Regular Article

Thrombin-activatable fibrinolysis inhibitor genetic polymorphisms as markers of the type of acute coronary syndrome

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Introduction: In patients with coronary disease at risk of acute coronary events it is unclear which biological factors could predict the type of acute coronary syndrome clinical presentation. The aim of the study was to investigate the role of genetic polymorphisms in key proteins in fibrinolysis in the type of acute coronary syndrome.

Materials and methods: 248 patients with acute coronary syndrome (unstable angina or myocardial infarction) (77% male, mean age 60.75 SD 13.30 years) were prospectively recruited. PAI-1 (type-1 plasminogen activator inhibitor) 4G/5G and TAFI (thrombin-activatable fibrinolysis inhibitor) Ala147Thr, C+1542G, and Thr325Ile polymorphisms were determined by PCR.

Results: 147 (59.3%) patients presented with ST-segment elevation acute coronary syndrome (all Q-wave myocardial infarction), and 101 (40.7%) with non-ST-elevation acute coronary syndrome (52 non-Q wave myocardial infarction, and 49 unstable angina). Homozygous TAFI +1542G and TAFI 325Ile genotypes were less prevalent in patients with ST elevation acute coronary syndrome (p < 0.001, OR: 0.22, 95% CI 0.10-0.50 and p < 0.001, OR: 0.25, 95% CI 0.11-0.55, respectively). There were no differences in TAFI Ala147Thr or PAI genotype distribution between ST elevation and non-ST elevation acute coronary syndrome. In the multivariate analysis including clinical variables, the best model for ST elevation acute coronary syndrome included TAFI +1542GG (p < 0.001, OR: 0.17, 95% CI 0.07-0.30), age (in years, p < 0.005, OR: 2.33, 95% CI 1.42-3.80) and dyslipidemia (p < 0.005, OR: 2.33, 95% CI 1.42-3.80).

Conclusion: TAFI polymorphism C+1542G and Thr325Ile are related to the type of acute coronary syndrome. Patients with coronary disease would benefit from individualized cardiovascular prophylaxis based on genetic risk.

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Acute coronary syndromes (ACS) result largely from thrombosis on the surface of an atheromatous coronary plaque. Plateau disruption or erosion with thrombosis resulting in a rapid change in plaque geometry may lead to acute occlusion or subocclusion, resulting in different clinical presentations of ACS, including myocardial infarction (MI), unstable angina and ischemic sudden death. It is known that local and circulatory factors can determine the extent and duration of thrombus and therefore the clinical presentation of ACS. In coronary patients at risk of ACS it is unclear whether biological factors can determine the different subsets of clinical presentation. Discovering the mechanisms that determine the grade or persistence of coronary thrombosis in ACS would have implications for the prevention and treatment of coronary atherosclerosis. The hemostatic system plays a central role in the pathogenesis of ACS and many studies suggest that vulnerability to atherothrombosis may be modulated by individual variations in the balance between coagulation and fibrinolysis [1]. Impaired fibrinolysis might be a risk factor for arterial thrombotic events [2] and a common feature of ACS in comparison with stable angina [3].

The main inhibitors of fibrinolysis are PAI-1 (type-1 plasminogen activator inhibitor) and TAFI (thrombin-activatable fibrinolysis inhibitor). PAI-1 reduces fibrinolysis by inhibiting t-PA, preventing the transformation of plasminogen to plasmin. TAFI is a procarboxypeptidase that attenuates fibrinolysis by removing carboxy-terminal lysine residues from partially degraded fibrin, inhibiting the assembly of fibrinolytic factors on the fibrin surface. Several studies have analyzed...
the relationship between PAI-1 and TAFI levels and the incidence of ACS, with contradictory results [4–6]. Patient characteristics (acute or chronic coronary disease), the time of blood sampling at which the study was performed (acute phase or not) and technical issues may explain the variability of the results. Non-modifiable factors such as polymorphisms in the genes encoding these proteins that may affect their structure, concentration, or function, might have an influence in the balance between coagulation and fibrinolysis and should be investigated to help identify a subset of patients at higher risk. Plasma PAI-1 levels have been found to be associated with a single base pair guanine deletion/insertion polymorphism (4G/5G) located in the promoter region, 675-bp upstream from the start of transcription of the PAI-1 gene [7]. Plasma TAFI levels or function have been related to several polymorphisms, of which the most-thoroughly-studied are two polymorphisms in the coding region that result in amino acid substitutions, Ala147Thr (505A/G), and Thr325lle (1040C/T) [8,9], and a polymorphism located in the 3′-UTR region C+1542G [10].

The objective of this study was to investigate whether genetic variations in the genes encoding key proteins in fibrinolysis are associated with the type of clinical presentation of ACS in a prospective cohort of patients.

Materials and methods

We prospectively recruited 248 consecutive patients with ACS (190 [77%] male, 58 [23%] female, mean age 60.75, SD 13.30 years) from the Cardiology Department of the Hospital Clinic of Barcelona (Spain). Inclusion criteria were unstable angina or myocardial infarction. Unstable angina was defined by chest pain at rest, or progressive angina, or cardiac ischemic symptoms of ≥10 minutes of duration at rest, and ST segment changes on the electrocardiogram suggestive of ischemia. Myocardial infarction (MI) was defined either by presence of ST-segment elevation (STE) or depression (non-STE) ≥1 mm in two or more contiguous leads, and troponin I levels >0.1 mg/mL. In the acute setting all the patients received aspirin, nitroglycerin and morphine. No pre-hospital thrombolysis was made in our series. Coronary angiography was conducted in 79% of patients, and significant coronary artery disease with, at least, one coronary artery showing >60% of luminal stenosis, was demonstrated in 95% of patients undergoing angiography. Overall 161 (65%) patients underwent PCI and 71 (29%) received thrombolytic therapy. One patient died intra-hospital, and at 30 days 5 more patients died.

The following variables were recorded: diabetes (defined as patients receiving insulin or oral hypoglycemic drugs or with fasting glycemia >200 mg/dL at admission or >126 mg/dL in two determinations); hypertension (defined as repeated blood pressure >140/90 mmHg or previous treatment with antihypertensive drugs); dyslipidemia (defined as total cholesterol >220 mg/dL, triglycerides >150 mg/dL or treatment with lipid lowering drugs), smoking and family history of cardiovascular disease.

The study was approved by the Human Experimental Committee of the Hospital Clinic and was performed according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Sampling

Venous blood samples were obtained at admission, within 48 hours of symptoms of cardiac ischemia. Samples for coagulation and fibrinolysis studies were obtained in tubes containing 3.8% trisodium citrate (1/9 volume/volume; Becton Dickinson, Rutherford, NJ), and platelet-free plasma was immediately obtained by double centrifugation, first at 2,000 g for 10 minutes at 22 °C, and then at 5,000 g for 10 minutes at 4 °C. Plasma was aliquoted, snap-frozen in a mixture of dry ice/ethanol (1/2 v/v), and stored. For genotype studies, samples were drawn in trisodium EDTA tubes (Becton Dickinson, Rutherford, NJ), and 100 µL of whole blood was immediately transferred into tubes containing lysis buffer (5 M guanidine thiocyanate, 1.3% [weight/volume] Triton X-100, and 50 mM Tris HCl, pH 6.4) and frozen at −80 °C.

Fibrinolysis parameters

TAFI antigen (TAFI Ag) levels were determined by an ELISA known to detect all TAFI isoforms [11] (Asserachrom TAFI, Diagnostica Stago, Asnieres, France). TAFI activity was determined by a chromogenic assay (Actichrome TAFI Activity Kit, American Diagnostica, Greenwich, CT).

Plasma levels of plasmin-alpha2-antiplasmin complexes (PAP) were quantified as a plasmin generation marker by ELISA (DadeBehring, Marburg, Germany).

Clot lysis time (CLT): Lysis of a thrombin-induced clot by exogenous t-PA was studied by monitoring changes in turbidity during clot formation and subsequent lysis. Plasma clot lysis time was done as previously described [12] with modifications. To 75 µL of citrated platelet poor plasma, 75 µL of a mixture containing thrombin (0.2 U/mL), 40 ng/mL t-PA (Actilyse, Boehringer Ingelheim, Germany), 12.5 mM CaCl2 and HEPES buffer (25 mM HEPES, 137 mM NaCl, 3.5 mM KCl, 3 mM CaCl2, 0.1% bovine serum albumin, pH 7.4) was added. After thorough mixing, turbidity at 405 nm was measured in time at 37 °C in a Multiskan Ascent (Thermolab Systems, Waltham, MA). Changes in optical density (OD) at 405 nm were monitored every 3 minutes. CLT was defined as the time from the midpoint of the clear to maximum turbid transition, which characterizes clot formation, to the midpoint of the maximum turbid to clear transition, which represents clot lysis. Samples were tested in duplicate.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>PCR conditions for the polymorphisms analyzed.</th>
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<td>Ref</td>
<td>Primers</td>
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<td>---------</td>
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<tr>
<td>PAI-1 4G/5G</td>
<td>Sense: 5′-CAG-AGG-GAG-AGG-TTC-TGT-GCC-CAC-TGG-3′</td>
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<td></td>
<td>Antisense: 5′-ATG-GCC-TAT-GAA-CCA-GAA-GT-3′</td>
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<tr>
<td>TAFI Ala 147Thr</td>
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</tr>
<tr>
<td></td>
<td>BaVi</td>
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<tr>
<td>TAFI C+1542G</td>
<td>58 °C</td>
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<tr>
<td></td>
<td>-</td>
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<td>TAFI Thr 325lle</td>
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**Results**

One hundred forty seven (59.3%) patients had ST-segment elevation (STE) ACS, and 101 (40.7%) had non-STE ACS. All patients with STE developed an Q-wave MI, while 52/101 (21%) patients with non-STE had non-Q wave MI and 49 (19.8%) had unstable angina. Patients with STE ACS and non-STE ACS (unstable angina or non-Q wave MI) were analyzed separately.

One hundred and one (40.7%) patients were smokers, 70 (28.2%) had diabetes, 143 (57.7%) had dyslipemia, 133 (53.6%) had hypertension, and 34 (13.7%) had a recorded family history of cardiovascular disease.

Mean TAFI Ag levels in ACS patients were 10.98 ± 3.01 μg/mL (normal values: 9.10 ± 1.51 μg/mL) and mean functional TAFI was 1.52 ± 0.21 μg/mL (normal values: 1.44 ± 0.32 μg/mL). Linear regression between both techniques was R = 0.79 (p < 0.001). Mean CLT was 641 ± 12.2 min. (normal values: 59 ± 6 min.), and mean PAP levels were 1487 ± 2152 μg/L (normal values: 415 ± 181 μg/L, p < 0.001).

The prevalence of PAI-1 and TAFI polymorphisms is shown in Table 2. All three TAFI polymorphisms were in linkage disequilibrium, with a stronger correlation between C + 1542G and Thr325Ile (C + 1542G/Thr325Ile: D = 0.0101, D′ = 0.0846, r² = 0.1989, p < 0.001; C + 1542G/Ala147Thr: D = 0.0317, D′ = 0.0054, r² = 0.0225, p < 0.001; Thr325Ile/Ala147Thr: D = 0.0432, D′ = 0.0104, r² = 0.040, p < 0.001)

The relationship between TAFI genotypes and plasma TAFI, PAP, and CLT values is shown in Table 3. The GG genotype of the C+1542G polymorphism was associated with lower TAFI Ag levels (p < 0.001, OR: 0.22, 95% CI 0.10-0.50), as was the TT (Ile/Ile) genotype of the Thr325Ile polymorphism (p < 0.001, OR: 0.25, 95% CI 0.10-0.60).

The relationship between TAFI, PAI-1 genotypes and plasma TAFI, PAP, and CLT values is shown in Table 3. The GG genotype of the C + 1542G polymorphism correlated with lower TAFI Ag and TAFI activity levels (p < 0.05 and p < 0.001, respectively). The TT (Ile/Ile) genotype of the Thr325Ile polymorphism also showed lower TAFI Ag and TAFI activity levels (p < 0.05 and p < 0.001, respectively). The GG (Ala/Ala) genotype of the Ala147Thr polymorphism was associated with lower TAFI Ag and TAFI activity levels (p < 0.001 and p < 0.05, respectively). No relationship was found between PAI-1 or genotypes evaluated or between the PAI-1 4G/5G polymorphism and PAP or CLT values.
95% CI 0.11-0.55). We found no differences in TAFI Ala147Thr or PAI genotype distribution between the two study groups.

In the multivariate analysis including only the genotypes, TAFI +1542GG was the only variable selected that had a protective effect for STE ACS (p < 0.001, OR: 0.22, 95% CI 0.08-0.55). When the TAFI C+1542G polymorphism was excluded, the TAFI 325Ile/Ile genotype was the only variable included (p < 0.001, OR: 0.25, 95% CI 0.09-0.75).

When clinical variables were also included in the multivariate analysis, the best model selected for STE ACS included TAFI +1542GG (Yes = 1, No = 0, p < 0.001, OR: 0.17, 95% CI 0.07-0.30), age (in years, p < 0.005, OR: 0.97, 95% CI 0.94-0.98) and dyslipidemia (Yes = 1, No = 0, p < 0.005, OR: 2.33, 95% CI 1.42-3.80).

**Discussion**

We analyzed the role of genetic variations in fibrinolysis inhibitors PAI-1 and TAFI in relation to the clinical presentation of ACS. TAFI alleles +1542G and 325Ile, associated with lower TAFI levels, were associated with non-STE ACS (non Q wave MI and unstable angina) in comparison with STE ACS.

In ACS, thrombus propagation over a disrupted arteriosclerotic plaque may occlude the coronary lumen to varying extents, resulting in different clinical manifestations. It is unclear why some patients develop an occluding thrombus causing myocardial necrosis and others do not. The progression of coronary atherosclerosis correlates poorly with the risk of plaque rupture and clinical presentation of an ACS, and studies have reported that over 75% of MI are due to rupture of only-mildly-stenosed coronary arteries [18]. Other factors play a central role in the risk of ACS, such as differences in blood thrombogenicity [17]. The search for coronary risk factors related to the fibrinolytic inhibitor TAFI is attractive because the persistence of hypofibrinolysis may limit the ability to reduce the size of the coronary thrombus. Moreover, genetic, non-modifiable factors are of particular interest because analysis of plasmatic TAFI levels in relation to the cardiovascular risk has shown contradictory results. In some studies, high TAFI levels were found to be protective against MI [18], while in others they were associated with increased risk of coronary disease [19,20] or no association with arterial thrombosis has been found [21,22]. A partial explanation for these discrepancies could be differences in the type of patient included (young survivors of a MI [18], patients with ACS [19,22], or angina [20]), the time of sampling (baseline [20,21] or acute phase [19,22]) or in the methods used to measure TAFI (antigenic [20] or functional [19]). The effect of TAFI polymorphisms on TAFI antigen levels is unclear, since variable antibody reactivity to TAFI isoforms in some TAFI antigen ELISA techniques underestimates real plasma TAFI levels when an Ile is located at position 325 [11,23]. When the results of the PRIME study, which found an association between TAFI plasma levels and the risk of angina in French patients [20] were reanalyzed using an ELISA capable of detecting both TAFI isoforms [24], they showed a relationship between several TAFI polymorphisms and TAFI levels, but showed no relationship between TAFI levels and the risk of coronary heart disease. We used an ELISA capable of detecting all TAFI isoforms, and, like the PRIME study, found a relationship between the three TAFI polymorphisms analyzed and plasma TAFI levels, but not between TAFI levels and the clinical presentation of ACS.

Most epidemiological studies have compared patients with ACS and healthy controls. However, this approach may not distinguish between factors that predict the development of coronary atherosclerosis in general and more-specific factors that might define vulnerability to STE ACS in patients with underlying coronary disease. We investigated specific risk factors for different types of ACS by comparing patients with STE ACS and non-STE ACS.

Several studies have analyzed the relationship between TAFI polymorphisms and coronary disease, with differing results. One study found that the 147Thr allele of the Ala147Thr polymorphism protected against MI [18], while in another study the 147Thr allele was associated with a higher risk of angina [20]. A prospective study found no clear relationship between TAFI gene haplotypes and the incidence of coronary heart disease [24].

In our study, the association found between TAFI genotypes and TAFI levels would predict a lower risk of STE ACS for the alleles related to lower TAFI levels (Ala147, +1542G and 325Ile) but a clear protective effect for STE ACS was only observed for the homozygous +1542G and 325Ile genotypes. We found no relationship between the Ala147Thr polymorphism and ACS type. A study analyzing recombinant TAFI proteins with different amino acid sequences found that the Thr147Ala polymorphism does not affect activation of TAFI, the intrinsic stability of TAFI, or its antifibrinolytic potential [25], which would help to explain our negative results for this polymorphism.

The role of individual SNPs on TAFI expression is unknown [26]. As previously reported [24], we found that the TAFI polymorphisms C+1542G, located in the 3′ untranslated region, and Thr325Ile, located in the coding region, were in strong linkage disequilibrium. Polymorphisms in the 3′ untranslated region may alter TAFI mRNA abundance by influencing mRNA processing or stability. However, amino acid substitutions in the TAFI protein could also play a role in determining plasma TAFI levels by influencing TAFI synthesis and secretion or by altering the plasma half-life of the protein. Specifically, the presence of Ile rather than Thr at position 325 results in a TAFI isoform that is twice as stable at 37 °C and meaning a higher antifibrinolytic capacity is expected [25]. However, the genotype corresponding to the more-stable TAFI variant (325Ile) was associated, both in our and another study [23], with lower plasma TAFI levels and TAFI activity. In two studies that examined TAFI as a risk factor for restenosis after coronary angioplasty or stenting, both lower plasma TAFI antigen concentrations [27] and the more-stable 325Ile/Ile genotype [28] were associated with a lower rate of restenosis. Alternatively, the C+1542G and Thr325Ile polymorphisms, which we found to be associated to the clinical presentation of ACS, may be in linkage disequilibrium with other polymorphisms that directly affect TAFI gene expression or activity.

In the present study we found a relationship of TAFI polymorphisms with clinical presentation of ACS, but not differences in TAFI levels or TAFI activity between STE and non STE ACS were found. Although TAFI genotype has been associated with TAFI levels, genes only explain a part of the TAFI protein level. The methods used in the present study detect total TAFI (reflecting mostly the proenzyme) or the global capacity of TAFI to be activated, but not the actual activated TAFI in the circulation. Very recently it has been suggested that TAFI activated/inhibited would have a better relationship with coronary artery disease than total TAFI [29]. Moreover, in the present series, an acute phase sample was analysed. It is known that in the acute phase of coronary disease increased thrombin generation may lead to TAFI activation and consumption so blunting the detection of previously increased plasma levels [22]. Finally, an additional explanation may be that little subtle changes in local coronary circulation may be relevant in the place of coronary thrombus formation and lysis but may not be reflected in venous circulation.

The significance of the 4G/5G polymorphism of PAI-1 as a thrombophilic factor is not completely clear [30–33]. We found no relationship between this polymorphism and the clinical presentation of ACS. We found no relationship between the other fibrinolysis parameters studied and the clinical presentation of ACS, with the exception of PAP, which, as expected, was higher in STE ACS, indicating the lysis of a higher burden of thrombus.

In conclusion, TAFI polymorphisms C+1542G and Thr325Ile are associated with the type of ACS clinical presentation. Confirmation of these results by independent studies will help to identify a non-modifiable cardiovascular risk factor that will predict the risk of STE ACS in patients with coronary artery disease, who would benefit from risk-individualized cardiovascular prophylaxis.
Conflict of interest statement

None declared.

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References