Dear Sir,

Activated protein C resistance (APC-R) is the most prevalent inherited cause of venous thrombosis, accounting for 37% of patients. More than 90% of the patients with APC-R are caused by a G to A point mutation in the codon for arginine 506 in the factor V gene, which is called factor V Leiden mutation. This mutation can be detected by the presence or absence of an Mnl I restriction site in a PCR product spanning codon 506. The incidence of this mutation is 3-7% in Caucasian population. Very few cases of Leiden mutation have been reported in South, Southeast, and East Asia. There are only 5 cases from India (1, 2), 1 case from Polynesians (3), and 2 cases from minor populations (4). The mutation has not been found in any other Asian countries such as Sri Lanka (2), Pakistan (4), Indonesia (2), Taiwan (2), Japan (5), Korea (5), China (5, 6), and Mongolia (2).

The low incidence of Leiden mutation in Asia seems to be consistent with the fact that venous thrombosis itself is rare in Southeast Asia (4). However, Thailand may be an exception. Thrombosis patients are often seen in hospitals in Thailand. A recent survey showed that the incidence of APC-R was 46.3% in patients with thrombosis from the indigenous Thai population (unpublished data). Interestingly, this incidence in Thai population was rather higher than 37% in Caucasian one (7). Therefore, we tried to detect the Leiden mutation in the indigenous Thai population.

One hundred and fourteen unrelated healthy volunteers without thrombosis and 14 unrelated patients with venous thrombosis were included in this study from the indigenous Thai population. The fourteen thrombosis patients were separated into two groups; 7, positive for APC-R test [normalized-APC-sensitivity ratio (n-APC-SR) < 0.7]; 7, negative for APC-R test (n-APC-SR ≥ 0.7).

PCR of the factor V gene was performed by a described method (8) using two primers 5'-ACCCACAGAAAATGATGCCCAG-3' and 5'-TGCCCCATTATTTGCGAGAGG-3' and AmpliTaq Gold DNA polymerase (Perkin Elmer) with a program of 1) preheating at 94°C for 12 min; 2) 50 cycles of amplification (91°C for 40 s, 55°C for 1 s, 72°C for 3 s); 3) final incubation at 72°C for 20 min. PCR product was digested with Mnl I enzyme (New England BioLabs) and electrophoresed on an agarose gel (Fig. 1).

Neither heterozygous nor homozygous Leiden mutation was detected in healthy volunteers. Surprisingly, 2 cases of heterozygous Leiden mutation were detected in 7 patients who were positive for APC-R test (Fig. 1, lanes 1 and 2), but homozygous one was not detected in the 7 patients. Neither heterozygote nor homozygote was detected in 7 patients who were negative for APC-R test.

Very few cases of Leiden mutation have been detected in Asia. So researchers think that the Leiden mutation occurred after Homo sapiens separated into Caucasians and Mongolians 40,000-60,000 years ago. Five cases of Leiden mutation detected in Indian and Polynesian populations seem like the results of admixture with colonizing Europeans (2, 3). However, we may have to think about it again at the moment. Thailand has never been colonized by the Europeans historically. So the admixture with Caucasians is hard to consider. Further study using haplotype analysis should be performed.

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References


Fig. 1 PCR analysis detecting factor V Leiden mutation. PCR products (223 bp) were digested with Mnl I and electrophoresed on an agarose gel. Lanes 1 and 2, heterozygous patterns (141, 104, 82, and 37 bp) from two patients who were found to possess factor V Leiden mutation; lanes 3-5, normal patterns (104, 82, and 37 bp) from healthy volunteers; lane 6, molecular markers

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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 FV Leiden mutation and Chinese APC resistance. Br J Haematol 1996; 93, (Suppl. 2): 3.

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 n (%)</th>
<th>TFPI antigen (ng/ml)</th>
<th>Venous thrombosis n (%)</th>
<th>TFPI antigen (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>130 (51.0)</td>
<td>80.7 ± 20.0</td>
<td>54 (48.6)</td>
<td>70.9 ± 24.4</td>
</tr>
<tr>
<td>CT</td>
<td>96 (37.6)</td>
<td>78.3 ± 20.6</td>
<td>46 (41.4)</td>
<td>72.6 ± 17.8</td>
</tr>
<tr>
<td>TT</td>
<td>29 (11.4)</td>
<td>77.3 ± 15.5</td>
<td>11 (10.6)</td>
<td>78.6 ± 22.2</td>
</tr>
</tbody>
</table>

Mean ± SD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n (%)</th>
<th>TFPI antigen (ng/ml)</th>
<th>n (%)</th>
<th>TFPI antigen (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>255 (100)</td>
<td>79.4 ± 19.8</td>
<td>111 (100)</td>
<td>72.2 ± 21.5</td>
</tr>
</tbody>
</table>

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

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