Tissue Factor and Coagulation Factor VII Levels During Acute Myocardial Infarction

Association With Genotype and Adverse Events

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Objective—We investigated in patients with ongoing myocardial infarction (MI) whether coagulation factor VII (FVII) and tissue factor (TF) levels are affected at admission by genetic components and whether they may predict subsequent cardiovascular events.

Methods and Results—256 patients admitted for MI were evaluated for FVII and TF antigen levels before any treatment at entry, and were genotyped for FVII and TF polymorphisms. FVII gene insertions at /H11002 323, 11293 and the /H11002 402G/A change predicted FVII levels and explained 14% of variance. The /H11002 603 TF gene polymorphism failed to affect significantly TF levels (P=0.07). These variables were correlated with the incidence of death (36 patients) and reinfarction (9 patients) after a median follow-up of 397 days. Events were independently predicted by FVII (HR 2.1, 95% CI 1.2 to 5.7) and TF (HR 4.1, 95% CI 2 to 11) levels. Composite end point was significantly worse when both parameters were above the receiver-operating characteristics (ROC) values (HR 8.3, 95% CI 5 to 18, compared with FVII and TF below), and above the ROC value of TF (>630 pg/mL) it differed among FVII genotype groups.

Conclusions—Admission FVII and TF antigen levels, partially predicted by polymorphisms, are independent predictors of mortality and reinfarction in patients with acute MI. (Arterioscler Thromb Vasc Biol. 2006;26:000-000.)

Key Words: myocardial infarction ■ tissue factor ■ factor VII ■ polymorphism ■ prognosis

Coagulation factor VII (FVII) and tissue factor (TF) play a key role in activating the extrinsic coagulation pathway and might be involved, as a single player or in combination, in triggering acute coronary syndromes (ACS). In healthy subjects and in patients with chronic ischemic heart disease, it has been demonstrated that FVII and TF levels are influenced by both environmental and genetic factors.1–4 Previous studies suggested that elevation of FVII may predict coronary events in healthy middle-aged subjects.5–6 However, others studies did not confirm these findings.7–9 In a consecutive group of patients undergoing diagnostic catheterization, TF levels were reported to be higher in those presenting with clinical instability and to predict the need for subsequent revascularization.9 Moreover, in a sub-study of the WARIS-II trial, involving patients with myocardial infarction (MI), increased levels of TF, measured 5 to 7 days after the acute event, were independently associated to the occurrence of future clinical events.10

The role of admission FVII and TF levels in patients with acute MI, however, has been less extensively studied. In particular, very limited data are available regarding the prognostic influence of FVII and TF levels at entry in patients admitted for acute MI. Moreover, whether FVII and TF levels, also during the acute phase of myocardial injury, are influenced by gene polymorphisms is unknown.

The purpose of the present article was to analyze whether FVII and TF levels at admission are affected by intragenic polymorphisms and assess their association to long-term outcome in patients with ongoing MI.

Methods

Patients

Between May 2003 and June 2004, 256 subjects with MI were enrolled in the present study. Patients were selected from a cohort of 378 patients consecutively referred to our Coronary Care Unit for ACS, based on the following inclusion criteria: prolonged chest pain occurring at rest accompanied by electrocardiographic ischemic changes defined as at least one of the following conditions: ST-segment elevation or depression ≥1 mm, T waves inversion, pseudo-normalization of previously negative T waves. Patients with symptoms onset >24 hours, or immunologic disorder or liver...
cirrhosis, use of oral anticoagulants or contraceptive were deemed ineligible for the study. The study was approved by the local Ethics Committee. All patients gave written informed consent. The median symptom onset-entry time was 5.3 hours (range 1 to 19 hours). Myocardial necrosis was defined as the elevation of CK-MB and/or Troponin I above the upper reference limit (respectively, 5 ng/mL and 0.1 ng/mL) in 2 or more consecutive samples.13

**Blood Sample Collection**

Blood withdrawal was performed from all patients on admission before any invasive procedure and the start of any therapy. Then, the samples were centrifuged for 20 minutes at 2000 g. All samples were stored at −80°C for DNA extraction and plasma determination.

**Factor VII and Tissue Factor Assays**

FVII antigen (ng/mL) was assayed in plasma by immunoenzymatic test (Asserachrom VII:ag, Diagnostica Stago). The intra-assay coefficient of variation was 6.9%, and the inter-assays coefficient of variation was similar (6.9%). Plasma TF antigen (pg/mL) was determined by ELISA method using IMUBIND Tissue Factor ELISA Kit (American Diagnostica Inc). The intra-assay coefficient of variation was 13.2%, and the inter-assays coefficient of variation was 14.7%.

**Mutation Analysis and Nomenclature**

DNA was extracted from peripheral blood lymphocytes by salting-out method. Three FVII gene polymorphisms were investigated: −402G/A (−323 decamer insertion (A1 and A2 alleles; A2 defined as presence of the insert)13; the 11293 to 11295insAA in the 3’ untranslated region (D and I alleles, I defined as the presence of the insert).14 Also the TF −603A/G promoter polymorphism, belonging to a specific haplotype with linked polymorphisms at positions −1812, −1332, −1208, was tested.13

**Clinical Follow-Up and End Point definitions**

Patients underwent outpatient visits every six months and no patient was lost to follow-up. Our composite end point was the cumulative incidence of death and reinfection. Reinfarction was diagnosed in the presence of new ischemic symptoms and recurrent elevation of biochemical myocardial necrosis markers (CK-MB and/or Troponin I) that were not due to a previous MI with confirmed electrocardiographic changes.

**Statistical Analysis**

Continuous data (normally distributed at Kolmogorov–Smirnov test) are presented as means ± SD, with the significance of differences judged by t-test. Categorical variables were summarized in terms of number and percentages and were compared using two-sided Fisher exact test. Spearman correlation coefficients were used to detect any association between variables. According to previous studies,8–10 it was hypothesized that patients with high (above median value) and low (below median value) levels of TF antigen would display a composite end point free survival rate of 75% and 90% at 1 year, respectively. Therefore, a final population of 583±193 versus 581±215 ng/mL, P=0.9) and it was not related to any of the variables listed in Table 1. No relationship was found between FVII and CK-MB release or left ventricular ejection fraction (LVEF). Gene frequencies of FVII polymorphisms were in Hardy–Weinberg equilibrium and were similar to those previously reported in the Italian population6 (Table 2). Homozygosis for the A allele was associated with 40% lower FVII levels, whereas homozygosis of the A allele of the −402 polymorphism was associated with 40% higher FVII levels (Table 2). 11293 polymorphism was in linkage disequilibrium with −323 polymorphisms and showed similar FVII antigen levels (Table 2). These polymorphisms explained 14% of the total variance of FVII levels (adjusted $R^2$: 0.1368). Figure 1 reports the distribution of FVII levels in patients grouped by −323 and −402 genotypes.

**Tissue Factor Plasma Levels and Gene Polymorphism**

As for FVII antigen, there was no difference in terms of TF levels between STEMI and NSTEMI groups (577±180 versus 576±118 pg/mL, P=0.9). Age showed a weak correlation with TF levels (r=0.16, P=0.01). Patients with previous MI had higher TF level (620±201 versus 570±150 pg/mL, P=0.05). No relationship was found between TF and CK-MB release or LVEF: Gene frequencies of ~603 polymorphism were in Hardy–Weinberg equilibrium and were similar to those previously reported in the European population4 (Table 2). The ~603 polymorphism genotypes failed to affect significantly TF levels (P=0.07, Table 2).

**Clinical Outcome**

After a median follow-up of 397 days (range, 312 to 435 days), 36 deaths (14%) and 9 reinfarction (3.5%) were observed. A total of 45 patients (17.5%) reached the composite end point (35 in the STEMI group and 10 in the NSTEMI group, P=0.2). Those satisfying the composite end point were on average older (73±8 versus 66±12 years, P<0.001), more often had previous MI (33% versus 16%, P=0.04), displayed higher Killip class at entry (31% versus 9%, P=0.002) and lower LVEF (44±10% versus 48±11%, P=0.03). Moreover, they tended to show higher levels of CK-MB, but the difference did not reach statistical significance (170.4±198 versus 140.7±178 ng/mL, P=0.3).

**Results**

Table 1 depicts demographic, clinical and biochemical baseline characteristics for the 256 patients studied. Interventional and pharmacological treatment in the two groups is shown in Table 1.

**Factor VII Plasma Levels and Gene Polymorphisms**

FVII plasma levels across the population are presented in Table 2. FVII did not differ in ST-segment elevation myocardial infarction (STEMI) patients as compared with non-ST-segment elevation myocardial infarction (NSTEMI) group (583±193 versus 581±215 ng/mL, P=0.9) and it was not related to any of the variables listed in Table 1. No relationship was found between FVII and CK-MB release or left ventricular ejection fraction (LVEF). Gene frequencies of FVII polymorphisms were in Hardy–Weinberg equilibrium and were similar to those previously reported in the Italian population6 (Table 2). Homozygosis for the A allele was associated with 40% lower FVII levels, whereas homozygosis of the A allele of the −402 polymorphism was associated with 40% higher FVII levels (Table 2). 11293 polymorphism was in linkage disequilibrium with −323 polymorphisms and showed similar FVII antigen levels (Table 2). These polymorphisms explained 14% of the total variance of FVII levels (adjusted $R^2$: 0.1368). Figure 1 reports the distribution of FVII levels in patients grouped by −323 and −402 genotypes.
In those patients who reached the composite end point, FVII and TF levels were higher than those with follow-up free from adverse events (687/11006 232 versus 560/11006 185 ng/mL, P < 0.0001, and 711/11006 206 versus 550/11006 135 pg/mL, P < 0.0001). Similar findings were found analyzing the incidence of death alone (673/11006 230 versus 568/11006 191 ng/mL, P < 0.003, and 675/11006 115 versus 562/11006 164 pg/mL, P < 0.0001).

FVII and TF gene polymorphisms were not significantly related with the clinical outcome. Also considering the genotype groups predicting significant differences in FVII levels (Figure 1), the rate of adverse events did not differ significantly (P < 0.4, log-rank test).

At Cox proportional hazards regression, age, LVEF, FVII, and TF antigen levels (evaluated both as single changes unit and as below versus above median value), together with Killip class and previous MI were predictors of composite end point.

By incorporating as putative predictors all significant variables at univariate analysis plus ST-segment elevation at entry, hyperlipidemia and fibrinogen, the independent predictors of composite end point were age, Killip class, FVII antigen, and TF antigen (respectively, HR 1.9, 95% CI 1.3 to 6.7; and HR 3.7, 95% CI 1.6 to 8).

FVII and TF antigen remained associated with composite end point and with death when the analysis was restricted to both STEMI and NSTEMI subgroups.

Receiver-Operating Characteristics

The cutoff value of FVII at entry for the prediction of composite end point was 618 ng/mL, as identified by ROC. This cutoff had 64% sensitivity and 67% specificity. The area under the ROC curve was 0.68 (95% CI 0.6 to 0.76). The cutoff value of TF was 363 pg/mL. This cutoff had 66% sensitivity and 79% specificity. The area under the ROC curve was 0.77 (95% CI 0.69 to 0.84).

Figure 3 shows differences in composite end point when patients were stratified according to FVII (3A) and TF (3B) levels (under versus above ROC value).

To explore the additive prognostic value of combining FVII and TF levels, Kaplan–Meier curves were constructed.
according to the combinations generated by having lower or higher identified ROC values (Figure 4A). Composite end point was significantly worse when both parameters were above the ROC values (HR 8.3, 95% CI 5 to 18, compared with patients with low FVII and TF).

Kaplan–Meier curves were constructed also combining the \( /H1 \) and \( /H2 \) FVII genotypes with TF levels (above versus below ROC value). Among patients with low TF, FVII genotype influence was negligible (\( P \geq 0.6 \), data not shown).

Differently, in the high TF group (Figure 4B), there was a significant difference in the composite end point free survival when the \( -323A2 \) carriers/\( -402GG \) were compared with all homozygotes for the \( -323A1 \) (\( P = 0.05 \)) or those who were carriers of the \( -402A \) (\( P = 0.05 \)).

**Discussion**

Although it is well known that FVII and TF are influenced by environmental and genetic factors,\(^1\)–\(^4\) their circulating levels in patients with ongoing MI and their relationship with environmental and genetic factors are poorly known. Moreover, although previous studies investigated the association between different hemostatic factors and the occurrence of coronary events,\(^5\)–\(^10\) it remains unclear whether TF and FVII levels, measured in patients with MI soon after symptoms onset, may carry valuable prognostic information.

The major findings of our study are:

1. Plasma protein levels, particularly FVII, are partially influenced by gene polymorphisms even in the first hours of a myocardial necrotic injury, with the investigated FVII gene polymorphisms explaining 14% of the total variance of FVII.

2. FVII and TF antigen levels, measured at entry, emerged as independent predictors of death and reinfarction.

### TABLE 2. FVII and TF Antigen Levels and Genotypes

<table>
<thead>
<tr>
<th>FVII Polymorphism</th>
<th>Alleles</th>
<th>n (%)</th>
<th>FVII antigen (ng/ml)</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All population</td>
<td>254</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-323 ) genotype</td>
<td>A1A1</td>
<td>168 (66)</td>
<td>622±203</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>A1A2</td>
<td>79 (31)</td>
<td>518±155†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2A2</td>
<td>7 (3)</td>
<td>377±156†</td>
<td></td>
</tr>
<tr>
<td>(-11293 ) genotype</td>
<td>DD</td>
<td>179 (70)</td>
<td>626±200</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>DI</td>
<td>72 (29)</td>
<td>499±158†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3 (1)</td>
<td>397±215‡</td>
<td></td>
</tr>
<tr>
<td>(-402 ) genotype</td>
<td>GG</td>
<td>164 (65)</td>
<td>542±187</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>82 (32)</td>
<td>647±198†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>8 (3)</td>
<td>758±204‡</td>
<td></td>
</tr>
<tr>
<td>TF Polymorphism</td>
<td>Alleles</td>
<td>n (%)</td>
<td>TF antigen (pg/ml)</td>
<td>( P ) Value*</td>
</tr>
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<td>All population</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(-603 ) genotype</td>
<td>GG</td>
<td>64 (25)</td>
<td>614±192</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>118 (47)</td>
<td>566±138</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>72 (28)</td>
<td>562±127</td>
<td></td>
</tr>
</tbody>
</table>

In two patients, determination of genotype was not possible for technical reasons.

*\( P \) values are for the overall comparison among patients with a given polymorphism and were calculated by analysis of variance. †\( P = 0.01 \) for the comparison with the FVII level in patients with the most frequent genotype. ‡\( P = 0.05 \) for the comparison with the FVII level in patients with DD genotype.

**Figure 1.** FVII antigen levels stratified according to \(-323 \) and \(-402 \) \( G/A \) polymorphisms. *\( P < 0.001 \) Probability values are for the overall comparison among patients with a given polymorphism and were calculated by analysis of variance. †\( P < 0.01 \) for the comparison with \(-323A1A1/-402A \) carriers. ‡\( P < 0.01 \) for the comparison with \(-323A1A1/-402A \) carriers.

**Figure 2.** Independent predictors of composite end point at multivariate analysis. ■=Hazard risk of composite end point. The horizontal lines indicate the 95% confidence interval.
3. Outcome was significantly worse when both studied factors were simultaneously elevated, based on the cut-off provided by ROC analysis. FVII genotype influenced outcome only in patients with high TF values. This is the first study to address the issue whether FVII and TF gene polymorphisms are associated with variations of FVII and TF antigen levels during the first hours of MI (within 24 hours from symptoms onset). With that respect, FVII gene polymorphisms showed a significant although weak influence on FVII plasma levels, whereas TF gene polymorphism failed to affect TF plasma levels.

Previous studies, including patients with stable coronary artery disease, showed that FVII and TF polymorphisms could influence the levels and the activity of these proteins, and as such being associated with a reduced risk of MI.\(^2\)\(^-\)\(^4\) Our data extend previous findings to the acute setting of MI. In these conditions upregulation of promoters could mask other environmental components, thus possibly explaining our findings.

Our analysis suggests that TF and FVII antigen are independent predictors for the composite end point of death and reinfarction. Importantly, the predictive value of FVII and TF antigens was not restricted to the composite end point but also predicted mortality alone. Moreover, finding that composite end point was significantly worse when both TF and FVII levels were simultaneously above the cut-off provided by ROC analysis further suggests their role to be additive, which is a biologically plausible observation.

The reasons behind the observed association between FVII-TF levels and outcome may lie in their influence in the size and stability of the thrombus, and hence in the consequent myocardial damage. Interestingly, in our study no smoking, which are known from previous studies to be associated with increased coagulation factor levels, did not show an appreciable influence both on FVII and TF levels during acute MI. In these conditions upregulation of promoters could mask other environmental components, thus possibly explaining our findings.

Figure 3. Kaplan–Meier analysis. Composite end point free survival for FVII (top, A, \(P<0.0001\), log-rank test) and TF (bottom, B, \(P<0.0001\)) levels. Low FVII and TF = under ROC value. High FVII and TF = above ROC value. FVII ROC value = 618 ng/mL. TF ROC value = 630 pg/mL. HR was 3.5 (95% CI, 2 to 6.7) and 6 (95% CI, 3.2 to 11) compared with patients with low FVII and TF levels, respectively.

Figure 4. Kaplan–Meier analysis. A, Composite end point free survival \((P<0.0001,\text{ log-rank test})\) according to combinations generated by having lower or higher ROC values for FVII and TF antigen. FVII and TF are defined low if under the ROC value, high if above the ROC value. FVII ROC value = 618 ng/mL. TF ROC value = 630 pg/mL. B, Composite end point free survival \((P=0.03,\text{ log-rank test})\) among patients with high TF grouped for the \(-323\) and \(-402G/A\) FVII genotypes. The \(-323A2/-402A\) carriers are not shown (6 patients).
relationship was found between FVII-TF levels and myocardial damage evaluated both as peak of CK-MB release or LVEF. Moreover, the FVII-activated TF complex may also play a role in the migration and proliferation of vascular smooth muscle cells, in vascular remodeling, and in plaque neovascularization and thereby in promoting plaque destabilization.

Prior studies have reported conflicting results. In a study by Malarstig and colleagues involving ACS patients, no association between plasma TF and outcome was observed. Other studies reported TF levels to be an independent predictor of cardiovascular events. FVII genotypes predicted FVII levels but they were significantly associated to the outcome only in the high risk subgroup of patients characterized by having elevated TF (above ROC value). However, it should be noted that the limited sample size of our study does not allow drawing definitive conclusions in this regard. Bearing in mind these limitations, our data suggest that at admission circulating levels of FVII or TF are more predictive than their polymorphisms, thus supporting the role of other genetic and/or acquired factors in the regulation of the expression of these genes.

**Study Limitations**

We have not measured the activity of FVII and TF forms. Both FVII and TF are present in plasma in different molecular forms reflecting activation or exposure on membranes. The analysis of FVII and TF antigen reflects total FVII, both zymogene and activated forms, and total TF, both membrane-bound and soluble forms. Although we are well aware that the various FVII and TF forms could play different roles in thrombus formation, we assumed, in accordance with other authors, that elevation of FVII and TF antigen reflects their procoagulant activity. Recently, it has been suggested that the TF determination with a commercially available immunoassay is not satisfactory. Nevertheless, this immunoassay has been extensively used in previous studies and our data are in the range of values obtained with the new improved assay. The sample size of our study was too small to allow an affordable estimate of predictive role of gene polymorphisms. In particular, to obtain a reliable estimate of the prognostic capability of gene polymorphisms and if they could be a simple method to stratify the patients, a larger prospectively collected study population is clearly in demand. Our study was powered to evaluate the role of TF in predicting outcome and all other analyses performed, particularly the prognostic influence of gene polymorphisms and the causal relationship between environmental factors-plasma proteins-myocardial damage-cardiac function, should be considered exploratory and hypothesis-generating. Additional clinical investigations are also needed to understand whether patients have elevated plasma levels also during hospitalization and follow-up. This could allow to understand the influence of acute event on levels variations and to select the ideal timing to identify patients with a blood prone to thrombosis and thus at high risk of adverse events. Our data may suggest that samples obtained at entry are suitable for such a purpose.

**Conclusions**

Admission FVII and TF antigen, partially predicted by polymorphisms, were independent predictors of composite end point (mortality and reinfarction), which was even worse in patients showing both parameters simultaneously elevated. FVII genotype influenced outcome only in patients with high TF values. Our findings may reinforce the interest to further evaluate, in the clinical setting, the benefit of using drugs able to interfere with the action of FVII-TF complex, which are currently being developed.

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**Disclosures**

None.

**References**


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