Protective Effect of a Thrombin Receptor (Protease-Activated Receptor 1) Gene Polymorphism Toward Venous Thromboembolism

Emmanuel Arnaud, Viviane Nicaud, Odette Poirier, Francine Rendu, Martine Alhenc-Gelas, Jean-Noël Fiessinger, Joseph Emmerich, Martine Aiach

Abstract—The human protease-activated receptor 1 (PAR-1) is activated by thrombin at the surface of platelets and endothelial cells, 2 cells that are implicated in hemostasis and thrombosis. We studied the PAR-1 gene in a large case-control study from the Paris Thrombosis Study (PATHROS), and the possible implication of polymorphisms in venous thromboembolism was evaluated. Two polymorphisms were found in the 5′ regulatory region. The first is a C to T transition that is 1426 nucleotides upstream from the translation start site (−1426 C/T), and the second is a 13-bp insertion repeating the preceding −506 5′-CGGCCGCGGAAG-3′ sequence (−506 I/D, where I indicates insertion and D indicates deletion), a putative cis-acting element of the Ets family. The third polymorphism is an A to T transversion in the intervening sequence (IVS) that is 14 nucleotides upstream from the exon 2 start site (IVS-14 A/T). The distribution of the 3 polymorphisms was otherwise similar in the 250 cases and the 1214 controls. A noteworthy sex heterogeneity led us to analyze men and women separately with regard to the −506 I/D polymorphism. We found that allele I was less frequent in male cases than in male controls (0.154 versus 0.247, P<0.01), with an odds ratio at 0.52 (95% CI 0.32 to 0.82, P<0.01). Furthermore, a reduction of prothrombin fragment 1+2 levels was observed in homozygous carriers of allele −506 I (P=0.04). Altogether, these data suggested a protective effect in men of −506 I/D polymorphism for venous thromboembolism. (Arterioscler Thromb Vasc Biol. 2000;20:585-592.)

Key Words: human thrombin receptor ■ gene polymorphism ■ venous thromboembolism

Thrombin is a serine-protease that plays a central role in hemostasis and thrombosis at molecular levels (by activating several coagulation factors to form fibrin clots) and at cellular levels (by activating platelets and vascular endothelial cells). Thrombin-induced cellular effects are mediated by a G-protein–coupled receptor activated by proteolysis of the amino-terminal exodomain, unmasking a new amino-terminus sequence acting as a tethered ligand.1 The peculiar mode of activation applies to all protease-activated receptors (PARs), with the thrombin receptor PAR-1 being the first cloned receptor of the family. Thrombin activates platelets to aggregate and secrete granules; this occurrence is the primary step to ensure hemostasis.2 Endothelial cell activation by thrombin leads to various responses, such as von Willebrand factor release, prostacyclin (PGI₂) and nitric oxide synthesis, and cellular proliferation.3–7 Endothelium-derived nitric oxide and PGI₂ act synergistically to inhibit platelet adhesion, aggregation, and secretion.8–10

Venous thromboembolism (VTE) is associated with several genetic risk factors, the most frequent being the factor V Arg506Gln and the prothrombin gene G20210A mutations, known to increase thrombin generation.11–14 Because thrombin activates endothelial cells and platelets through PAR-1, the density of this receptor at the cellular surface could have consequences involving hemostasis or thrombosis. At present, no human PAR-1 genetic variation associated with bleeding or thrombosis has been reported.

The PAR-1 gene comprises 2 exons separated by a large intron (∼22 kb) and is located on chromosome 5q11.2 to q13.3.15–18 The first sequence analysis of the regulatory region reveals the lack of evident TATA and CAAT sequences in the appropriate locations, a frequent feature of G-protein–coupled receptor genes,19 and the presence of several putative regulatory motifs (SP1, Ets, transcriptional enhancer factor-1, and GATA).20 Promoter functional analysis showed that 2 clusters, SP1 and activator protein 1, are important for basal activity.21,22

The present study was undertaken to establish whether genetic variations of PAR-1 and their possible involvement in basal and/or induced transcription could have consequences involving the occurrence of thrombosis. For this purpose, we searched for polymorphisms in the regulatory regions of the

Received March 17, 1999; revision accepted July 6, 1999.
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PAR-1, ie, the promoter and the 2 exon/intron boundaries that could influence the gene expression. Three polymorphisms were identified. Among them, a repeat of the putative Ets motif in the promoter was associated with lower fragment 1+2 (F1+2) levels in healthy controls and was underrepresented in male cases, suggesting a sex-dependent VTE protective effect of the insertion.

Methods

Study Population

A case-control study, the Paris Thrombosis Study (PATHROS), was begun in our center in November 1995 to seek the genetic risk factor for VTE. Two hundred fifty patients aged <61 years were included if they had experienced at least 1 episode of objectively diagnosed deep venous thrombosis (by compression and ventilation lung ultrasonography or venography) and/or pulmonary embolism (by perfusion and ventilation lung scan, conventional pulmonary angiography, or computed tomographic angiography).

Controls were 1214 healthy subjects age- and sex-matched recruited from a healthcare center to which they had been referred for a routine checkup. On the basis of a medical questionnaire, subjects with a history of VTE, arterial disease (stroke, myocardial infarction, angina, or peripheral vascular disease), or known malignancy were excluded. Most of the subjects (85% of controls and 88% of cases) were born in Europe, and non-European subjects were similarly distributed in the 2 groups.

Blood was taken into a tube containing 0.11 mol/L sodium citrate (1:10). DNA was prepared from white blood cells by a standard technique and stored at 4°C until analysis. Factor V Arg506Gln and prothrombin gene G20210A mutations were identified as previously described.

For a subset of 383 healthy controls (195 men and 188 women), plasma was stored at −80°C until analysis. The mean age of these subjects (43±9 years) was not statistically different from that of the whole population of controls. Von Willebrand factor was measured by use of a commercial kit (Asserachrom vWF, Diagnostica Stago). Prothrombin F1+2 was measured with an Enzygnost F1+2 kit (Dade Behring).

Molecular Biology Techniques

Determination of Exon/Intron Boundary Sequences

Because the sequences of the exons/introns boundaries were not known for more than a few nucleotides, we used a commercial kit (the promoter finder DNA Walking, Clontech) to sequence the genomic DNA flanking exons 1 and 2 in their 3′ and 5′ extremities, respectively. The different primers used for amplification and sequencing the exon/intron boundaries are listed in Table 1. Five amplified fragments were submitted to a second PCR with a nested PAR-1 gene–specific primer (GSP1) and an outer adaptor primer (AP1). These 5 amplified fragments were submitted to a second PCR with a nested PAR-1 gene–specific primer (GSP2), and a second adaptor primer (AP2). The products of the nested PCR were electrophoresed in 3% agarose and stained with ethidium bromide. Positive amplicons were purified on Sephadex G-25 spin columns (Pharmacia Biotech Inc) and directly sequenced with an ABI prism dye terminator cycle sequencing reaction kit and loaded on the automated sequencer 310 capillary system (PE Applied Biosystems).

PCR—Single-Strand Conformation Polymorphism

For PCR—single-strand conformation polymorphism (PCR-SSCP) analysis, 40 patients (20 men and 20 women) with deep venous thrombosis from the PATHROS study were screened. Seven overlapping fragments <320 bp in length covering the described 5′ regulatory region were amplified. Two fragments were amplified to analyze the 2 newly sequenced exon/intron junctions. The sequences of primers are listed in Table 1.

Each amplification was performed by using a solution of 100 ng in a total volume of 50 μL containing 20 mmol/L Tris-HCl (pH 8.8), 10 mmol/L KCl, 1.5 mmol/L MgCl2, 0.1% Triton X-100, 0.2 mg/mL BSA, 200 μmol/L dNTP, 50 pmol of each primer, and 0.1 U Taq polymerase (ATGC). To amplify PCR fragments 6 and 7, 10% (vol/vol) dimethyl sulfoxide was added.

For SSCP analysis, 0.3 μCi of [α-32P]CTP was added to the PCR mix. PCR products were diluted 2-fold in a solution containing 95% deionized formamide, 10 mmol/L EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol. After denaturation at 94°C for 5 minutes, the samples were cooled on ice, and 4-μL samples were loaded onto nondenaturing 6% polyacrylamide gel (acylamide to bis-acrylamide ratio 39:1). Two different SSCP conditions were used for each fragment: 7.5% and 0% glycerol at 40 mA for 6 hours at 4°C, with use of a cooling fan. Then, gels were dried and autoradiographed overnight at −80°C with an intensifying screen. The DNA of patients presenting different single-strand conformation patterns of migration was reamplified, purified, and sequenced as described above.

Identification of Genotype in Study Population

A fragment encompassing the polymorphic site at position −1426 was amplified with primers TR1U and TR2Lmod, a primer modified to create a restriction site for BstNI (Biolabs, Ozyme) for the presence of the nucleotide T at position −1426. A second polymorphism at position −506 was genotyped after amplification of fragment 6 and cleavage with HinfI (Biolabs) to detect the 13-bp insertion. The digested PCR products were electrophoresed in 3% agarose and stained with ethidium bromide.

The IVSs-14 polymorphism was genotyped by using allele-specific oligonucleotides. The PCR product (15 μL) was denatured in 150 μL of 0.5 mol/L NaOH and 1.5 mol/L NaCl and blotted onto nylon membranes (ICN). Allele-specific oligonucleotides for each allele were labeled with 50 μCi of [γ-32P]ATP by T4 kinase (Biolabs) at 37°C for 20 minutes. Membranes were incubated for 4 hours with 20 pmol of labeled probe at 29°C, washed twice at room temperature in 1× SSC for 5 minutes followed by 5 minutes in 0.5× SSC at 31°C, and autoradiographed overnight at −80°C with an intensifying screen. Primers and allele-specific oligonucleotides are listed in Table 1.

Statistical Analysis

Data were analyzed with SAS Statistical software (SAS Institute Inc). Clinical characteristics of cases and controls were compared by a χ² test with 1 df, except for age; ANOVA was used.

Pairwise linkage disequilibrium coefficients were estimated in controls by use of log-linear model analysis. The extent of the disequilibrium has been reported as the ratio of the unstandardized coefficients to their minimal/maximal value, [D*], and varies between 0 and 1. The sign in front of the coefficients indicates whether the linkage disequilibrium is positive (rare alleles preferentially associated) or negative (rare alleles preferentially associated with frequent alleles).

Hardy-Weinberg equilibrium was tested by a χ² test with 1 df in cases and controls separately. Allele frequencies were deduced from the genotype frequencies, and their differences between cases and controls were tested by a χ² test (1 df).

The odds ratios (ORs) and 95% CIs for thrombosis associated with the −506 allele I (ID or II carriers versus DD carriers, where I indicates insertion and D indicates deletion) were calculated by a logistic regression procedure (SAS-PROC LOGIST, SAS Institute Inc). The homogeneity of the ORs associated with −506 I/D was tested separately in men and women and across age by entering the corresponding interaction term in the logistic regression.

Differences in F1+2 levels according to −506 I/D genotype were tested by a general linear procedure (SAS-PROC GLM, SAS Institute Inc) adjusted for age and sex. Arithmetic means are presented in Table 5, but tests were performed on log-transformed F1+2 to remove the skewness of the distribution.

The significance level was taken to be P<0.05.
Results

Identification of Polymorphisms in Promoter Domain and Exon/Intron Boundary Region

The 5' domain of the PAR-1 gene was sequenced by Schmidt et al.20 at 1732 nucleotides upstream from the translation start site. Because the exon/intron boundary sequences were not determined in this pioneer work,20 we used the human promoter finder DNA Walking kit to establish the sequences of these junction domains possibly involved in the splicing process. The nested PCR performed on each of the DNA library products obtained after the first amplification yielded a unique fragment encompassing ~800 bp of the 5' intronic region (after exon 1). This fragment allowed us to sequence the first 236 nucleotides. The same approach was used to sequence the 3' intronic region (preceding exon 2). The 4-kb fragment obtained was sequenced over 205 nucleotides. The sequences of both exon/intron junctions are shown in Figure 1.

Both exon/intron junctions of the single intron of the PAR-1 gene as well as the 1732 nucleotides 5' to exon 1 were amplified in 9 different fragments (see Methods) and submitted to SSCP analysis. We analyzed 80 alleles from patients belonging to the PATHROS case-control study. Three different single-strand conformation patterns of migration were

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**Table 1.** Amplifiers, Allele-Specific Oligonucleotides, and Reaction Conditions Used for Intron Sequencing, SSCP Scanning, and Genotyping of Sequence Variation of PAR-1 Gene

<table>
<thead>
<tr>
<th>Position From Translation Start Codon</th>
<th>Amplifiers (5' to 3')</th>
<th>Size, bp</th>
<th>Annealing Temperature, °C</th>
<th>Allele-Specific Oligonucleotide</th>
<th>Hybridation Temperature, °C</th>
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<tr>
<td>Determination of intron/exon boundary sequence</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Exon 1/intron</td>
<td>4 to 23</td>
<td>GSP1: GGG CCG CGG CGG CTG CTG CT</td>
<td>319</td>
<td>56</td>
<td>...</td>
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<tr>
<td></td>
<td></td>
<td>GSP2: CCC CCC CTG CTT CAG TCT GT</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>27 to 46</td>
<td>ISP: GCG GCC CGC TGT TGT CTG CC</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Intron/exon 2</td>
<td>468 to 449</td>
<td>GSP1: GGG GAG CAC AGA CAC AAA CA</td>
<td>302</td>
<td>56</td>
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<tr>
<td></td>
<td>258 to 238</td>
<td>GSP2: TGC AGG AAG TTT TTT TTG AAG</td>
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<td>...</td>
<td>...</td>
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<tr>
<td></td>
<td>142 to 121</td>
<td>ISP: TCA TCC TCC CA AAT GGT TCA TA</td>
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<td>...</td>
<td>...</td>
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<td>PCR-SSCP fragments</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>−1818 to −1798</td>
<td>1U: AAC TGG GTA CTT CCG CAA TT</td>
<td>319</td>
<td>56</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1L: ATG GGT CCC CAA ATA GCT TT</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2</td>
<td>−1496 to −1516</td>
<td>2U: TGA GCT TTC TCA GAA ACA GC</td>
<td>264</td>
<td>56</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>2L: CCT CCT GCC TCA GGC TCC TA</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>3</td>
<td>−1317 to −1298</td>
<td>3U: GCG GTG ATC ACC GCC TGT AA</td>
<td>302</td>
<td>56</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3L: AGG AGA GAA ATG GCC TGG TA</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>4</td>
<td>−1015 to −1034</td>
<td>4U: ATC GCT TCA AGA AGC TG</td>
<td>313</td>
<td>56</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4L: CCT TGA GTC TGG ACA GAA TT</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>5</td>
<td>−757 to −776</td>
<td>5U: GCG GCC CGC TGT TGT CTG GA</td>
<td>304</td>
<td>56</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>5L: CCA TCC TTC GAG AAT GAC AC</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>−572 to −551</td>
<td>6U: TCC TGG CGG CGG GCT CCA CT</td>
<td>316</td>
<td>70</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>6L: CCG GCC TGC AGT GAG AGT CTC TG</td>
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<td>...</td>
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</tr>
<tr>
<td>7</td>
<td>−45 to −64</td>
<td>7U: GAG CCG CTG CAC CGG GC</td>
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<td>70</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>7L: TCC TTC GGC TCC TGG ACT TCC</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>Exon 1/intron</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>8U: GTT GTG TCG CAC CGC CG</td>
<td>267</td>
<td>56</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8L: CCC CTG GCA GCC GCT GCA CC</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Intron/exon 2</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>TTA GAG TAT GCT AAT TAA GC</td>
<td>270</td>
<td>56</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TTA CTC TTT GGG GGG GGG TGG</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>Genotyping</td>
<td></td>
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</tr>
<tr>
<td>−1426 C/T</td>
<td>1U: AAC TGG GTA CTT CCG CAA TT</td>
<td>430</td>
<td>56</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>TR2Lmod: TGC TAA GGG CCC CCA GGG GGG GCG CGT CGT GGG GTG GCC</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>IVS-14 A/T</td>
<td>9U: TTA GAG TAT GCT AAT TAA GC</td>
<td>270</td>
<td>56</td>
<td>ATTTTTTTATTTTATT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9L: TTA CTC TTT GGG TGG TGG CTG AG</td>
<td>29</td>
<td>ATTTTTTTATTTTATT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
detected. The corresponding fragments were sequenced bidirectionally, which allowed the identification of 3 sequence variations: (1) a C to T transition 1426 upstream from the translation start site (\(2^{1426} \text{C/T}\)), (2) a 13-bp insertion repeating the preceding 5’-CGGCCGCGGGAAG-3’ sequence (\(2^{506} \text{I/D}\)), and (3) an A to T transversion in the intervening sequence (IVS) 14 nucleotides upstream from the exon 2 start site (IVS-14 A/T).

The frequency of each polymorphism was determined in the 1214 controls, as described in Methods. An example of the genotyping is presented in Figure 2. The allelic frequencies of \(2^{1426} \text{T}\), \(2^{506} \text{I}\), and IVS-14T were 0.041, 0.256, and 0.185, respectively. The 3 polymorphisms were in moderate linkage disequilibrium in 1214 controls. Pairwise linkage disequilibrium coefficients (\(D^*\)) were \(-0.55\) (\(P<0.05\)) and 0.40 (\(P<0.001\)) between \(-1426\) C/T and \(-506\) allele I and between \(-1426\) C/T and IVS-14T, respectively, and \(D^*\) was \(-0.43\) (\(P<0.001\)) between \(-506\) I/D and IVS-14T.

### Frequency of PAR-1 Polymorphisms in PATHROS Study

The main features of the studied population are presented in Table 2. Cases and controls did not significantly differ according to age and sex. Women taking oral contraception were significantly more frequent in the case group than in the control group. Primary thrombosis (in association with the absence of contraception, recent [<1 month] surgery or trauma, pregnancy or childbirth, immobilization, or cancer) and recurrent thrombosis occurred in 59.3% and 27.9%, respectively. Pulmonary embolism was diagnosed in 31.9% of cases. A family history of thrombosis was recorded in 38% of cases. The 2 common genetic risk factors for venous thrombosis, factor V Arg506Gln, and prothrombin gene G20210A mutations were found within the expected range of the European population. The factor V Arg506Gln mutation was observed in 21.2% of cases and 3.7% of controls (\(P<0.001\)), and the prothrombin G20210A mutation was observed in 12.4% of cases and 2.9% of controls (\(P<0.001\)).

Distribution of genotypes and of allelic frequencies of the 3 PAR-1 polymorphisms is depicted in Table 3. No significant deviation from Hardy-Weinberg equilibrium was noted for the polymorphisms investigated in the population studied. The prevalence of \(-1426\) T (10.4% of cases versus 8.1% of controls), \(-506\) I (40.4% of cases versus 44.9% of controls), and IVS-14T (29.6% of cases...
versus 33.3% of controls) alleles was not significantly different between cases and controls. By contrast, the significant heterogeneity according to sex for the −506 I/D polymorphism ($P<0.01$) led us to compare men and women separately (Table 4). Allele I was significantly less frequent in male cases than in male controls (0.154 versus 0.247, $P=0.008$). Females were analyzed according to the use of oral contraceptives. No difference was observed between cases and controls in the subset group of women with or without oral contraception.

The OR associated with the −506 I allele was $0.52$ (95% CI 0.32 to 0.82, $P<0.01$), pointing to a protective effect of this allele in males. The protective effect of the −506 I allele in men was conserved after exclusion of subjects bearing factor V Arg506Gln or prothrombin gene G20210A mutations; OR was $0.48$ (95% CI 0.29 to 0.80, $P<0.01$). No association with the clinical status was observed by testing heterogeneity according to the occurrence of pulmonary embolism, the existence of an acquired risk factor, the recurrence, and the age of first thrombosis even after analyzing males and females separately.

### Association of PAR-1 Gene Polymorphism With Plasma Phenotype Variation in Controls

To establish whether PAR-1 polymorphisms were correlated with hemostasis circulating markers, we measured von Willebrand factor plasma concentration, released by endothelial cells in response to thrombin stimulation, and prothrombin $F1\_2$, a marker of thrombin generation, in a subset of 383 controls. No detectable variations of von Willebrand factor concentrations according to the genotype were found (not shown). By contrast, $F1\_2$ levels were associated with −506 I/D polymorphism but not with the 2 other polymorphisms. As expected, $F1\_2$ levels were higher in females and increased with age ($P<0.0001$, not shown). After adjustment for these covariates, a significant reduction of $F1\_2$ was observed, confined to homozygous −506 I carriers, with a mean value of 1.04 nmol/L compared with 1.21 nmol/L and 1.25 nmol/L in heterozygous carriers and noncarriers, respectively ($P=0.04$). Of note, there was no significant heterogeneity between men and women in the lowering effect of carrying 2 I alleles (Table 5). The results suggested an overall reduction in the basal level of hemostasis activation in control subjects bearing −506 I.

### Discussion

The present study was designed to seek an association of PAR-1 genetic polymorphisms with VTE in a case-control...
study with a population comparable to other European populations for genetic risk factors, as shown by the similar frequency of the factor V Leiden and the prothrombin gene G20210A mutations.

The PAR-1 gene was screened for sequence variations that could modify the gene expression. In addition to the promoter, intronic sequences may be involved in gene expression by modification of the splicing process. Therefore, we analyzed the 5′ regulatory domain and the domains flanking exons 1 and 2 of the unique PAR-1 intron. Because only a few nucleotides of these exon/intron junctions were known, we first sequenced 236 nucleotides upstream and 205 nucleotides downstream from the extremities of this large 22-kb intron. We confirmed that the intron started with dinucleotide GT (donor site) and ended with dinucleotide AG (acceptor site) according to the splice donor/acceptor rules. The branch site is usually located within 30 bases (range 18 to 40) upstream from the 3′ end of the intron. The sequence −35 UUUUUAC −29, determined in the present study, could correspond to the consensus sequence 5′-YNYRAY-3′ (Y=CorT; N=A, T, CorG; R=AorG) (see Figure 1).

Screening the promoter and the exon/intron junctions by PCR-SSCP allowed us to identify 3 novel polymorphisms in the PAR-1 gene. One polymorphism was found in the 3′ end of the large intron near a putative acceptor site (IVS-14), and the 2 others were a C to T transition at position −1426 and a 13-bp insertion repeating the preceding 5′-CGGCC-GCGCCAAG-3′ sequence at position −506 in the promoter.

Potential transcription factor binding sites were searched with the Transcription Factor Database program. The polymorphism located at −1426 did not create or disrupt a cis-acting putative element. Interestingly, inside the 13-nucleotide sequence repeat at position −506, a potential recognition site (5′-GCGCCGGAACG-3′) for the known activating factor ets was found. The ets proteins are members of a large family with a conserved 84-amino acid sequence (the ets domain) allowing their binding to the GGA core as monomers.

The ets proteins play a major role in the formation of the initiation complex in genes lacking the canonical TATA box sequence, such as the PAR-1 gene promoter. In such promoters, the ets binding motifs help to determine the selectivity for transcription factors. PAR-1 gene promoter deletion and expression in human endothelial cells showed that the nucleotide sequence −702 to −4, a region encompassing several putative cis-activating sequences, had the highest expression of the reporter gene. Because the −506 I/D polymorphism duplicates a putative binding site for Ets-1/Ets-2 and is located within the active part of the promoter, it might influence the gene expression level in basal conditions or in response to different stimuli.

Experimental data, such as transient transfection studies, have not yet been completed to support the functional implication of this polymorphism on the promoter activity.

The primary goal of the present study was to search for an association of the PAR-1 genotype with thrombosis. The large number of controls allowed a reliable estimation of the allele frequencies in our French population: 0.185, 0.041, and 0.256 for the IVS-14T, −1426 T, and −506 I alleles, respectively. In the 250 patients, similar frequencies were observed, suggesting no overall association of these polymorphisms with VTE. However, comparison of cases with controls showed a strong heterogeneity according to sex for the distribution of the −506 I/D polymorphism (P<0.01), which prompted us to analyze men and women separately. Men carrying one or two −506 I alleles had a reduced risk of developing thrombosis with an OR at 0.52 (95% CI 0.32 to 0.82, P<0.01), inferring that the I allele might protect men from VTE. The absence of a protective effect in women might be related to an influence of female hormones on PAR-1 expression, with estrogens at pharmacological concentrations being risk factors for venous thrombosis. However, this is unlikely because the distribution of the polymorphism in women without oral contraception remained similar in cases and in controls.

PAR-1 is expressed on endothelial cells and on platelets, 2 key cells for hemostasis. Platelet activation by low thrombin concentrations via PAR-1 results in different cellular responses, such as aggregation, intragranular component release, and phosphatidylserine exposure with subsequent procoagulant activity. The latter response is crucial for prothrombinase complex assembly and thrombin generation. Endothelial PAR-1 activation may downregulate platelet aggregation by increasing PGI2 synthesis and, conversely, contribute to thrombin generation by inducing tissue factor. Thrombin-activated endothelial cells may themselves express a procoagulant phenotype and/or release proinflammatory cytokines with subsequent monocyte tissue factor induction. Thus, several mechanisms may explain an involvement of PAR-1 in the regulation of thrombin generation reflected by F1+2 concentration. Thus, it will be important to determine whether the I/D polymorphism influences the density of PAR-1 receptors on both platelets and endothelial cells and to evaluate the putative consequences on each cellular response. It is also

---

**TABLE 5. F1+2 Levels According to −506 I/D in Controls**

<table>
<thead>
<tr>
<th>−506 Genotype</th>
<th>Males</th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
<th>All</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean±SEM*</td>
<td></td>
<td>n</td>
<td>Mean±SEM*</td>
<td></td>
<td>n</td>
<td>Mean±SEM†</td>
</tr>
<tr>
<td>DD</td>
<td>115</td>
<td>1.08±0.04</td>
<td></td>
<td>109</td>
<td>1.41±0.04</td>
<td></td>
<td>224</td>
<td>1.25±0.03</td>
</tr>
<tr>
<td>ID</td>
<td>68</td>
<td>1.04±0.06</td>
<td></td>
<td>61</td>
<td>1.39±0.06</td>
<td></td>
<td>129</td>
<td>1.21±0.04</td>
</tr>
<tr>
<td>II</td>
<td>12</td>
<td>0.96±0.13</td>
<td></td>
<td>18</td>
<td>1.12±0.11</td>
<td></td>
<td>30</td>
<td>1.04±0.08</td>
</tr>
</tbody>
</table>

Tests (on log of F1+2) are as follows: overall genotype effect, males and females pooled, P=0.04; test of heterogeneity of genotype effect between males and females, P=0.67.

*Adjusted for age. †Adjusted for age and sex.
difficult to explain why the −506 I allele was significantly associated with decreased F1+2 levels in both male and female subjects homozygous for the −506 I allele but that the protective effect was restricted to male patients. The number of −506 I homozygote carriers was very low in the subset of subjects in whom F1+2 was measured (12 males and 18 females), which reduces the power of the statistical analysis.

To our knowledge, this is the first report involving human PAR-1 gene polymorphisms. One of them, an insertion of a putative Ets cis-acting element, could play a role in gene regulation. The protective effect of the −506 I/D polymorphism argues for an involvement of PAR-1 in the pathological process leading to thrombosis, although we cannot exclude the possibility that the −506 I allele is genetically linked to other causative gene variations.

Acknowledgments
This study was supported by a grant No. A094031 (Evaluation Clinique et Biologique du Risque Thrombotique) from Program Hospitalier de Recherche Clinique and by a grant from Center Claude Bernard de Recherche sur les Maladies Vasculaires Périphériques. We thank Véronique Remones and Richard Casseron for their technical assistance and José Bon-Deguingand for her secretarial assistance.

References


Protective Effect of a Thrombin Receptor (Protease-Activated Receptor 1) Gene Polymorphism Toward Venous Thromboembolism
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Arterioscler Thromb Vasc Biol. 2000;20:585-592
doi: 10.1161/01.ATV.20.2.585
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

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