PAI-1 Gene 4G/5G Genotype: A Risk Factor for Thrombosis in Vessels of Internal Organs

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Although the common 4G/5G polymorphism in the promoter of the PAI-1 gene was suggested to be a risk factor for some of the thrombotic disorders, its significance in the development of thrombosis is still controversial. This study presents the data on a total of 357 patients with different types of thrombosis and 281 unrelated healthy controls. It was found that the 4G/4G genotype is associated with a higher risk of thrombosis (OR, 1.7; 95% CI, 1.1–2.5). Patients were divided into five distinct groups according to the site of thrombosis. Both 4G/4G and 4G/5G genotypes were associated with a higher risk of thrombosis development in a group of 69 patients with internal organ thrombosis (OR, 6.35; 95% CI, 2.5–16.1 and OR, 4.85; 95% CI, 2.0–12.1, respectively). Interestingly, this association was even stronger in a subgroup of 33 patients with portal vein thrombosis (PVT) and 4G/4G and 4G/5G genotypes conferred more than 10- and 6-fold increases in the risk of developing PVT (95% CI: 2.3–47.1 and 1.4–28.8), respectively. No statistically significant association was found between 4G/4G genotype and the groups of deep vein thrombosis (126 patients), cerebral thrombosis (80 patients), retinal thrombosis (72 patients), and purpura fulminans (16 patients). Factor V Leiden or prothrombin G20210A mutations did not emerge as additional risk factors for thrombosis in any of the groups studied. To conclude, this study suggests that there may be an association between 4G/4G and 4G/5G genotypes and the thrombosis in vessels of internal organs especially in the portal veins. Am. J. Hematol. 71:89–93, 2002. © 2002 Wiley-Liss, Inc.

Key words: PAI-1 gene; 4G/5G polymorphism; thrombosis; internal organ vessels; genetic risk factor

INTRODUCTION

Plasminogen activator inhibitor-1 (PAI-1) is the major inhibitor of tissue type plasminogen activator (tPA). Reduced fibrinolytic capacity due to increased plasma PAI-1 levels was postulated to play an important role in the pathogenesis of disorders associated with thrombosis [1]. Recently, a common functional deletion/insertion polymorphism (4G/5G) in the promoter of the PAI-1 gene located 675 bp upstream from the transcription start site was reported to result in the elevated expression of PAI-1 gene [2]. Individuals homozygous for the 4G allele had increased plasma PAI-1 concentrations compared to the ones with 5G allele [3]. To date, this polymorphism has been studied extensively, and in some studies, the prevalence of 4G allele was found to be higher in disorders like coronary artery disease, meningococcal septic shock, osteonecrosis, severe pre-eclampsia, type 2 diabetic nephropathy, pulmonary thromboembolism (PTE), and arterial thrombosis associated with hereditary protein S deficiency [1,4–8]. Other studies have failed to show such an association [9,10]. The purpose of this study was to elucidate the significance of this polymorphism in development of thrombosis in various
internal organ vessels, deep, cerebral, retinal veins, and purpura fulminans.

PATIENTS AND METHODS

A total of 357 unrelated consecutive Turkish patients (211 males/146 females) who were admitted to the Hacettepe University Hospitals in Ankara, between April 1997 and September 2000 for evaluation of a recent thrombotic event and 281 unrelated healthy controls without family history of thrombosis who were sampled at the same time period were the subjects of this study. All of the subjects were coming from Ankara and its surroundings and were suitable for inclusion to the study. The study was approved by the Hacettepe University ethics committee on human research, and informed consent was received from all of the subjects participating to the study.

The peripheral and internal organ thrombi were diagnosed by Doppler ultrasonography, computed tomography, and magnetic resonance imaging; and retinal thrombi were diagnosed by fundoscopic examination. Patients who had a history of an in-dwelling umbilical artery catheter were excluded from the study.

Patients were divided into four groups according to the site of the thrombosis: group A—internal organ thrombosis (IOT), 69 patients (33 M/36 F) with hepatic, splenic, cardiac, portal, renal, and pulmonary vein thrombosis; group B—deep vein thrombosis (DVT), 126 patients (73 M/53 F) with thrombosis at femoral/popliteal, mesenteric veins, vena cava superior/inferior, and DVT combined with other thrombotic events like pulmonary vein thrombosis; group C—cerebral thrombosis (CT), 80 patients (49 M/31 F) with cerebral infarct, cerebral, and sinovenous thrombosis; group D—retinal thrombosis (RT), 72 patients (45 M/27 F) with mostly retinal vein and few retinal artery thrombosis; group E—purpura fulminans (PF), 16 patients including two newborn babies (8 M/8 F).

High molecular weight DNA was extracted from peripheral blood by standard procedures. Part of the PAI-1 gene promoter flanking 4G/5G polymorphism was amplified by using the primers described earlier [9]. The PCR products of 163-bp DNA fragments were digested with BseL1 (MBI Fermentas) at 55°C overnight. Digestion products were subjected to electrophoresis on 3% agarose gel. The presence of 107- and 56-bp DNA fragments indicated the 4G allele, while the 74-, 56-, and 34-bp fragments indicated the 5G allele. Patients were also screened for Factor V Leiden and prothrombin G20210A mutations as described before [11,12].

The statistical significance of differences between each of the patient groups and the control group were estimated by logistic regression analysis on SPSS statistical package (version 10). 5G/5G was taken as reference category and odds ratios (ORs) of 4G/4G or 4G/5G versus 5G/5G were calculated and are given within 95% confidence intervals (CI). Statistical analyses were also performed after FV Leiden and prothrombin G20210A-positive patients were eliminated from the study. The possible association of 4G/5G genotype with FV Leiden and prothrombin G20210A mutations in the generation of thrombosis within each group of patients was analyzed by the χ² test (2-sided).

RESULTS

Of the 357 patients [age 25 ± 19 (mean ± SD) years at sampling] that were included in this study, 32% had the 4G/4G, 43% the 4G/5G, and 25% 5G/5G genotype. On the other hand, of the 281 healthy controls 26% had 4G/4G, 40% 4G/5G, and 34% 5G/5G genotype. The difference in the frequencies of the 4G/4G genotype was statistically significant, and the presence of 4G/4G was associated with an increased risk of thrombosis (OR, 1.7; 95% CI, 1.1–2.5) (Table I). As expected, the prevalence of the 4G allele was higher among patients (53%) than controls (46%).

The results of the statistical analysis of each group are given in Table II. Of the 69 patients (mean age 23 ± 19 years) with internal organ thrombosis in group A, 29 (42%) had the 4G/4G, 34 (49%) 4G/5G, and only 6 (9%) had the 5G/5G genotype (Table II). The allele frequency of 4G was 67%, while that of 5G was 33% for this group. The frequencies of both the 4G/4G and the 4G/5G genotypes were significantly higher in this group compared to controls, and the presence of these genotypes showed an association with an increased risk of thrombosis in vessels of internal organs (OR, 6.4; 95% CI: 2.5–16.1 and OR, 4.9; 95% CI, 2.0–12.1, respectively).

Thirty-three of 69 patients with thrombosis in vessels of internal organs had portal vein thrombosis (mean age 25 ± 18 years; age range 2–64 years; 13 M/20 F); 16 of these patients (48%) had the 4G/4G, 15 (45%) the 4G/5G, and only 2 (6%) had 5G/5G genotype (Table III). The presence of the 4G/4G and 4G/5G genotypes showed an association with 10.5-fold (95% CI: 2.3–47.1) and 6.4-fold (95% CI: 1.4–28.8) increased risk of devel-

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of patients (%)</th>
<th>No. of controls (%)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>4G/4G</td>
<td>114 (32)</td>
<td>73 (26)</td>
<td>1.7</td>
<td>1.1–2.5</td>
</tr>
<tr>
<td>4G/5G</td>
<td>152 (43)</td>
<td>112 (40)</td>
<td>1.4</td>
<td>1.0–2.1</td>
</tr>
<tr>
<td>5G/5G</td>
<td>91 (25)</td>
<td>96 (34)</td>
<td>1.0*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>357</td>
<td>281</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reference category 5G/5G.
The control group given in Table I is used in the statistical analysis.

CI: 1.3

years; 19 M/17 F) also had a 4-fold increased risk of

± 4.7 years; age range 3 days

retinal thrombosis (group D), 16 patients (mean age 4.5

– 17 years) with deep vein thrombosis (group B), 80 pa-

ments (mean age 19 ± 19 years) with cerebral thrombosis

notype and Factor V Leiden mutation contradicts to the

results of a previous study in this respect [15]. In some studies, the 4G/4G genotype has been

shown to be associated with some thrombotic disorders; in others, however, no association was found [8,10]. There are even reports on the selection of 5G/5G geno-

myle in some of the thrombotic disorders [13,14].

The positive associations remained the same when Factor V Leiden or prothrombin G20210A-positive cases were eliminated from the study. The co-existence of ei-

ther Factor V Leiden or prothrombin G20210A mutation with 4G/4G genotype was not found to be statistically significant in any of the groups studied (P > 0.05 for all) (Table IV).

**DISCUSSION**

Although there are many reports indicating that the 4G/5G polymorphism of the PAI-1 gene promoter is a risk factor for some diseases related to thrombosis, the exact role of this functional polymorphism in the develop-

ment of these disorders still remains to be controvers-

ial [1]. In some studies, the 4G/4G genotype has been shown to be associated with some thrombotic disorders; in others, however, no association was found [8,10]. There are even reports on the selection of 5G/5G geno-

myle in some of the thrombotic disorders [13,14].

The results of this study indicated that the difference in the frequencies of the 4G/4G genotype is statistically significant between the patients of all groups and the control subjects (Table I). When the study groups were analyzed separately, the presence of a significant asso-

ciation was shown between carriers of the 4G deletion polymorphism in the PAI-1 gene promoter and the thrombosis of various organ vessels (Table II). When the subgroup of patients with portal vein thrombosis (PVT) were analyzed as an independent group, the difference between the patients with PVT and control group became even more prominent (Table III). FV Leiden and pro-

thrombin G20210A mutations seem not to be additional risk factors for development of thrombosis since the positive associations were remained the same even after positive cases for these mutations were eliminated from the study. To date, there was no report on the role of PAI-1 promoter polymorphism for the development of portal vein thrombosis. In this respect, information given about the presence of a possible association between car-

riers of the 4G deletion polymorphism and the risk of development of PVT is important. However, since the number of patients was quite small, further studies are

opring PVT, respectively. The remaining 36 patients of this group (mean age 21 ± 20 years; age range 1–73

years; 19 M/17 F) also had a 4-fold increased risk of

thrombosis for both 4G/4G and 4G/5G genotypes (95%

CI: 1.3–13.7 and 1.3–12.4, respectively) (Table III).

The frequency of the 4G/4G genotype did not show any significant difference in 126 patients (mean age 31 ± 17 years) with deep vein thrombosis (group B), 80 pa-

ents (mean age 19 ± 19 years) with cerebral thrombosis (group C), 72 patients (mean age 32 ± 16 years) with

retinal thrombosis (group D), 16 patients (mean age 4.5 ± 4.7 years; age range 3 days–13 years) with purpura

fulminans (group E) and the control group (Table II). Although the number of patients in the PF group was too small to obtain reliable results from the statistical analy-

sis, it is included here only to provide preliminary data.

**TABLE II. Distribution of PAI-1 4G/5G Polymorphism in Various Groups**

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Thrombosis site</th>
<th>Total no. of patients</th>
<th>Genotype</th>
<th>No. of patients (%)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Internal organ</td>
<td>69</td>
<td>4G/4G</td>
<td>29 (42)</td>
<td>6.4</td>
<td>2.5–16.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4G/5G</td>
<td>34 (49)</td>
<td>4.9</td>
<td>2.0–12.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5G/5G</td>
<td>6 (9)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Deep vein</td>
<td>126</td>
<td>4G/4G</td>
<td>35 (28)</td>
<td>1.4</td>
<td>0.8–2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4G/5G</td>
<td>57 (45)</td>
<td>1.4</td>
<td>0.9–2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5G/5G</td>
<td>34 (27)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Cerebral</td>
<td>80</td>
<td>4G/4G</td>
<td>24 (30)</td>
<td>1.2</td>
<td>0.6–2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4G/5G</td>
<td>29 (36)</td>
<td>0.9</td>
<td>0.5–1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5G/5G</td>
<td>27 (34)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Retinal</td>
<td>72</td>
<td>4G/4G</td>
<td>25 (35)</td>
<td>1.6</td>
<td>0.8–3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4G/5G</td>
<td>26 (36)</td>
<td>1.1</td>
<td>0.6–2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5G/5G</td>
<td>21 (29)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>
| E             | Purpura fulmi-
|               | nans            | 16                    | 4G/4G    | 4 (19)              | 0.8| 0.2–3.4 |
|               |                 |                       | 4G/5G    | 8 (50)              | 1.4| 0.4–4.3 |
|               |                 |                       | 5G/5G    | 5 (31)              | 1.0|        |

*Control group given in Table I is used in the statistical analysis. 

Reference category 5G/5G.

**TABLE III. Frequencies of PAI-1 4G/5G Genotype in Portal Vein Thrombosis and Thrombosis of Other Internal Organs**

<table>
<thead>
<tr>
<th>Thrombosis site</th>
<th>Total no. of patients</th>
<th>Genotype</th>
<th>No. of patients (%)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal vein</td>
<td>33</td>
<td>4G/4G</td>
<td>16 (48)</td>
<td>10.5</td>
<td>2.3–47.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4G/5G</td>
<td>15 (45)</td>
<td>6.4</td>
<td>1.4–28.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5G/5G</td>
<td>2 (6)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Others*</td>
<td>36</td>
<td>4G/4G</td>
<td>13 (36)</td>
<td>4.3</td>
<td>1.3–13.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4G/5G</td>
<td>19 (53)</td>
<td>4.1</td>
<td>1.3–12.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5G/5G</td>
<td>4 (11)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

*Control group given in Table I is used in the statistical analysis. 

Reference category 5G/5G.

*Includes hepatic, splenic, cardiac, renal, and pulmonary thrombosis.

The positive associations remained the same when Factor V Leiden or prothrombin G20210A-positive cases were eliminated from the study. The co-existence of ei-

ther Factor V Leiden or prothrombin G20210A mutation with 4G/4G genotype was not found to be statistically significant in any of the groups studied (P > 0.05 for all) (Table IV).
However, the results of this study indicated that the 4G/5G polymorphism of the PAI-1 gene had no significant contribution to the development or prevention of cerebral thrombosis probably because the patients with younger age range were used in this study.

The role of PAI-1 4G/5G genotype on development of retinal thrombosis has not been reported previously. Although a slightly higher frequency of 4G/4G genotype was observed in patients (35%) than in controls (26%), no statistically significant association could be found between PAI-1 genotype and retinal thrombosis in this study (Table II).

Although no difference was observed in the frequency of 4G/5G polymorphism between the purpura fulminans and the control group, the number was too small to draw any statistical conclusion. Therefore, this study would only provide preliminary data for future studies on this subject.

In conclusion, the results of this study suggest that 4G allele of the PAI-1 gene promoter may be an increased risk factor for development of thrombosis in the vessels of several internal organs especially in the portal veins. However, further studies are needed to draw a definite conclusion.

ACKNOWLEDGMENT

We are grateful to Associate Prof. Dr. Osman Saracbası for his valuable comments in the statistical analysis of this study.

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