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, 7.2; 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 (FXIIag) were measured in survivors of an acute myocardial infarction (MI) and healthy subjects, the former had significantly raised levels in one study15 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 characteristics20 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.20 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 trasylol, EDTA, and a chloromethylketone thrombin inhibitor (Byk-Sangtec). Plasma levels of prothrombin fragment F1 \(_2\), in blood collected in this anticoagulant are in excellent agreement with those in samples taken into an anticoagulant mixture of acid citrate dextrose, EDTA, adenosine, and heparin.21 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 (FVIIla)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 the use of a standard plasma (Immuno) calibrated against a World Health Organization (WHO) international standard (89/644). Prothrombin fragment F1 \(_2\), and fibrinopeptide A (FPA) were measured in plasma collected into the commercial anticoagulant. Plasma F1 \(_2\) was measured by double-antibody radioimmunoassay25 and FPA was measured by commercial radioimmunoassay (Byk-Sangtec) as indexes of prothrombin and fibrinogen turnover, respectively. Plasma apparent FXIIa was measured by an ELISA with a monoclonal antibody specific for FXIIa26 (Axis-Shield). Serum cholesterol and triglyceride concentrations were measured by automated enzyme procedures (Sigma and Wako Chemicals [Alpha Laboratories], respectively).

For apparent FXIIa, the within-person coefficient of variation of measurements made during the study was 10.5%, while the within-run coefficient of variation of assays of split samples was 5.4%. The respective coefficients of variation for other hemostatic indexes were as follows: FVIIc, 15.0% and 2.2%; FXIIa, 23.0% and 11.1%; FVIIag, 11.1% and 3.5%; F1 \(_2\), 25.0% and 7.6%; and FPA, 38.8% and 24.0%.

Tests were performed to determine whether the FXIIa assay was influenced by the quality of venepuncture,20 length of sample storage, and 24.0%.

Follow-Up and End Points

The principal end points were first CHD events defined as any of the following: (1) acute CHD, defined as sudden coronary death or fatal and non-fatal MI (History, ECG, cardiac enzymes, and pathology were assessed by an independent reviewer who classified events by WHO criteria27); (2) silent MI, defined as new major Q waves on the ECG (Minnesota codes 1 1 ,1 2.1 through 1 2.7 , and 1 2.8 plus 5 1 or 5 2 )28; and (3) surgery for angina pectoris with CHD angiographically demonstrated. Acute CHD alone was a subsidiary end point. Any CHD event was 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>
<thead>
<tr>
<th>TABLE 1. Hemostatic Factors by FXII Genotype</th>
</tr>
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<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>Mean FXIIa, ng/mL†</td>
</tr>
<tr>
<td>Mean FVIIc, % standard</td>
</tr>
<tr>
<td>Mean FPA, nmol/L§</td>
</tr>
</tbody>
</table>

*Number with results also for BMI and triglyceride concentration. †In 1745 subjects (CC =971; CT =665; TT =108). ‡0.045 after adjustment for BMI and triglyceride concentration. §Logarithmic means. Results excluded in samples with FPA ≥10 nmol/L.20
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>FVII 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. ²⁰

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, 1.70 (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 F₁₋₂ 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, F₁₋₂, 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 F₁₋₂ 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 groups (P=0.12). These findings were essentially unchanged when the analysis was repeated on acute CHD alone.

In the 1745 men who were genotyped, had FXIIa determined, and were followed up, 92 (5.3%) had a CHD event and 56 (3.2%) had acute CHD. Median (interquartile range) FXIIa was 2.0 ng/mL (1.4 to 2.4 ng/mL) in the group of 92 compared with 1.7 ng/mL (1.2 to 2.2 ng/mL) in those remaining free of the disorder (P=0.005, Kruskal-Wallis test). Incidence rates of CHD per 1000 person-years by thirds of the distribution of FXIIa were as follows: low (<1.5 ng/mL), 7.2; middle, 7.2; and high (>2.0 ng/mL), 13.6. The respective hazard ratios with the low third as reference group were 1.01 (95% CI, 0.58 to 1.77) and 1.96 (95% CI, 1.20 to 3.20) (P=0.007, likelihood ratio test). Thus, there was strong evidence for an increased risk of CHD when FXIIa was >2.0 ng/mL. Table 3 shows that the estimate of hazard in the high third remained significantly increased after adjustment for genotype in a multivariate Cox model, whereas genotype was unrelated to risk when FXIIa was taken into account. After allowance for genotype, cholesterol, triglycerides, fibrinogen, FVIIc, FPA, systolic BP, and BMI, the adjusted hazard ratios in the middle and high thirds of FXIIa (relative to low third) were 1.03 (95% CI, 0.56 to 1.90) and 2.05 (95% CI, 1.11 to 3.75; P=0.02), respectively. For acute CHD alone, the respective estimates were 1.06 (95% CI, 0.51 to 2.19) and 2.14 (95% CI, 1.13 to 4.04; P=0.03).

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 satisfactorily) 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, 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

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>0.002</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>0.23</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.
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.

**Acknowledgments**

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**References**


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