A Locus on Chromosome 2 Influences Levels of Tissue Factor Pathway Inhibitor
Results From the GAIT study


Objective—Levels of tissue factor pathway inhibitor (TFPI) have been associated with arteriosclerosis and thrombotic disease. Although a genetic component to variation in TFPI levels is well-documented, no systematic genome-wide screens have been conducted to localize genes influencing levels of TFPI.

Methods and Results—We studied TFPI levels in 397 individuals in 21 Spanish families participating in the Genetic Analysis of Idiopathic Thrombosis (GAIT) study. Twelve families were selected through a proband with idiopathic thrombosis and 9 were ascertained without regard to phenotype. A genome scan was performed using microsatellite markers spaced at approximately 10 cM intervals. Standard multipoint variance component linkage methods were used. The heritability of TFPI levels was 0.52 ($P<0.0001$), with no evidence for shared household effects. In the genome screen, only 1 LOD score >2 was observed. On chromosome 2q, the maximum multipoint LOD score was 3.52 near marker D2S1384. This is near the structural gene for TFPI, which is located at 2q32. In follow-up association analyses, marginal evidence of association ($P=0.04$) was observed with the TFPI promoter variant C-399T.

Conclusion—These results suggest that polymorphisms in and around the TFPI structural gene may be the major genetic determinants of variation in TFPI levels. (Arterioscler Thromb Vasc Biol. 2005;25:1489-1492.)

Key Words: TFPI • linkage • association • heritability

Tissue factor pathway inhibitor (TFPI) is a Kunitz-type protease inhibitor that inhibits fibrin clot formation through regulation of the extrinsic pathway of coagulation. TFPI was originally called extrinsic pathway inhibitor (EPI) or lipoprotein-associated coagulation inhibitor (LACI) as it is found in association with very low-, low-, and high-density plasma lipoproteins. The extrinsic pathway of coagulation begins with the exposure of tissue factor due to tissue damage and the formation of a complex between tissue factor and factor VII. TFPI, in conjunction with activated factor X, inhibits the activated factor VII-tissue factor complex, regulating fibrin clot formation.

Levels of TFPI have been associated with both atherosclerotic and thrombotic disease. Associations have been demonstrated between peripheral artery disease and decreased total TFPI antigen levels, between myocardial infarction and increased TFPI activity levels, and between increased TFPI and measures of subclinical cardiovascular disease, including internal carotid artery stenosis and carotid wall thickness. Decreased TFPI levels have also been associated with ischemic stroke, deep vein thrombosis, and portal vein thrombosis in patients with cirrhosis. To our knowledge, this is the first reported genome-wide screen for loci influencing variation in TFPI levels.

Methods

The GAIT project has been extensively described in previous publications. The sample consists of a total of 398 individuals in 21 extended Spanish pedigrees composed of 3 to 5 generations. Subjects ranged in age from less than 1 year to 88 years with a mean of 37.7 and approximately equal numbers of males (46%) and females (54%). Twelve families were selected through a proband with idiopathic thrombophilia and the remaining 9 families were randomly selected without regard to phenotype. Thrombophilia was defined as early onset thrombosis or recurrent episodes of thrombosis at least one of which was spontaneous. The probands' thrombophilia was considered idiopathic because those biological causes of thrombosis recognized at the time of family recruitment (1995-1997) had been excluded.

Blood was obtained from fasting subjects and was collected in 1/10 volume of 0.129 mol/L sodium citrate and stored at −80°C until use. Thrombophilic participants were not taking anticoagulants at the time of sampling. TFPI was measured by a functional method as described by Sandset et al. Assays were performed in duplicate and the average value was taken for each person. All procedures were reviewed by the Institutional Review Board of the Hospital de
la Santa Creu i Sant Pau (Barcelona, Spain). Adult subjects gave informed consent for themselves and for their minor children, when applicable.

Subjects were genotyped for a genome-wide scan including 363 highly informative autosomal DNA markers. DNA extraction was performed according to standard protocols. Microsatellites consisted primarily of the ABI-Prism genotyping set MD-10. Linkage mapping was undertaken with the PE LMS II fluorescent marker set with multiplex polymerase chain reaction (PCR) as described; in a few instances, nearby Genethon markers were substituted for LMS II markers to improve robustness (http://www.cng.fr/). PCR products were analyzed on PE 310, PE 377 and PE 3700 automated sequencers, and genotyped using the PE Genotyper software. The average heterozygosity of the microsatellite markers was 0.79 and the average interval between markers was 9.5 cM.

Markers in or near several hemostasis-related candidate genes were used to augment this genome scan. The C-399T, P151L and the V264M polymorphisms in the TFPI gene were genotyped as previously reported with minor modification (10, 11, and 12).

The genotypic data were analyzed for discrepancies (i.e., violations of Mendelian inheritance), using the PEDSYS program INFER. Discrepancies were checked in the laboratory for mistyping, and markers for discrepant individuals were either corrected or excluded from the analysis. Allele frequencies were estimated from the GAIT sample using maximum likelihood techniques. Marker maps for multipoint analyses were obtained from ABI-Prism (http://www.appliedbiosystems.com/molecularbiology/) and from the Marshfield Medical Research Organization (http://research.marshfieldclinic.org/genetics/).

Standard multipoint variance component linkage methods, as implemented in SOLAR, were used to assess linkage between autosomal markers and quantitative levels of plasma TFPI. Age, sex, cigarette smoking and oral contraceptive use were screened as potential covariates. Linkage conditional on measured genotype was used to test whether associated single nucleotide polymorphisms (SNPs) could account for the observed linkage signal.

Previous studies have suggested that variance component linkage methods may be vulnerable to deviations from multivariate normality and particularly to high levels of kurtosis in the trait distribution. TFPI levels in this sample were normally distributed with no significant kurtosis (0.57). Recent statistical genetic theory demonstrates that this level of kurtosis will not affect the distribution of LOD scores and that the standard nominal probability values for LOD scores are appropriate for the TFPI linkage screen.

As 12 of the families were ascertained through thrombophilic probands, all analyses included an ascertainment correction achieved by conditioning the likelihood of these pedigrees on the likelihood of their respective probands. Genome-wide probability values were calculated using the method of Feingold et al.

**Results**

As previously reported, the mean TFPI levels in the GAIT sample were 91.60%. TFPI values ranged from 35% to 170%, excluding 1 individual with an extreme measurement of 209%. TFPI levels increased with age ($P<10^{-7}$), but were not influenced by sex, cigarette smoking, or oral contraceptive use. Conditional on age, the heritability of TFPI levels was $0.52 \pm 0.09$ ($P<10^{-7}$). Effects of nongenetic familial environment were tested using a household component of variance through which individuals living in the same household at the time of examination may be correlated. This component was not significant ($P>0.20$) and was not included in the linkage model. Lipoprotein levels were also investigated as potential covariates. Levels of TFPI were increased in individuals with

![Figure 1](https://example.com/figure1.png)

*Figure 1. Results of the genome-wide screen. Each chromosome is represented by a line, with the top of the line being the p-terminus and the bottom of the line the q-terminus.*
higher low-density lipoprotein levels ($P<0.00001$), but did not differ by levels of very low-density lipoprotein or high-density lipoprotein ($P>0.10$).

In the initial linkage screen (Figure 1), the highest LOD score observed was 1.3 on chromosome 2q in the region of the TFPI structural gene. Addition of another microsatellite marker in this region, D2S1384, raised the multipoint LOD score to 3.52 (Figure 2), corresponding to a genome-wide $P$ value of 0.01. The only other LOD score over 1.0 in the genome scan was a LOD of 1.1 on chromosome 19 between the markers D19S420 and D19S902.

Three SNPs in the TFPI gene were tested for association with TFPI levels, C-399T, P151L, and V264M. The 2 SNPs causing amino acid changes, V264M and P151L, had very low frequencies of the variant alleles ($<0.02$) and showed no evidence of association with TFPI levels ($P>0.20$). The C536T variant was present in only 1 family and the V264M variant occurred in 4 families. C-399T, polymorphic in 16 families, showed somewhat higher allele frequencies (0.88 and 0.12) as well as suggestive evidence of association with TFPI levels ($P=0.04$). TFPI levels were lower in carriers of the T allele. However, in linkage analyses conditional on this polymorphism, significant evidence of linkage remained (LOD = 3.25), suggesting that this variant is either in disequilibrium with another functional site or is only one of a group of functional SNPs.

**Discussion**

TFPI levels are strongly influenced by genetic factors ($h^2=0.52$) but not by shared household effects. To our knowledge, this genome scan is the first attempt to systematically localize the genes influencing variation in levels of TFPI. In a genome-wide linkage screen, only 1 significant or suggestive region was identified, on chromosome 2q near the TFPI structural locus (LOD = 3.52). This suggests that polymorphisms in the TFPI structural gene may be the major genetic determinant of normal variation in TFPI levels.

Of 3 SNPs tested in the TFPI gene, one, C-399T, showed suggestive evidence of association with TFPI levels ($P=0.04$). C-399T is located in the distal activator protein-1 binding site of the 5′ regulatory region of the gene.$^{20,21}$ Whereas some studies suggest an association between the rare allele and decreased TFPI levels, results have been conflicting, with the strongest associations observed in clinical populations.$^{10,22}$ Previous examinations of the V264M and P151L variants have been limited,$^{11,12}$ and the relationship between these polymorphisms and variation in TFPI levels is unclear. The relatively low frequency of the V264M and P151L variants in the GAIT sample is consistent with the low minor allele frequencies reported for these variants in other European samples.$^{12,23}$

It is clear that these 3 polymorphisms cannot account for the observed linkage of variation in TFPI levels to the region of the TFPI structural gene. In fact, they can only account for a small portion of the observed linkage signal. This suggests that there are other variants in or near the TFPI structural gene that influence variation in TFPI levels. These may include the recently identified T-287C promoter variant$^{22}$ or other as yet undiscovered polymorphisms. We are currently in the process of resequencing the TFPI gene and surrounding regulatory and conserved regions to catalog the full extent of genetic variation present in this population and identify the functional polymorphisms responsible for the observed linkage signal.

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**References**


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