other studies have convincingly shown evidence of ongoing activation among patients with thrombotic CVD.39–41

Conclusions

The Arg/Gln polymorphism was a significant determinant of factor VII levels in the Framingham Heart Study, such that individuals with the Gln allele had lower levels. The strength of this association suggests that genetic variations play an important role in determining factor VII levels. Although factor VII plays a pivotal role in the coagulation system, we did not find a significant association between the Arg/Gln polymorphism and prevalent CVD. Further prospective evaluations are needed.

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