Epidemiological and Genetic Associations of Activated Factor XII Concentration With Factor VII Activity, Fibrinopeptide A Concentration, and Risk of Coronary Heart Disease in Men

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Background—The relations of plasma activated factor XII (FXIIa) concentration and a common polymorphism (C46T) of the factor XII gene with hemostatic status and risk of coronary heart disease (CHD) were examined by prospective surveillance.

Methods and Results—Genotyping for the C46T variant was performed in 2624 men 50 to 61 years of age who were free of CHD at baseline. The genotype distribution was as follows: CC, 56.7%; CT, 36.9%; and TT, 6.6%. Plasma FXIIa was measured by ELISA on 1745 samples collected 1 year after baseline; median levels were (ng/mL) CC, 2.0; CT, 1.4; and TT, 0.8 (P<0.0001). Respective values for plasma fibrinopeptide A (FPA, nmol/L) were 1.52, 1.35, and 1.15 (P<0.0001); for factor VII coagulant activity (FVIIc, % standard), 114.5, 116.2, and 109.3 (P=0.02). Group differences in FVIIc were unchanged by adjustment for body mass index and serum triglycerides. Whereas CHD incidence did not differ significantly by genotype, rates (per 1000 person-years) by thirds of FXIIa distribution were for <1.5 ng/mL, 1.5; for 1.5 to 2.0 ng/mL, 7.2; and for >2.0 ng/mL, 13.6. Respective hazard ratios with the low third as reference group were 1.01 and 1.96 (P=0.007), which were essentially unchanged after allowance for genotype, blood lipids, blood pressure, body mass index, FVIIc, and FPA.

Conclusions—The C46T polymorphism is a determinant of FXIIa, FPA, and possibly FVIIc, suggesting that FXII influences the activity state of the coagulation pathway and FPA cleavage from fibrinogen in vivo. Plasma FXIIa is increased in middle-aged men at high risk of CHD. (Circulation. 2000;102:2058-2062.)

Key Words: coronary disease ■ genes ■ coagulation ■ epidemiology

The contact system consists of the plasma proteins factor XII (FXII), prekallikrein, factor XI, and high-molecular-weight kininogen.1 The step initiating activity is the conversion of FXII to its derivative enzyme FXIIa, achieved in vitro (but not yet demonstrated unequivocally in vivo) by contact with substances having a negatively charged surface.2,3 Human endothelial cells possess receptors for FXII4 and high-molecular-weight kininogen.5 Production of FXIIa creates the potential for dissemination of reactions along several pathways concerned with tissue defense and repair,6–11 including the conversion of plasminogen to the fibrinolytic enzyme plasmin12 and activation of factor IX (FIX)13 and factor VII (FVII)14 in the coagulation system. Some of these actions have been demonstrated only in vitro, however, and their relevance for health is poorly understood.

When FXII coagulant activity (FXIIC) and FXII antigen (FXIICg) were measured in survivors of an acute myocardial infarction (MI) and healthy subjects, the former had significantly raised levels in one study,15 but not in another.16 A clinical study reported FXIIa to be significantly higher in survivors of MI than in healthy control subjects.17 An earlier analysis of the present study showed that FXIIa was positively and independently associated with the major conventional risk factors for coronary heart disease (CHD).18 Here, the relations of plasma FXIIa concentration and a common (C46T) polymorphism of the FXII gene19 with risk of CHD have been explored by prospective surveillance.

Methods

Subjects and Procedures

Details of the survey, the Second Northwick Heart Study, have been reported previously.20 Briefly, 4600 men 50 to 61 years of age belonging to 9 general medical practices were screened for eligibil-
ity. Those with evidence of MI on the ECG, a history of unstable angina, MI, regular medication with aspirin or anticoagulants, cerebrovascular disease, life-threatening malignancy, or other infrequently specified characteristics were excluded, leaving 2951 for study. Recruitment began before the assay for FXIIa became available, so those measurements were made on samples stored at the first annual reexamination.

Participants attended for examination in a nonfasting state, having been requested not to smoke or undertake vigorous exercise from the midnight before. A 23-mL blood sample was taken and processed as described elsewhere. Silicon-coated tubes were used to collect 4.5 mL of blood into 0.5 mL of 0.106 mol/L trisodium citrate anticoagulant and 13.5 mL into 1.5 mL of a commercial anticoagulant containing trasylool, EDTA, and a chloromethylketone thrombin inhibitor (Byk-Sangtec). Plasma levels of prothrombin fragment F1,12 in blood collected in this anticoagulant in excellent agreement with those in samples taken into an anticoagulant mixture of acid citrate dextrose, EDTA, and heparin. A 5-mL blood sample was taken for serum.

Assays were performed on citrated plasma except when indicated. FVII coagulant activity (FVIIc)22 and activated FVII (FVIIa)23 were measured by 1-stage bioassay. FVII antigen (FVIIag) concentration was measured by ELISA (Novo Nordisk, Bagsvaerd). Fibrinogen concentration was measured by a thrombin-clotting method24 with those in samples taken into an anticoagulant mixture of acid citrate dextrose, EDTA, adenosine, and heparin.21 Plasma levels of prothrombin fragment F1,12, in the presence of the T allele, digestion does not occur. Digested polymerase chain reaction fragments were separated as described elsewhere.30

Statistical Analysis

Univariate analysis of continuous variables was by 1-way ANOVA. Variables were log transformed when needed to normalize distributions and stabilize variance. Plasma FXIIa levels were highly skewed; therefore, a Kruskal-Wallis nonparametric test was used in preference to 1-way ANOVA. Survival analysis used Cox’s proportional-hazards model, with the significance of the parameters in the model assessed by log likelihood ratio test. All results were exponentiated as hazard ratios (95% CIs).

Results

Of 2951 European respondents qualifying for follow-up, 2624 were genotyped. At the first annual reexamination, 2471 genotyped respondents were seen and known to be free of a CHD event. Stored plasma for determination of FXIIa at this reexamination was available for 1745 men who were followed up beyond this point.

Plasma FXIIa concentration was not different in the 23 men with an FPA of 10 nmol/L and those with lower FPA levels (P = 0.16). However, although FXIIa concentrations were similar in those with satisfactory and less than satisfactory venepunctures, they appeared raised in 27 men with unsatisfactory venepunctures; the mean levels were 1.65, 1.49, and 2.07 ng/mL, respectively (P = 0.004). Exclusion of these 27 had no effect on the results of the statistical analysis; therefore, the findings presented are for all 1745 respondents. The assay result was not affected by storage up to 12 months or by holding samples at room temperature for up to 6 hours.

Clotting Factors and Genotype

The distribution of genotypes for FXII was in Hardy-Weinberg equilibrium, with frequencies as follows: CC, 56.7%; CT, 36.9%; and TT, 6.6%. The relative frequency of the T allele was 25% (95% CI, 24 to 27). Table 1 presents the mean or median of characteristics significantly related to genotype. Median FXIIa in the TT genotype was 40% of that in the CC group, with that in the CT genotype intermediate (P < 0.0001), suggesting codominance. Similarly, mean FPA in the TT genotype was 24% lower than that for the CC group, with men of CT genotype intermediate (P < 0.0001). Mean FVIIc was lower by ≈5% of standard in the TT genotype compared with others (P = 0.02), the difference remaining essentially unchanged (P = 0.045) after allowance for body mass index (BMI) and triglyceride concentration.

**TABLE 1.** Hemostatic Factors by FXII Genotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n*</td>
<td>1359</td>
<td>890</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>Median FXIIa, ng/mL†</td>
<td>2.0</td>
<td>1.4</td>
<td>0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean FVIIc, % standard</td>
<td>114.5</td>
<td>116.2</td>
<td>109.3</td>
<td>0.02‡</td>
</tr>
<tr>
<td>Mean FPA, nmol/L§</td>
<td>1.52</td>
<td>1.35</td>
<td>1.15</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Number with results also for BMI and triglyceride concentration.
†1745 subjects (CC = 971; CT = 665; TT = 109).
‡0.045 after adjustment for BMI and triglyceride concentration.
§Logarithmic means. Results excluded in samples with FPA ≥ 10 nmol/L.
TABLE 2. Distributions of Selected Characteristics in Men Genotyped and Free of CHD

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n*</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>2471</td>
<td>57.6</td>
<td>3.4</td>
</tr>
<tr>
<td>BMI, kg/m²†</td>
<td>2404</td>
<td>26.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>2404</td>
<td>133.2</td>
<td>18.2</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>2404</td>
<td>81.9</td>
<td>11.0</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>2386</td>
<td>5.60</td>
<td>0.98</td>
</tr>
<tr>
<td>Triglyceride, mmol/L‡</td>
<td>2386</td>
<td>1.98</td>
<td>1.21</td>
</tr>
<tr>
<td>FVIIa coagulant activity, % standard</td>
<td>2384</td>
<td>114.8</td>
<td>29.1</td>
</tr>
<tr>
<td>Activated FXII, ng/mL‡§</td>
<td>1745</td>
<td>1.83</td>
<td>0.92</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL†</td>
<td>2384</td>
<td>275.7</td>
<td>54.4</td>
</tr>
<tr>
<td>Prothrombin F1,₂, nmol/L¶</td>
<td>2344</td>
<td>0.76</td>
<td>0.34</td>
</tr>
<tr>
<td>FPA, nmol/L¶</td>
<td>2346</td>
<td>1.65</td>
<td>1.02</td>
</tr>
</tbody>
</table>

*Numbers are <2471 for variables because of missing values.
† Logarithmic mean with approximate SD.
‡ Nonfasting: median (interquartile range)=1.70 (1.19–2.39).
§ Median (interquartile range)=1.70 (1.20–2.20).
¶ Result excluded in samples with FPA ≥10 nmol/L.

Data were available for FVIIc, FVIIag, and FXIIa at baseline, with associations with FXII genotype being similar to that for FVIIc at 1 year of follow-up: mean FVIIc (% standard): CC, 108; CT, 107; and TT, 101 (P=0.055); mean FVIIag (% standard): CC, 127; CT, 128; and TT, 123 (P=0.087); mean FXIIa (ng/mL): CC, 2.05; CT, 1.80; and TT, 1.50 (P=0.067).

Mean diastolic blood pressure (BP) was similar in the CC and CT genotypes but was 2.5 mm Hg lower in the TT group (P=0.03). Smoking status, systolic BP, BMI, cholesterol, triglycerides, fibrinogen, and F₁₂ were unrelated to FXII genotype.

FXII and Conventional Risk Factors for CHD

Table 2 presents the distributions of age, BMI, BP, cholesterol, triglycerides, FVIIc, FXIIa, fibrinogen, F₁₂, and FPA in the 2471 subjects genotyped and free of CHD at 1 year. When these distributions were divided into thirds, clear trends to increasing FXIIa concentration were observed with increasing values of all variables (P<0.001 for linear trend) except F₁₂ and fibrinogen concentrations. The associations of FXIIa with FVIIc and FPA were independent of cholesterol and triglyceride concentrations (r=0.08, P=0.002 and r=0.19, P<0.0001 after adjustment, respectively). Furthermore, the associations of FXIIa with BMI, BP, cholesterol, triglycerides, FVIIc, and FPA were independent of its association with risk of CHD (P<0.001 after adjustment except for systolic BP, P=0.005).

FXII and CHD

Among the 2590 men genotyped and followed up, 139 (5.4%) had a CHD event during 16 163 person-years of surveillance, and 97 (3.8%) had acute CHD. In the CC group, 74 (5.1%) of 1462 men had a CHD event compared with 60 (6.3%) of 969 in the CT group and 5 (3.0%) of 169 with the TT genotype. In a Cox proportional-hazards model with TT as the reference group, the hazard ratio did not differ significantly between genotype and risk of CHD (P=0.001 after adjustment except for systolic BP, P=0.005).

TABLE 3. Independent Associations of FXIIa and FXII Genotype With Risk of CHD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio* (95% CI)</th>
<th>p (Likelihood Ratio Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXIIa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle third</td>
<td>1.15 (0.63–2.08)</td>
<td>0.58</td>
</tr>
<tr>
<td>High third</td>
<td>2.45 (1.39–4.32)</td>
<td>0.002</td>
</tr>
<tr>
<td>FXII genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>1.05 (0.40–2.77)</td>
<td>0.23</td>
</tr>
<tr>
<td>CC</td>
<td>0.70 (0.25–1.91)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

*Hazard ratios estimated from the same model. Those in the low third of the distribution of FXIIa with the TT genotype were the reference group.

Discussion

The assay of apparent FXIIa appeared to be affected by an unsatisfactory venepuncture, but inclusion of these results did not alter the findings of the statistical analysis. With respect to sample handling, storage up to 12 months and standing at room temperature for up to 6 hours did not affect FXIIa concentration. Thus, although samples were collected with venepunctures of differing quality (93% were coded satisfactory) and stored frozen for varying periods, collection and handling procedures appeared not to have introduced errors that could lead to misinterpretation of the findings.

As reported by others,19,31 the CC genotype was associated with a significantly higher FXIIa concentration than homozygosity for the rare allele of the FXII genotype (TT). The C-T change creates a novel AUG methionine start-of-translation codon upstream of the correct AUG site, shown in vitro to result in less efficient translation of the T-mRNA compared.
with the C-mRMA. Unimpaired translation in the CC genotype results in relatively high FXII and FXIIa levels.

Novel findings in this study were the significantly lower levels of FVIIc and FPA in the FXII TT genotype compared with the CT and CC groups. On average, FVIIa was 27% lower in the TT group than the CC group ($P=0.055$). Studies in vitro have shown activation of FVII by FXIIa, but whether the same occurs in vivo is uncertain. Although the higher FVIIc and FXIIa levels in the CC group, who had the higher FXIIa levels, could have occurred by chance, they may indicate that the genotypically determined status of FXII has consequences for FVII activity in vivo. This conclusion agrees with findings in patients with hereditary angioedema, in whom C1 inhibitor is very low and the contact system is activated. Plasma FXIIa concentrations in such patients are significantly higher than in healthy control subjects, suggesting modest yet biologically important activation of FVII when FXIIa activity is raised. Furthermore, activation of FVII has been observed in rhesus monkeys after implantation of a porous chamber containing immobilized human FXIIa.

The strong association between FXIIa and FPA levels, according to FXII genotype, suggested that an increase in FXIIa has consequences for the cleavage of FPA from fibrinogen. Unexpectedly, this effect was not accompanied by any increase in F$_1$$_2$. Although there are no data within the study to address this observation further, the paradox could be explained by a reduction in antithrombin, which would result in lengthening of the half-life of thrombin. Similar apparent discrepancies have been observed in other studies. For example, in patients who suffered an acute MI but did not receive heparin, fibrin monomer was increased on the second day after the event in those who died or had a new MI within 90 days, whereas F$_1$$_2$ concentration was normal at this time point.

The genotype for FXII determines FXII concentration and therefore will contribute to the FXIIa level by mass action. This study has also shown that serum cholesterol and triglyceride concentrations account for some additional variability in FXIIa. Thus, plasma FXIIa concentration appears to be determined partly by FXII genotype, partly by plasma lipid levels, and partly by nonlipid factors that explain its association with CHD. The independent association of FXIIa with CHD and its relations with several major CHD risk factors suggested that FXIIa concentration serves as a marker of the extent and severity of atherosclerosis. Coppola et al reported a marked increase in FXIIa in patients with MI who had received thrombolytic therapy. Kohler et al found FXIIa concentration to be strongly associated with the extent of coronary artery stenosis.

The origin of an increased FXIIa in men at high risk of CHD is uncertain. One possible mechanism might be that activation of vascular endothelium or other cells involved in the atherosclerotic process enhances FXII activation. This would help explain why a low FXIIa concentration is not protective against CHD; increased concentrations are primarily a consequence rather than a cause of underlying vascular disease, although high FXIIa levels may conceivably play a secondary role in the disease process.

Six percent of men were of the TT genotype. Compared with the CC and CT genotypes, TT carried a crude hazard ratio of 0.42, although with a wide 95% CI (0.13 to 1.35), and in a Cox proportional-hazards model, there was no significant difference in risk of CHD by genotype. Larger studies are needed to state with confidence whether the TT genotype confers protection against CHD because of its relatively infrequent association with a high FXIIa concentration. However, if as suggested a high FXIIa level is an effect rather than a cause of the underlying pathology in CHD, such an inherited form of protection would seem a remote possibility.

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_Circulation_. 2000;102:2058-2062
doi: 10.1161/01.CIR.102.17.2058

_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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