Dear Sir,

Tissue factor pathway inhibitor (TFPI) is a plasma protease inhibitor with three tandem inhibitory domains that inhibits the initial step of extrinsic coagulation pathway (1, 2). TFPI gene consists of 9 exons separated by 8 introns (3-5). In the course of genetic analysis of the TFPI gene, we found a polymorphism at position -399 [where the upstream site of the translation initiation sites reported by van der Logt et al. (3) is considered to be +1]. This polymorphism is present within the putative activator protein-1 (AP-1) binding site in the human TFPI gene promoter (3, 4). Here, we describe the prevalence of this polymorphism and the relationship between the polymorphism and the plasma TFPI antigen level.

The normal control population in this study consisted of 255 healthy individuals who participated in the Kisei-cho cohort study in Japan (6). Patients with deep venous thrombosis in the lower legs (n = 111) were referred to Osaka University Medical School. Informed consent was obtained from all subjects. Diagnosis of deep vein thrombosis was made by ultrasonography, radioisotope venography, and magnetic resonance imaging angiography. Genomic DNA was prepared from the peripheral blood using standard methods (7). To distinguish the mutant allele from the normal allele, a Hinf I site into the amplified DNA derived from the normal allele, a mutant allele was 262 bp. For measurement of plasma TFPI levels, venous blood samples were collected in a 1/10 vol of 3.8% (wt/vol) trisodium citrate. Samples were centrifuged at 3000 × g for 15 min at room temperature. Plasma was obtained in stored in plastic tubes at -80°C until the TFPI measurement was performed. TFPI antigen level was measured using a one-step Total TFPI ELISA kit (Chemo-Sero-Therapeutic Research Institute, Japan) (8). The kit consists of the rabbit anti-TFPI polyclonal antibody immobilized to the microplate well and the horseradish peroxidase-conjugated monoclonal antibody that can recognize the specific conformation formed between the Kunitz 1 and Kunitz 2 domains. The results are expressed as means ± one standard deviation (SD). Statistical analysis was performed with the Mann-Whitney U test. Probability values less than 0.05 were accepted as significant.

A polymorphism at position -399 was present in the putative binding site (TGTCCTCA: polymorphic site underlined) for AP-1 in the promoter region of the human TFPI gene. To investigate whether the genetic variation was associated with the plasma TFPI antigen level, we determined the genotype for the polymorphism and measured the plasma TFPI antigen levels in 255 healthy individuals (Table). We identified 130 individuals (51.0%) with CC genotype, 96 (37.6%) with CT genotype, and 29 (11.4%) with TT genotype. The plasma TFPI antigen levels in 255 healthy individuals (Table). We identified 130 individuals (51.0%) with CC genotype, 96 (37.6%) with CT genotype, and 29 (11.4%) with TT genotype. The plasma TFPI antigen levels in the CC genotype group were significantly lower than in the other genotype groups (p < 0.05).

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Table. Genotype frequency of C-399T polymorphism in normal Japanese subjects and patients with deep venous thrombosis and TFPI antigen level of genotype groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normal individuals</th>
<th>Venous thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>TFPI antigen (ng/ml)</td>
</tr>
<tr>
<td>CC</td>
<td>130 (51.0)</td>
<td>88.7 ± 20.0</td>
</tr>
<tr>
<td>CT</td>
<td>96 (37.6)</td>
<td>78.3 ± 20.6</td>
</tr>
<tr>
<td>TT</td>
<td>29 (11.4)</td>
<td>77.3 ± 15.5</td>
</tr>
</tbody>
</table>

TFPI antigen levels were not significantly different (p>0.05) among the genotype groups.

Thromb Haemost 1998; 80: 345–6

C-399T Polymorphism in the Promoter Region of Human Tissue Factor Pathway Inhibitor (TFPI) Gene Does not Change the Plasma TFPI Antigen Level and Does not Cause Venous Thrombosis

CT genotype, and 29 (11.4%) with TT genotype. Therefore, allele frequencies of C and T in the healthy Japanese population (510 alleles tested) were 69.8% and 30.2%, respectively. The mean ± SD TFPI antigen level in total was 79.4 ± 19.8 ng/ml. Individuals with the CC genotype had mean ± SD TFPI levels of 80.7 ± 20.0 ng/ml, whereas individuals with the CT or TT genotype had mean ± SD TFPI levels of 78.3 ± 20.6 ng/ml or 77.3 ± 15.5 ng/ml, respectively, and the difference was not statistically significant.

To determine the prevalence in patients with venous thrombosis, we also examined the genotype of patients with venous thrombosis (n = 111) (Table). We identified 54 patients (48.6%) with CC genotype, 46 (41.4%) with CT genotype, and 11 (10.0%) with TT genotype. Therefore, allele frequencies of C and T in patients with venous thrombosis (222 alleles tested) were 69.4% and 30.6%, respectively, and were not significantly different from that obtained from the normal population. We also measured the plasma TFPI levels in patients with venous thrombosis (Table). The mean ± SD TFPI antigen levels in patients with deep vein thrombosis (n = 51) was 72.2 ± 21.5 ng/ml. Deep vein thrombotic patients with the CC, CT or TT genotype had mean ± SD TFPI levels of 70.9 ± 24.4 ng/ml (n = 27), 72.6 ± 17.8 ng/ml (n = 20) or 78.6 ± 22.2 ng/ml (n = 4), respectively, and the difference was not statistically significant.

We concluded from our analysis that the C-to-T polymorphism at position -399 in the TFPI promoter region is not related to the plasma antigen level of TFPI, and the prevalence was the same between normal Japanese subjects and patients with deep venous thrombosis.

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References

Received August 14, 1997 Accepted after resubmission April 7, 1998

Sodium Salicylate Enhances Expression of Tissue Factor (TF) in Human Adherent Monocytes and in Whole Blood

Dear Sir,

Sodium salicylate (NaSal) has been reported to inhibit LPS-induced tissue factor (TF) expression in monocytes (MO), an effect which may be attributed to inhibition of NFκB-mediated signalling (1–3). In marked contrast, our group has found that pre-treatment of human MO or whole blood (HWB) with NaSal actually enhances LPS-induced increases in TF expression (Table 1).

White blood cells were isolated from whole blood (~60% MO) and incubated overnight, in culture plates. The following day, MO were

| Table 1 | Effects of pre-treatment with NaSal (5 mM; 30 min) on TF expression in MO and whole blood (5 h). The readout in the chromogenic assay was rate of change of absorbance at 405 nm, following hydrolysis of S-2765, by newly formed factor Xa, in the presence of TF and factor VIIa. Clotting in citrated (4.9 mM) blood was initiated by the addition of 150 μl of CaCl2 (20 mM) to 100 μl aliquots of blood, and clotting measured in a thrombotrack 4. Control clotting was deemed to be 100%. Data are mean ± SEM of 3-6 exps |
|---------------------------|---------------------|---------------------|---------------------|
| ASSAY | MO (Chromogenic assay) | MM (ELISA) | Whole blood (Clotting assay) |
| Treatment | Response (% LPS control) | pg TF/mg protein | Clotting time (% control) |
| LPS 100ng/ml NaSal + LPS 100μg/ml | 100 | 192 ± 14.4 | 257 ± 70.5 | 45.3 ± 6.7 |
| 992 ± 14.4 | 1160 ± 442 | 79.3 ± 6.8 |

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