Platelets

Identification of polymorphisms in the 5′-untranslated region of the TAFI gene: relationship with plasma TAFI levels and risk of venous thrombosis

RENDRIK F. FRANCO,* MARIA G. FAGUNDES,*
JOOST C.M. MEIJERS,@ PIETER H. REITSMA,^ DAYSE
LOURENÇO,° VÂNIA MORELLI,° FRANCISCO H. MAFFEI,#
IRACEMA C. FERRARI,# CARLOS E. PICCINATO,*
WILSON A. SILVA-JR,* MARCO A. ZAGO*

Correspondence: R.F. Franco, M.D., Ph.D., Laboratory of Hematology, Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, 14048-900, Ribeirão Preto, SP, Brazil. Phone: international: + 55-16-6022223 – Fax: international + 55-16-6331144 - E-mail: rendri@hotmail.com

Background and Objectives. Thrombin activatable fibrinolysis inhibitor (TAFI) plays an important role in hemostasis, functioning as a potent fibrinolysis inhibitor. TAFI gene variations may contribute to plasma TAFI levels and thrombotic risk.

Design and Methods. We sequenced a 2083-bp region of the 5'-regulatory region of the TAFI gene in 127 healthy subjects searching for variations, and correlated identified polymorphisms with plasma TAFI levels. TAFI polymorphisms were examined as risk factors for venous thrombosis by determining their prevalence in 388 patients with deep venous thrombosis (DVT) and in 388 controls.

Results. Seven novel polymorphisms were identified: -152 A/G, -438 A/G, -530 C/T, -1053 T/C, -1102 T/G, -1690 G/A, and -1925 T/C. -152 A/G, -530 C/T and -1925 T/C were found to be in strong linkage disequilibrium, as were the -438 A/G, -1053 T/C, -1102 T/G and -1690 G/A. Plasma TAFI levels were higher in -438GG/-1053CC/-1102GG/-1690AA homozygotes than in -438AG/-1053TC/-1102TG/-1690GA heterozygotes, and -438AA/-1053TT/-1102TT/-1690GG homozygotes had the lowest TAFI levels (*p*=0.0003). TAFI concentrations in -152AA/-530CC/-1925TT homozygotes were somewhat higher but not significantly different from levels observed for -152AG/-530CT/-1925TC heterozygotes. Taken in combination, -438AG/-1053TC/-1102TG/-1690GA and -438AA/-1053TT/-1102TT/-1690GG yielded an OR for DVT of 0.8 (95%CI: 0.6-1). In subjects aged <35 years the OR was 0.7 (95%CI: 0.5-1.1). The OR for -152AG/-530CT/-1925TC was 1 (95%CI: 0.5-2.2) in the whole group of patients and controls, whereas in subjects aged <35 years the OR was 0.1 (95%CI: 0.02-0.9).

Interpretation and Conclusions. Polymorphisms in the TAFI promoter determine plasma antigen levels and may influence the risk of venous thrombophilia.
©2001, Ferrata Storti Foundation

*Center for Cell-based Therapy, School of Medicine of Ribeirão Preto, University of São Paulo (USP), °Federal University of São Paulo (UNIFESP), #State University of São Paulo (UNESP), Brasil; @Department of Vascular Medicine, ^Department for Experimental Internal Medicine, Academic Medical Center, University of Amsterdam, the Netherlands

TAFI (thrombin-activatable fibrinolysis inhibitor, also referred to as plasma carboxypeptidase B, procarboxypeptidase U or procarboxypeptidase R; EC 3.4.17.20) is a plasma zymogen that plays an important role in hemostasis, functioning as a potent fibrinolysis inhibitor.[1-8] TAFI is activated by thrombin, trypsin and plasmin, and activated TAFI inhibits fibrinolysis by removing carboxy terminal lysine residues from fibrin during the clot lysis process, thereby suppressing the cofactor properties of partially degraded fibrin in plasminogen activation.[1,2] Recently, elevated plasma levels of TAFI were associated with a mild (1.7-fold) but significantly increased risk of deep venous thrombosis (DVT) in a large, population-based, case-controlled study, the Leiden Thrombophilia Study (LETS).[3]

Recently, Boffa *et al.* have cloned and characterized the gene encoding human TAFI.[4] The TAFI gene has been mapped to 13q14.11, spans approximately 48 kb of genomic DNA and is organized in 11 exons separated by 10 introns. The TAFI promoter lacks a consensus TATA sequence, and transcription starts from multiple sites. Two polymorphisms in the coding region have been described so far: a G to A transition at nucleotide 505 (which results in the Ala147Thr amino acid substitution) and the silent C to T polymorphism at nucleotide 678.[9]

In the present investigation we used DNA sequencing to search for polymorphisms in the 5'- regulatory region of the TAFI gene. We report on several previously unknown sequence variations in the TAFI promoter, and describe their relation with plasma TAFI antigen levels and the risk of DVT.

Design and Methods

Healthy control subjects

Blood was collected after informed consent from 127 unrelated blood donors (75 males and 52 females;

median age 33 years, range 17-59). Individuals were carefully selected so as to include only subjects who denied a history of venous thromboembolism, arterial disease (myocardial infarction, angina, peripheral artery disease or cerebral vascular disease), hypertension, diabetes, smoking, dyslipidemia, acute infection, or use of any medication in the two months preceding blood collection. Based on phenotype characteristics and information on ethnic background of the parents and grandparents, each subject included was classified as Caucasian (114 subjects), Mulatto (9 subjects), Black (2 subjects) or Asian (2 Japanese descendents). Plasma from these individuals was used for measurements of TAFI antigen levels and DNA was used to search for polymorphisms in the TAFI promoter (see below).

Patients with DVT and matched controls

The BRATROS (Brazilian Thrombosis Study) is an ongoing case-controlled study aimed at investigating the contribution of genetic and acquired factors to the risk of venous thrombosis in the Brazilian population. Between October 15, 1996 and June 15, 2000, blood samples were obtained from 388 unrelated patients (239 females and 149 males; median age 41 years, range 2-70 years) admitted to a University Hospital with an episode of DVT. The diagnosis of the DVT was established on the basis of objective documentation of the thrombotic event by ultrasonography or phlebography. Patients aged more than 70 years or with an evidence of malignant disease were not enrolled. Patients included were consecutively admitted to three University Hospitals in the same geographic area (State of São Paulo, South-Eastern Brazil): School of Medicine of Ribeirão Preto (University of São Paulo, USP), School of Medicine of Botucatu (State University of São Paulo, UNESP) and Federal University of São Paulo (UNIFESP). From each patient, we collected data on exposure to *classical* acquired risk factors for DVT, i.e., periods of immobilization, surgery, trauma, and, in the case of women, data on pregnancy, puerperium, use of oral contraceptive and hormone replacement therapy (HRT). A family history of thrombosis was investigated in all cases and considered to be positive when the proband referred a medical diagnosis of venous thrombosis or pulmonary embolism in a first-degree relative. A subject was considered White, Black, Mulatto or Asian on the basis of phenotype characteristics and information about ethnic origin of the parents. While the patients were being included in the study, unrelated healthy blood donor candidates without a personal history of venous or arterial thrombosis were invited to participate as controls. Each control was matched for gender, age (± 3 years), race, and time of recruitment to a patient. Final response rates were 93% and 85% among patients and controls, respectively. This study was approved by the Institutional Ethics Committee and informed consent was obtained from the participants.

Plasma TAFI antigen levels

Blood from the volunteer donors was taken from the antecubital vein in the morning, after overnight fasting, and collected into 3.8% trisodium citrate (0.129 M) (9:1, v/v). Platelet-poor plasma was obtained after centrifugation (2000 g for 30 min at +4°C) and frozen at -80°C until use. Plasma TAFI antigen levels (%) were determined by enzyme-linked immunosorbent assay (ELISA) as reported elsewhere,[5] without knowledge of gene analysis results (see below). The results are expressed as a percentage, with 100% equivalent to the TAFI antigen level in pooled plasma samples from 150 healthy volunteers.

DNA automatic sequencing

DNA from all individuals was isolated from peripheral leukocytes employing standard methods. TAFI promoter was screened for the presence of polymorphisms by automatic sequencing of the 5′-untranslated region in both directions, in 127 healthy subjects (blood donors). Five different regions (referred to as A, B C, D and E in the present study), spanning a region of 2083-bp of the TAFI promoter, were specifically amplified by polymerase chain reaction (PCR). Table 1 shows the sequence of the oligonucleotide primers employed, the region in the TAFI promoter under analysis, the size (bp) of the fragment obtained after PCR amplification using each set of primers, and the annealing temperature used in PCR reactions. PCR products were sequenced with the Big Dye terminator kit and analyzed on an ABI 377-96 automated sequencer (Applied Biosystems). All chromatograms were visually checked for detection of ambiguous bases and to correct misreads.

Restriction-digestion analysis of polymorphisms

Genotyping for the factor V Leiden (1691 G/A) and FII 20210 G/A mutations was performed by PCR amplification followed by *Mnl*I and *Hind*III restriction digestion, as previously reported.[11,12] Protocols of restriction enzyme digestion for detection of novel polymorphisms identified in the TAFI promoter were also established (see below).

Statistical analysis

Allele frequencies were calculated by gene counting. Odds ratios (OR), as a measure of relative risk, and 95% confidence intervals (95% CI) were calculated using standard methods. The Mann-Whitney test was used to test for statistical difference of plasma TAFI levels between two groups of genotypes and between two subgroups of patients. One-way ANOVA was used to

Table 1. Sequence of oligonucleotide primers and amplified fragments in the TAFI gene promoter.

Primer	Oligonucleotide Sequence (5' – 3')	Primer Position*	Annealing temp.(°C)	Product size (bp)
TAFI-E1	GGG CAG CAG ATT CCT ACC AT	640-659	45	844
TAFI-E2	ACT TTT CCT TTT GAG ATT TA	1483-1464		
TAFI-D1	CCC TCC CTG TCT CTT CTA CC	1251-1270	51	619
TAFI-D2	TAA ATC AGC CTG CCC ACA AT	1869-1850		
TAFI-C1	CTT GGG CTA CAG AGT GAG AC	1761-1780	51	675
TAFI-C2	GCC AAG AAT AAA AGG GTA GT	2435-2416		
TAFI-B1	GGG GGT GGG CCT ATC ATT CC	2135-2154	51	478
TAFI-B2	GTT CCC TCT TGT TCC TGT GG	2612-2593		
TAFI-A1	TTT ATT CTT GGC CCT ACC TT	2424-2443	51	299
TAFI-A2	GCT TCA TCC CAA CAG CAA TT	2722-2703		

Sequences obtained from Genbank (access number AF080222).

test for differences in TAFI levels between three different genotypes. The Spearman non-parametric correlation test was used to analyze the correlation between age and plasma TAFI levels. A *p* value ≤ 0.05 was taken as statistically significant.

Results

Patients with DVT and control subjects

Table 2 lists the general characteristics of the 388 patients. Three hundred and twenty-nine patients were Whites, 30 were Blacks, 28 were Mulattos and one was a Japanese descendent. The episode of DVT was considered spontaneous in 210 patients (54.1%), whereas in 178 (45.9%) patients one or more acquired circumstantial risk factor for venous thrombosis was recognized. A family history of venous thromboembolism was present in 84 patients (21.6%). FVL was present in 41 patients (39 heterozygous, 2 homozygous) and in 9 controls (heterozygous only), yielding an OR for DVT of 5 (95%CI: 2.4-10.4). FII G20210A was detected in a heterozygous state in 27 patients and in 5 controls (OR: 5.7; 95%CI: 2.2-15). Inherited antithrombin, protein C and protein S deficiency was detected in 4 (1.1%), 4 (1.1%) and 15 (3.9%) patients, respectively.

The general characteristics of younger patients (i.e. age <35 years, n=180) were as follows. The male/female ratio was 0.5; 154 patients were Whites, 16 were Blacks and 10 were Mulattos. A family history of thrombosis was present in 35/180 subjects (19.4%) and the thrombotic episode was spontaneous in 78 (43.3%). Hormone use was identified in 30 women, the acquired risk factor was pregnancy or puerperium in 31 women, and it was surgery, trauma or immobilization in 74 patients (20%). The prevalence of FVL and FII G20210A in this subgroup of patients was 10% and 5.6%, respectively.

Table 2. General characteristics of patients with DVT and controls.

Variable	Patients (n=388)	Controls (n=388)
Median age (range)	41 (2-70)	41 (2-70)
Male/female ratio	0.6	0.6
Ethnicity		
Whites	329 (84.8%)	329 (84.8%)
Blacks and Mulattos	58 (14.9%)	58 (14.9%)
Asian	1 (0.3%)	1 (0.3%)
Family history of VTE	84 (21.6%)	---
Spontaneous DVT	210 (54.1%)	–
Acquired risk factor for DVT#		
Hormone use (women)*	58 (14.9%)	---
Pregnancy/puerperium	39 (10.1%)	---
Surgery/trauma/immobilization	74 (19.1%)	---
Genetic risk factor for DVT		
Factor V Leiden	41 (10.6%)	9 (2.3%)
Factor II G20210A	27 (7%)	5 (1.3%)
Inherited inhibitor deficiency§	23 (6%)	---

*#Ten patients were exposed to more than one circumstantial risk factor. *Oral contraceptives or hormone replacement therapy. §Antithrombin, protein C and protein S deficiencies taken together.*

An inherited deficiency of a natural anticoagulant was present in 10% of these patients.

Plasma TAFI antigen levels in healthy blood donors

Mean TAFI levels (± SD) in 127 blood donors were 89.2±16.8%. In females the levels were 89.7±14.7% and in males 88.8±18.2% (difference not significant; *p*=0.63). TAFI levels of 89.5±16.9% were detected in Caucasians. In the 11 Mulattos and Blacks the concentrations were 85.6±17.5%, and in the two Asians mean TAFI levels were 91±4.2%. No correlation between age and plasma TAFI levels could be detected (r=-0.13, *p*=0.13).

Identification of novel polymorphisms in the TAFI gene promoter by DNA sequencing

DNA sequencing of the TAFI promoter in 127 subjects revealed the presence of seven novel polymorphisms when sequences were compared to published nucleotide sequence (Figure 1): an A to G substitution at position –152, an A to G substitution at position –438, a C to T substitution at position –530, a T to C substitution at position –1053, a T to G substitution at position –1102, a G to A substitution at position –1690, and a T to C substitution at position –1925 (nucleotide positions numbered following Boffa *et al.*[4]). The –152 A/G, –530 C/T and –1925 T/C transitions were found to be in complete
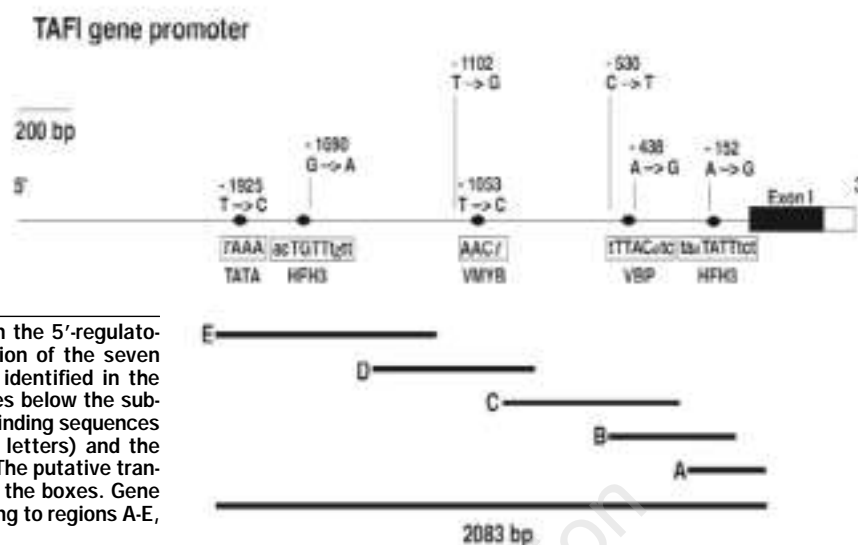
Figure 1. Novel polymorphisms in the 5'-regulatory region of the TAFI. The position of the seven variations of the TAFI promoter identified in the present study are depicted. Boxes below the substitutions indicate the potential binding sequences for transcription factors (capital letters) and the point of the substitution (italic). The putative transcription factors are given below the boxes. Gene fragments amplified, corresponding to regions A-E, are also shown.

linkage disequilibrium. A strong linkage disequilibrium was also observed between the –438 A/G, –1053 T/C, –1102 T/G and –1690 G/A polymorphisms: as shown in Table 3, only in one case was a discrepant genotype detected. Five heterozygotes for the –152 A/G, –530 C/T and –1925 T/C polymorphisms were found among 127 subjects analyzed (carrier frequency 3.9%; allele frequency 0.019). The –438 A/G, –1053 T/C, –1102 T/G and –1690 G/A substitutions were detected in 57 (6 homozygous and 51 heterozygous) of 127 subjects (carrier frequency 44.9%; allele frequency 0.248).

To search for potential binding sites in the TAFI promoter, we used MatInspector 2.2 (as found on *http://transfac.gbf.de/cgi-bin/matSearch/matsearch.pl*). As shown in Figure 1, five of the seven polymorphisms identified are located within (–1053 T/C and –1925 T/C) or very close to (–152 A/G, –438 A/G, –1690 G/A) the

potential binding sites of several transcription factors. The –152 A/G transition is located one base upstream of the potential binding site for the HFH3 transcription factor. The –438 A/G polymorphism is located 1-bp (downstream) of the binding site for the VBP transcription factor. The –1053 T/C transition alters the binding sequence for the VMYB transcription factor. The –1690 G/A transition is located 2-bp downstream of another potential binding site for HFH3, and the –1925 T/C transition disrupts the potential binding sequence for a TATA transcription factor. In contrast, the –530C/T and –1102 T/G variations do not disrupt potential binding sequences.

Plasma TAFI antigen levels in relation to promoter polymorphisms

Mean plasma TAFI levels (± SD) were 84±9.9 for five –152AG/–530CT/–1925TC heterozygotes and 89.3±17.1 for one hundred and twenty-two –152AA/–530CC/–1925TT homozygotes, a difference that did not reach statistical significance (p=0.52) (Table 4). The –438 A/G, –1102 T/G, –1053 T/C and –1690 G/A polymorphisms significantly influenced plasma TAFI levels (Table 4). The 70 homozygotes for the –438GG/–1053CC/–1102GG/ –1690AA genotype had a mean TAFI level of 94±16.5, which was significantly higher than the levels found for fifty-one –438AG/–1053TC/–1102TG/–1690GA heterozygotes (84.6±15.5) (p<0.01) and six homozygotes for the –438AA/–1053TT/ –1102TT/–1690GG genotype (72.2±8.8) (p<0.01). When the three groups were compared for differences, a p value of 0.0003 was obtained.

Table 3. Prevalence of TAFI genotypes in 127 healthy blood donors.

			TAFI Promoter genotype				
-152	-530	-1925	-438	-1053	-1102	-1690	n (%)
AA	CC	TT	GG	CC	GG	AA	67 (52.8)
AA	CC	TT	AG	TC	GT	AG	48 (37.8)
AA	CC	TT	AA	TT	TT	GG	6 (4.7)
AG	CT	TC	GG	CC	GG	AA	3 (2.4)
AG	CT	TC	AG	TC	GT	AG	2 (1.6)
AA	CC	TT	AG*	CC	GG	AA	1 (0.8)

Indicates the discrepant base (see text).
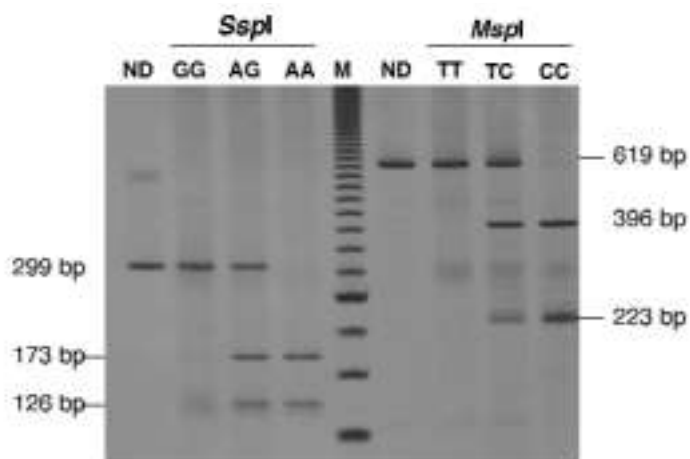
PCR genotyping of TAFI promoter polymorphisms in patients with DVT and controls

When the analysis of the TAFI promoter polymorphisms was extended to a random sample of 180 patients with DVT and 180 matched controls, the −152 A/G, −530 C/T and −1925 T/C transitions were confirmed to be in tight linkage disequilibrium, as were the −438 A/G, −1102 T/G, −1053 T/C and −1690 G/A polymorphisms. In this sample of 360 subjects, no discrepant genotype was observed. Hence, a restriction digestion procedure was developed to detect the −152 A/G and −1053 T/C polymorphisms, employing *Ssp*I and *Msp*I enzymes, respectively (Figure 2). In total, 400 samples were tested for the presence of the two polymorphisms using both DNA sequencing and the restriction-digestion approach, with agreement of the results between the two methods in all cases.

By employing this restriction-digestion protocol, the frequencies of the −152 A/G and −1053 T/C promoter variations were determined in the whole sample of 388 patients with DVT and 388 matched controls. Given that the two groups of polymorphisms were found to be in strong linkage disequilibrium, the results of the analyses for the −152 A/G and −1053 T/C were taken as representative of each group of genotypes.

The −152AG/−530CT/−1925TC genotype was found in 13 patients (all heterozygotes) and in 13 controls (12 heterozygotes and one heterozygotes), yielding a neutral OR for DVT of 1 (95%CI: 0.5-2.2) (see Table 5). The OR for heterozygotes was 1.1 (95%CI: 0.5-2.4) and for homozygous −152GG/−530TT/−1925CC the OR was zero. The OR in females was 0.4 (95%CI: 0.1-1.6) and in males it was 1.7 (95%CI: 0.6-4.8). Among 84 patients with a positive family history of venous thromboem-

Table 4. Plasma TAFI antigen levels in blood donors, in relation to polymorphisms in the TAFI promoter.

TAFI promoter genotype	No. of Subjects	TAFI levels (%)	p value
-152AA/-530CC/-1925TT	122	89.4±17.1	0.52
-152AG/-530CT/-1925TC	5	84±9.9	
-152GG/-530TT/-1925CC	0	–	
-438GG/-1053CC/-1102GG/-1690AA	70	94±16.5	< 0.0003
-438AG/-1053TC/-1102TG/-1690GA	51	84.6±15.5	
-438AA/-1053TT/-1102TT/-1690GG	6	72.2±8.8	

Table 5. TAFI promoter polymorphisms and the risk of venous thrombosis.

TAFI Genotype	No. of pts. n(%)	Controls n(%)	OR (95% CI)
-152AA/-530CC/-1925TT	374 (96.4)	375 (96.6)	1*
-152AG/-530CT/-1925TC	13 (3.4)	12 (3.1)	1.1 (0.5-2.4)
-152GG/-530TT/-1925CC	0	1 (0.3)	0
-152AG/-530CT/-1925TC + -152GG/-530TT/-1925CC	13 (3.4)	13 (3.4)	1 (0.5-2.2)
-438GG/-1053CC/-1102GG/-1690AA	222 (57.2)	201 (51.8)	1*
-438AG/-1053TC/-1102TG/-1690GA	139 (35.8)	157 (40.5)	0.8 (0.6-1)
-438AA/-1053TT/-1102TT/-1690GG	27 (7)	30 (7.7)	0.8 (0.5-1.4)
-438AG/-1053TC/-1102TG/-1690GA + -438AA/-1053TT/-1102TT/-1690GG	166 (42.8)	187 (48.2)	0.8 (0.6-1)

*Reference category.

bolism, the OR for DVT was 0 (95%CI: 0-2.4). In subjects aged less than 35 years (n=180), the OR for DVT linked to −152AG/−530CT/−1925TC was 0.1 (95%CI: 0.02-0.9). Taken together, the −438AG/−1053TC/−1102TG/−1690GA and −438AA/−1053TT/−1102TT/−1690GG genotypes were detected in 166 patients and in 187 controls, yielding an OR for DVT of 0.8 (95%CI: 0.6-1) (Table 5). The OR for −438AG/−1053TC/−1102TG/−1690GA was 0.8 (95%CI: 0.6-1) and for homozygous −438AA/−1053TT/−1102TT/−1690GG the OR was also 0.8 (95% CI: 0.5-1.4). In females (n=239) the OR was 0.9 (95%CI: 0.6-1.3), whereas in males it was 0.7 (95%CI: 0.4-1). Among 84 patients with a positive family history of venothromboembolism, the OR was 0.6 (95%CI: 0.3-1). In subjects aged less than 35 years the OR was 0.7 (95%CI: 0.5-1.1).

Discussion

We report on the existence of polymorphisms in the 5′-untranslated regulatory region of the TAFI gene. As shown in Figure 1, five of the seven polymorphisms identified are located within (-1053 T/C and −1925 T/C) or very close to (-152 A/G, −438 A/G, −1690 G/A) the potential binding sites of several transcription factors. Thus, it might be hypothesized that the proximity to these putative regulatory sites may influence gene transcription and, ultimately, plasma TAFI levels. However, our results do not permit us to conclude which of the sequence variations directly contribute to TAFI levels. Indeed, we have to consider the alternative possibility that the promoter polymorphisms described here might be in linkage disequilibrium with variations located in either regulatory or coding regions in the TAFI gene, which would directly influence gene activity. In any of these scenarios, the data show a clear association between circulating levels of the protein and the −438 A/G, −1102 T/G, −1053 T/C and −1690 G/A polymorphisms, which in this sense may be considered genetic determinants of plasma TAFI levels. These findings give support to the recent hypothesis, raised by Juhan-Vague *et al.,* that TAFI antigen levels are mainly under genetic control, since environmental factors do not seem to have a significant role in affecting plasma TAFI concentrations.[13]

During the preparation of this manuscript, Crainich *et al.* published an abstract describing the −438 A/G polymorphism and its relation with TAFI levels.[14] Henry and colleagues also published a paper describing the −438 G/A, −1102 G/T and −1690 G/A promoter variations; the authors found that these polymorphisms determine TAFI levels and are in strong linkage disequilibrium with a previously described polymorphism in the coding region (Ala147Thr).[15] This last aspect was not assessed in our study as polymorphisms in the coding region were not investigated. Interestingly, Henry *et al.* did not report either on the −1053 C/T or on the −152 A/A, −530 C/T and −1925 T/C variations. It remains to be demonstrated whether ethnic variations in the distribution of the polymorphisms may explain these different findings.[15]

Since the −152 A/G, −530 C/T and −1925 T/C transitions were found to be in strong linkage disequilibrium, as were the −438 A/G, −1102 T/G, −1053 T/C and −1690 G/A polymorphisms, we took one base change from each group (-152 A/G and −1053 T/C) and determined its prevalence in patients with DVT and matched controls. A trend towards protection against DVT was observed in subjects with the −438AG/ −1053TC/ −1102TG/−1690GA and −438AA/−1053TT/ −1102TT/ −1690GG genotypes when compared with subjects bearing the −438GG/−1053CC/−1102GG/ −1690AA genotype, as revealed by the OR for DVT of 0.8. Although this trend just missed being statistically significant (95%CI included the unity), it was more evident in the group of patients with a positive family history of venous thromboembolism, in which the OR was 0.6 (95% CI: 0.3-1), and in subjects aged less than 35 years (OR 0.7; 95% CI: 0.5-1.1). It should be noted that this trend towards decreased risk parallels the lower plasma TAFI levels detected in −438AG/−1053TC/−1102TG/−1690GA and −438AA/−1053TT/−1102TT/−1690GG carriers and agrees with the recent data showing that plasma TAFI levels influence the risk of DVT.[3] It would therefore be interesting to test for this polymorphism in a large group of thrombotic patients and controls, in order to clarify its role in venous thrombophilia further. The −152AG/−530CT/−1925TC genotype was not found to influence TAFI plasma levels significantly. However, the relative rarity of this sequence variation in the sample of 127 subjects in whom TAFI concentrations were available precludes us from ruling out that this genotype also contributes to TAFI levels. In this sense, it should be noted that our findings suggest that −152AG/−530CT/−1925TC might influence the risk of DVT in young subjects, since a significant protective effect against the occurrence of DVT (8-fold decreased risk) was observed in subjects aged less than 35 years. Since both groups of polymorphisms seem to have a more obvious impact on thrombotic risk in young subjects, it might be speculated that in older patients the putative protective effect conferred by TAFI polymorphisms does not counterbalance the prothrombotic effect of acquired circumstantial risk factors, including age. This remains to be assessed in further studies. Indeed, to test the prevalence of the TAFI polymorphisms in large studies will be necessary to understand the apparent paradox that polymorphisms clearly related to TAFI levels are not statistically related to thrombotic risk whereas the rare polymorphisms (for which no

significant influence on TAFI levels was observed) seem to influence the risk of DVT in young subjects.

Plasma TAFI levels, as well as allele frequencies of the several TAFI polymorphisms here described, did not significantly differ between subjects with different ethnic backgrounds. Our study was not, however, intended to address the issue of ethnic variations in TAFI levels and therefore further investigation is necessary not only to establish the prevalence of the TAFI promoter polymorphisms in different populations, but also to determine their contribution to the recently described variance of TAFI levels in different ethnic groups.[10] Based on our data it is only possible to infer that both polymorphisms are probably very old in human evolution as they were found in both Caucasians and Blacks. The absence of an age effect on TAFI levels most likely reflects the fact that the healthy blood donors investigated here were all relatively young and belonged to a similar age group. Thus, the hypothesis that TAFI levels may vary according to age was not tested in the present investigation.

In conclusion, we report on the extensive sequencing analysis of the 5′-regulatory region of the TAFI gene. Two groups of polymorphisms were identified, one of which significantly influences TAFI levels. This finding indicates that physiologic control of TAFI levels is regulated, at least in part, by genetic variations. The data also suggest that the two groups of polymorphisms described may confer a protective effect against the occurrence of DVT, a hypothesis that should be tested in additional studies. Further investigation on the role of TAFI promoter polymorphisms in venous and arterial thrombosis is therefore warranted.

Contributions and Acknowledgments

Disclosures

Manuscript processing

Potential implications for clinical practice

TAFI gene variations determine plasma levels of the protein. Polymorphisms in the TAFI promoter may influence thrombotic risk.

References

1. Nesheim M, Wang W, Boffa M, Nagashima M, Morser J, Bajzar L. Thrombin, thrombomodulin and TAFI in the molecular link between coagulation and fibrinolysis. Thromb Haemost 1997; 78:386-91.
2. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. J Biol Chem 1996; 271:16603-8.
3. Van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. Blood 2000; 95:2855-9.
4. Boffa MB, Reid ST, Joo E, Nesheim ME, Koschinsky ML. Characterization of the gene encoding human TAFI (thrombin-activatable fibrinolysis inhibitor; plasma procarboxypeptidase B). Biochemistry 1999; 38:6547-58.
5. Mosnier LO, von dem Borne PA, Meijers JC, Bouma BN. Plasma TAFI levels influence the clot lysis time in healthy individuals in the presence of an intact intrinsic pathway of coagulation. Thromb Haemost 1998; 80: 829-35.
6. Bajzar L, Nesheim M, Tracy PB. The profibrinolytic effect of activated protein C in clots formed from plasma is TAFI-dependent. Blood 1996; 88:2093-100.
7. Bajzar L, Nesheim M, Morser J, Tracy PB. Both cellular and soluble forms of thrombomodulin inhibit fibrinolysis by potentiating the activation of thrombin-activatable fibrinolysis inhibitor. J Biol Chem 1998; 273:2792-8.
8. von dem Borne PA, Bajzar L, Meijers JC, Nesheim ME, Bouma BN. Thrombin-mediated activation of factor XI results in a thrombin-activatable fibrinolysis inhibitor-dependent inhibition of fibrinolysis. J Clin Invest 1997; 99:2323-7.
9. Zhao L, Morser J, Bajzar L, Nesheim M, Nagashima M. Identification and characterization of two thrombin-activatable fibrinolysis inhibitor isoforms. Thromb Haemost 1998; 80:949-55.
10. Chetaille P, Alessi MC, Kouassi D, Morange PE, Juhan-Vague I. Plasma TAFI antigen variations in healthy sub-

jects. Thromb Haemost 2000; 83:902-5.

11. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369:64-7.

12. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 10:3698-703.

13. Juhan-Vague I, Renucci JF, Grimaux M, et al. Thrombin-activatable fibrinolysis inhibitor antigen levels and car-
diovascular risk factors. Arterioscler Thromb Vasc Biol 2000; 20:2156-61.

14. Crainich P, Tang ZH, Macy EM, et al. A polymorphism at position –438 in the promoter region of thrombin-activatable fibrinolysis inhibitor (TAFI) is strongly associated with plasma antigen levels in healthy older men and women. Circulation (Suppl S)2000; 102:4155.

15. Henry H, Aubert H, Morange PE, et al. Identification of polymorphisms in the promoter and the 3' region of the TAFI gene: evidence that plasma TAFI antigen levels are strongly genetically controlled. Blood 2001; 97:2053-8.