The endothelial cell protein C (PC) receptor (EPCR) facilitates PC activation by the thrombin-thrombomodulin complex. A soluble form of this receptor (sEPCR) found in plasma inhibits both activated PC (aPC) activity and PC activation by competing for PC with membrane-associated EPCR. Elevated sEPCR levels are found in approximately 20% of healthy subjects, but the mechanisms underlying this interindividual variability are unknown. We measured sEPCR levels in 100 healthy male volunteers, and observed 2 phenotypic groups of subjects. The temporal stability of sEPCR levels suggested genetic control. Extensive analysis of the EPCR gene in these subjects revealed 13 polymorphisms in complete linkage disequilibrium; these defined 3 haplotypes, 1 of which (A3) was strongly associated with high sEPCR levels. The high constitutive sEPCR levels observed in A3 haplotype carriers might reduce the efficiency of the PC system and predispose these subjects to venous thrombosis. By studying 338 patients with venous thrombosis and 338 age- and sex-matched healthy subjects, we found that the A3 haplotype was overrepresented in the patients. In multivariate analysis, subjects carrying the A3 haplotype had an increased risk of thrombosis (odds ratio [OR] = 1.8; \textit{P} = .004). Thus, the A3 haplotype, which is associated with elevated plasma sEPCR levels, is a candidate risk factor for venous thrombosis. (Blood. 2004;103:1311-1318)
Dysfunctional EPCR-dependent activation of PC would potentially be thrombogenic. A loss of function could result from mutations leading to decreased expression of membrane EPCR. A 23-base pair (bp) insertion has been reported to impair EPCR functions by leading to the synthesis of a truncated protein that is not expressed on endothelial surfaces. Although initially identified in thrombophilic subjects, the role of this mutation in thrombosis is difficult to assess because its allelic frequency is low.

Point mutations were recently described within the promoter region of the gene in 4 thrombophilic subjects, but the involvement of these mutations in gene regulation could not be clearly demonstrated.

Another possible mechanism leading to dysfunction of the EPCR-mediated coagulation-regulating mechanism consists of mutations (or polymorphisms) leading to increased levels of sEPCR. Indeed, increased sEPCR levels may be prothrombotic, as sEPCR can inhibit aPC activity, as well as PC activation, by competing for PC with membrane-associated EPCR.

Recently, 2 studies have shown that sEPCR levels vary widely among healthy subjects. While sEPCR levels are between 75 and 178 ng/mL in 80% of subjects, the remaining 20% of subjects have values between 200 and 700 ng/mL. This bimodal distribution has repeatedly been reported in both French and Italian populations.

The aim of this study was to seek a genetic explanation for the bimodal distribution of sEPCR levels. We first measured plasma sEPCR levels in 100 healthy male volunteers and confirmed the expected bimodal distribution. We then extensively analyzed the EPCR gene of the same subjects. We identified several polymorphisms that were in complete linkage disequilibrium, defining 3 haplotypes. One of these haplotypes was associated with increased sEPCR levels, offering the first evidence that interindividual variations in sEPCR levels are genetically regulated.

As sEPCR can inhibit both aPC generation and aPC activity, we examined whether the haplotype associated with high sEPCR levels carried an increased risk of venous thrombosis. On comparing 338 subjects with thrombosis and 338 age- and sex-matched healthy controls, we observed a significantly higher allelic frequency of this haplotype in the cases, suggesting that it may be a risk factor for venous thrombosis.

Patients, materials, and methods

Materials

Evacuated tubes were from Becton Dickinson (Le Pont de Claix, France). The Qiamp Maxi kit was from Qiagen (Courtaboeuf, France). The sEPCR Asserachrom kit was kindly supplied by Stago Laboratories (Asnières, France). The DNA sequencing kit (Big Dye Terminators V3.0 Cycle Sequencing Ready Reaction with AmpliTaq DNA Polymerase FS) and the ABI Prism 3700 sequencer were from Applied Biosystems (Applera, Courtaboeuf, France). The deoxynucleoside triphosphate (dNTP) mix was from Amersham Biosciences Europe (Orsay, France). Oligonucleotides were from Proligo (Paris, France). The DNA sequencing kit (Big Dye Terminators V3.0 Cycle Sequencing Ready Reaction with AmpliTaq DNA Polymerase FS) and the ABI Prism 3700 sequencer were from Applied Biosystems (Applera, Courtaboeuf, France). The deoxynucleoside triphosphate (dNTP) mix was from Amersham Biosciences Europe (Orsay, France). Oligonucleotides were from Proligo (Paris, France). Plates for amplification product purification were from Millipore (Saint-Quentin en Yvelines, France). PstI restriction endonuclease was from New England Biolabs (Ozyme, Saint Quentin en Yvelines, France). Agarose was from Life Technologies (Invitrogen, Cergy-Pontoise, France).

Healthy subjects and patients

Recruited and studied at the Clinical Investigations Center of Hôpital Européen Georges Pompidou were 100 unrelated healthy white male volunteers aged from 18 to 35 years. This population has been described in detail elsewhere. Briefly, the volunteers were nonsmokers and had not taken any medication for at least 10 days before blood sampling. Volunteers with a personal or family history of excessive bleeding or thrombosis were excluded. The subjects underwent a physical examination and routine laboratory tests, including C-reactive protein and F1+2 assay.

Blood was collected from all volunteers by venipuncture in tubes containing 0.11 M sodium citrate (1 vol/9 vol) on day 1 (visit 1) and day 7 (visit 2). Plasma was obtained by centrifugation at 2300g for 20 minutes, and was immediately subjected to routine laboratory tests or stored at −80°C until use. Genomic DNA was isolated from peripheral blood mononuclear cells using the Qiamp Maxi kit according to the manufacturer’s instructions.

A group of 338 patients, matched for age and sex with 338 controls, was studied in a second phase. These subjects had participated in a case-control study, the PAris THRombosIs Study (PATHROS), designed to seek genetic risk factors for venous thromboembolism (VTE). The inclusion and exclusion criteria applied to cases, and their clinical and biologic characteristics, have been extensively described elsewhere.

Briefly, the patients had had at least one episode of objectively diagnosed deep venous thrombosis (documented by compression and ventilation lung ultrasonography or venography) and/or pulmonary embolism (documented by perfusion and ventilation lung scanning, convention pulmonary angiography, or computed tomographic angiography). The controls were healthy European subjects recruited from a health care center to which they had been referred for a routine checkup. Subjects with a history of VTE, arterial disease, or known malignancy were excluded on the basis of a medical questionnaire. To avoid a possible bias due to the fact that controls came from a health care center, we checked that the frequencies of factor V and prothrombin 20210G>A mutations were similar to those observed in other control populations.

The study protocols were approved by our local ethics committee. DNA was extracted from white blood cells using a standard method.

Soluble EPCR assay

Soluble EPCR (sEPCR) levels were determined in plasma by using sEPCR Asserachrom enzyme-linked immunosorbent assay (ELISA) kits from the same batch, according to the manufacturer’s instructions.

EPCR gene screening for polymorphisms

The nucleotides (nt) of the EPCR gene were numbered according to the sequence available under GenBank accession number AF106202. As shown in Figure 1, 7 amplification fragments spanned more than 99% of the EPCR gene. The location of the amplification primers relative to the EPCR gene sequence, as well as their nucleotide sequences, is indicated in Figure 1 and its legend. Each amplification product was purified by filtration on Millipore plates and sequenced with a DNA sequencing kit according to the manufacturer’s instructions; the sequencing products were analyzed on ABI Prism 3700 sequencer.

Screening of 40 subjects from a given population is sufficient to identify polymorphisms having a frequency of 5% or more, with a confidence interval (CI) of 95%. Thus, the entire EPCR gene of the first 48 consecutive healthy volunteers was screened for polymorphisms as described above. Then, the polymorphic sites identified in these 48 subjects were screened for in 52 additional healthy subjects, with primers targeting these sites.

Haplotype A3 identification in the PATHROS population

A rapid method of A3 haplotype identification was developed using the G at nt 6932 of the EPCR gene as marker. The region surrounding nucleotide 6932 was amplified using a mutagenic 35-mer 6936 mutagen (5′-CCTACACTTGCGCTGGCGTGGCCGTCCTTGCGTGC-3′) as upstream primer, and a 22-mer 7190Rv (5′-CAGATCTTTGTCACGCCTTCCT-3′) as downstream primer. The upstream primer bore 2 foreign nucleotides (underlined lowercase characters in the preceding sequence), thereby
allowing amplified fragments bearing an A at position 6936 of the EPCR gene (thus corresponding to haplotype A1 or A2) to be cleaved by the restriction endonuclease PstI, whereas amplified fragments bearing a G (and corresponding to haplotype A3) remained undigested. With 20 units of PstI, 20/9262L of the 290-bp amplification product was incubated overnight at 37°C, and digestion was checked by electrophoresis on 2% agarose gel.

Statistical analysis

Continuous variables are reported as means and standard deviation or as medians and range (according to their distribution), and categoric variables are reported as counts and percentages. Skewed variables were log-transformed before analysis. Individual subjects’ sEPCR plasma concentrations at visits 1 and 2 were compared using a concordance test. The chi-square test was used to compare the observed genotype frequencies with the Hardy-Weinberg equilibrium prediction. The association between the genotype and the biologic phenotype (sEPCR level) was tested using analysis of variance. Comparisons between case and control subjects were based on student unpaired t test for continuous variables, and the chi-square test or Fisher exact test for categoric variables. Multivariate analysis was used to determine the odds ratio (OR), based on multiple logistic regression. Statistical tests were run on Statview5 statistical software (SAS, Cary, NC), and differences with P values less than .05 were considered statistically significant.

Results

To assess the intraindividual variability of plasma sEPCR levels, we tested 2 blood samples, obtained one week apart, from each of 100 healthy male volunteers, except for 2 subjects who did not attend visit 2. At least 2 phenotypic groups were identified. Values in both groups had a gaussian distribution (Figure 2, upper panel); sEPCR levels were less than 137.5 ng/mL (3 nM) at both visits in 84 subjects, and more than 138.5 ng/mL at both visits in 14 subjects. Plasma sEPCR levels concorded between the 2 visits (R² = 0.95, P < .0001) (Figure 2, lower panel). One subject had a very high sEPCR level (854 ng/mL) at both visits.

As plasma sEPCR levels may be influenced by inflammation, C-reactive protein (CRP) levels were also determined in all the subjects. The results (CRP values always less than 5 mg/mL) ruled out a role of inflammation in the bimodal distribution of sEPCR levels. F1 + 2 levels in the 84 subjects with lower sEPCR levels (median, 1.03 nM; range, 0.56-3.45 nM) were similar to those in the 14 subjects with higher sEPCR levels (median, 1.28 nM; range,
The existence of different phenotypic groups of sEPCR expression, together with the stability of individual levels over time, pointed to genetic control of the sEPCR level. We therefore analyzed the EPCR gene in 48 consecutive healthy volunteers, from nucleotides 80 to 8100 (ie, 99% of the available AF106202 sequence, corresponding to 2300 nucleotides upstream of the ATG codon, the exons, the introns, and 1500 nt downstream of the stop codon). We found 16 single nucleotide polymorphisms (SNPs) located throughout the gene. The first was a C to G transition at nucleotide (nt) 1651, located within the promoter region. There were 6 other SNPs located in intron 1 that affected nt 3610 (T to C transition), nt 3787 (T to C transition), nt 3877 (A to G transition), nt 4216 (C, G, or A), nt 4414 (T to C transition), and nt 4868 (C to T transition). There were 4 SNPs located in intron 2 that affected nt 5233 (A to G transition), nt 5760 (C to T transition), nt 6146 (G to A transition), and nt 6333 (C to T transition). Exon 4 contained 2 SNPs, with an A to G transition at nt 6936, changing Ser 219 to Gly,12 and a C to G transition at nt 7014, in the noncoding part of exon 4. Finally, 3 SNPs were located in the 3′-UTR part of the gene; they consisted of a C to G transition at nt 7966, an A to G transition at nt 7968, and an A to G transition at nt 7999. These polymorphisms had allelic frequencies higher than 0.05, except for 4414C and 6146A (0.041 and 0.036, respectively).

Using primers targeting the 14 frequent polymorphic positions, we amplified and sequenced the corresponding regions of the EPCR gene in the other 52 healthy volunteers in order to determine the allelic frequencies of the polymorphisms. All but one (nt 7966) of the 14 frequent SNPs were in complete linkage disequilibrium. These 13 SNPs defined 3 haplotypes, which we designated A1, A2, and A3. As shown in Figure 3, A1 and A2 were major haplotypes, with allelic frequencies of 0.48 and 0.45, respectively.

The A1 haplotype consisted of a combination of T at nt 3787, A at nt 3877, C at nt 4216, C at nt 4868, A at nt 5233, C at nt 5760, C at nt 6333, C at nt 7014, G at nt 7968, and A at nt 7999. The A2 haplotype was a combination of C at nt 3787, G at nt 3877, G at nt 4216, T at nt 4868, G at nt 5233, T at nt 5760, T at nt 6333, G at nt 7014, A at nt 7968, and G at nt 7999. A3, the least common haplotype (allelic frequency 0.07), differs from the A2 haplotype at 4 nucleotide positions (G at nt 1651, C at nt 3610, A at nt 4216, and G at nt 6936). The allelic frequencies of the 3 haplotypes were in Hardy-Weinberg equilibrium (χ2 test, P > .05).

To establish whether the plasma sEPCR level is genetically regulated, we compared the plasma sEPCR level with the EPCR genotype (Figure 4). As plasma sEPCR levels were stable between the 2 visits, we used the mean value for each subject. As shown in Figure 4, sEPCR levels were significantly higher in subjects carrying one A3 allele (A1 A3 or A2 A3) than in subjects carrying no A3 allele. No significant difference in sEPCR levels was observed between A1 A1 or A2 A2 homozygotes and A1 A2 heterozygotes. The mean sEPCR level in subjects having at least one A3 allele was 264 ± 174 ng/mL (range, 138.5 to 854 ng/mL; 218.9 ± 39.36 ng/mL [range, 138.5 to 274.9 ng/mL] after excluding the subject with a value of 854 ng/mL), compared with 83.6 ± 17.2 ng/mL (range, 50.5 to 137.5 ng/mL) in the other subjects (P < .0001, 95% CI). It is important to underline that all the subjects carrying the A3 haplotype had elevated sEPCR levels at both visits, one week apart. Interestingly, none of the 100 volunteers was an A3 A3 homozygote.

To evaluate the possible influence of sEPCR levels on the risk of venous thromboembolism, we investigated a cohort of 338 patients matched for age and sex with 338 healthy controls from the PATHROS study. The patients were 162 men (47.9%) and 176 women (52.1%). Mean age was 46 ± 13 years in the control group and 48 ± 15 years in the patient group (P = .06). The main characteristics and clinical events of the patients and controls are shown in Table 1.

The factor V Arg506Gln mutation was detected in 18.6% of cases and 4.2% of controls (P < .0001), and the prothrombin 2010G>A mutation was detected in 11.9% of cases and 4.3% of controls (P = .0003). The observed frequencies of these polymorphisms in our control population and the associated risks for venous thromboembolism disease are similar to those reported in other independent studies. Therefore, the recruitment of control subjects in a health care center did not appear to be a major selection bias for the study of genetic risk factors.

To identify subjects bearing an A3 allele, we developed a rapid screening method for this haplotype (for details see Figure 5). Using this method, we identified 89 patients (26.3%) carrying at least one A3 allele (4 were homozygous and 85 “heterozygous”); 60 controls (17.7%) carried an A3 allele (2 were homozygous and 58 “heterozygous”) (P = .099) (Table 2). The allelic frequency of

0.68-3.12 nM) (P = .57), ruling out elevated thrombin generation in the latter group.
Table 1. Characteristics of the PATHROS case-control study population

<table>
<thead>
<tr>
<th></th>
<th>Cases, n = 338</th>
<th>Controls, n = 338</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of men/women</td>
<td>162/176</td>
<td>162/176</td>
<td>—</td>
</tr>
<tr>
<td>Age, y</td>
<td>48 ± 15</td>
<td>46 ± 13</td>
<td>.06</td>
</tr>
<tr>
<td>OC or HRT in females</td>
<td>28.9</td>
<td>40.2</td>
<td>—</td>
</tr>
<tr>
<td>Pulmonary embolism, %</td>
<td>43.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Recurrent thrombosis, %</td>
<td>33.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Primary thrombosis, %</td>
<td>25.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Factor V Arg506Gln, %</td>
<td>18.6</td>
<td>4.2</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Factor II 20210G&gt;A mutation, %</td>
<td>11.9</td>
<td>4.5</td>
<td>.0003</td>
</tr>
</tbody>
</table>

OC indicates oral contraceptive; HRT, hormonal replacement treatment; and —, no event.
*Excluding the following acquired risk factors: pregnancy, cancer, surgery, and immobilization.

To determine whether the A3 haplotype is associated with an increased risk of thrombosis, we studied 338 patients with a history of deep venous thrombosis and 338 age- and sex-matched healthy subjects.

The A3 haplotype was overrepresented in the patients, with an allelic frequency of 0.138 compared with 0.092 in the controls (P = .009), suggesting that the A3 haplotype may increase the risk of thrombosis. Indeed, the OR was 1.7 in univariate analysis (95% CI, 1.2 to 2.4; P = .007) and 1.8 in multivariate analysis, taking into account sex, age, and the factor V Leiden and prothrombin 20210A mutations (95% CI, 1.2 to 2.6; P = .004) (Table 3). When restricted to subjects with a first thrombotic event, similar results were obtained: the ORs were 1.6 (95% CI, 1.1 to 2.5; P = .017) and 1.7 (95% CI, 1.1 to 2.6; P = .014) in univariate and multivariate analysis, respectively. Conversely, no statistically significant effect was observed on thrombosis recurrence.

Figure 5. Rapid A3 haplotype identification method. Upper panel: schematic representation of the part of the human EPCR gene exon 4 containing G 6936, which identifies the A3 haplotype. The 6936 mutagen primer contains 2 foreign nucleotides at positions n -4 and n -3 from the 3' end (indicated by asterisks) in order to create a restriction site for the endonuclease Pst I when the amplified fragment contains an A at position 6936, which corresponds to haplotype A1 or A2; the amplified fragment containing a G, which corresponds to haplotype A3, remains undigested. After genomic amplification using this primer and the 7190Rv primer, the PCR-amplified fragment contains a Psf site (CTGGA/G; underlined) when nucleotide 6936 is an A. In the amplified fragment, the part corresponding to the primer is shown in lower letters. Lower panel: 2% agarose gel electrophoresis of digested PCR products obtained using 6936 mutagen and 7190Rv primers. Lanes i, ii, iii: subjects homozygous for an A at position 6936 (A1/A1 and A2/A2); lanes iv, v, vi: subjects heterozygous A/G at position 6936 (A1/A3 or A2/A3, ie, A3 "heterozygotes"); both patterns are visible, corresponding to the undigested (290 bp) and digested (254 bp) amplified fragments. Lane vii: undigested PCR-amplified fragment.
Surprisingly, the distribution of the A3 haplotype was sex-related, being significantly more frequent in male cases than in male controls (28.4% versus 16%, $P = .01$), whereas no such difference was found between female cases and female controls (24.4% versus 19.3%, $P = .3$). Men carrying 1 or 2 A3 alleles were at an increased risk of thrombosis, with an OR of 2.1 (95% CI, 1.2 to 3.6; $P = .008$) in univariate analysis and 2.5 (95% CI, 1.4 to 4.5; $P = .0017$) in multivariate analysis; the corresponding OR values in females were 1.4 (95% CI, 0.8 to 2.3; $P = .25$) in univariate analysis and 1.3 (95% CI, 0.8 to 2.2; $P = .37$) in multivariate analysis. Thus, the A3 haplotype appeared to increase the risk of thrombosis in men but not in women, although the sEPCR level is also increased in women carrying the A3 haplotype.

It is noteworthy that the frequency of hormonal treatment (oral contraception or replacement therapy) was higher in the female cases than in female controls. This hinders the interpretation of our results in the absence of data on the possible interaction of the A3 allele with hormonal treatment. In addition, because of subgroup analysis with sample size reduction and decreased statistical power, a true effect in women may have been missed. However, we cannot exclude a hazard effect, the women cases having by chance a few percent less carriers than the male cases, and the women controls a few percent more carriers than male controls, which reduced the power to find a significant effect in women.

The mechanism linking elevated sEPCR levels to venous thrombosis remains to be determined. In healthy subjects, the mean physiologic sEPCR concentration being around 3 nM, and thus well below the concentration (~70 nM) of circulating PC and the $K_d$ (30 nM) of the EPCR/PC and EPCR/aPC interactions, elevated sEPCR concentrations are unlikely to markedly affect the interaction with PC. However, in subjects with increased circulating sEPCR levels due to the A3 haplotype (250-854 ng/mL represents ~6-20 nM sEPCR), the local sEPCR concentration at the endothelial surface may approach or exceed the $K_d$ of the PC interaction, especially in the presence of other factors such as inflammation and thrombin generation, which can trigger sEPCR production. Increased sEPCR concentrations result in decreased aPC generation and inhibit generated aPC, with possible implications for the regulation of coagulation, since low circulating aPC level has been shown to be a risk factor for venous thromboembolism.37

Several polymorphisms belonging to the haplotypes described here have already been identified separately in other studies. The A>G polymorphism affecting nt 6936 has been described in 2 different studies, both of which showed a very similar frequency of the G allele (corresponding to the A3 haplotype) in thrombophilic and control white subjects.38,39 This apparent discrepancy may be due to the heterogeneity of the populations studied, or to the fact that the subjects studied were not matched for sex and age.

In a recent study, Espana et al38 reported a second polymorphism (7763C>G) corresponding to nt 7014 polymorphism described here. Interestingly, they found that patients carrying the

Table 2. Repartition of A3 haplotype in cases and controls in the PATHROS study, in the whole population or according to sex

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A3 carriers, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No A3 allele</td>
<td>26.3*</td>
<td>17.7</td>
<td>116</td>
<td>136</td>
<td>176</td>
<td>176</td>
</tr>
<tr>
<td>1 A3 allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 A3 alleles</td>
<td>2.6</td>
<td>2.6</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*indicates $P = .009$; †, $P = .011$; and ‡, $P = .3$.

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>0.138</td>
<td>0.092</td>
<td>0.145</td>
<td>0.08</td>
<td>0.13</td>
<td>0.1</td>
</tr>
<tr>
<td>A1 + A2</td>
<td>0.862</td>
<td>0.908</td>
<td>0.855</td>
<td>0.992</td>
<td>0.87</td>
<td>0.9</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>No A3 allele</th>
<th>Mean sEPCR (ng/mL)</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>One A3 allele</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Two A3 alleles</td>
<td>600</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. Plasma sEPCR levels according to the presence or absence of A3 alleles, in a series of 176 healthy female controls from the PATHROS case-control study. Upper panel: distribution of sEPCR levels. Lower panel: correlation between the genotype and the sEPCR level.

Table 3. OR in total population, or according to sex

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
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</thead>
<tbody>
<tr>
<td>Univariate analysis ($P$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[95% CI]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No A3 allele</td>
<td>1.7</td>
<td>2.1</td>
<td>1.4</td>
</tr>
<tr>
<td>One A3 allele</td>
<td>2.1</td>
<td>2.5</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Multivariate analysis for sex, age, factor V Leiden mutation, and prothrombin 20210A mutation. CI indicates confidence interval.
residue, and these 2 adjacent Gly residues may destabilize the helical transmembrane domain and thus might change the exposure of the cleavage site, resulting in a protein that is more sensitive to metalloproteinase cleavage.

One final hypothesis is that 1 (or several) of the 4 nt changes characterizing the A3 haplotype stabilizes the mRNA generated by the A3 allele, leading to increased protein synthesis.

Several acquired factors, such as sepsis and systemic lupus erythematosus, as well as thrombin generation, are reported to increase plasma sEPCR levels. The present study offers the first evidence that genetic control, mediated by a specific haplotype of the EPCR gene, contributes to sEPCR levels. The A3 haplotype is the least frequent of the 3 possible EPCR haplotypes. From an evolutionary standpoint, the fact that the A3 haplotype differs from the A2 haplotype by only 4 nucleotides indicates that this allele is the youngest of the 3. This could explain observed differences in allele distribution according to ethnic origin. The A3 allele is probably mainly observed in white subjects; indeed, Hayashi et al never observed a Gly-encoding sequence at position 219 of the EPCR gene in a study of 30 Japanese subjects.

Our results thus confirm an in vivo function of EPCR in the regulation of coagulation in humans. In particular, we found that the A3 haplotype was associated both with increased plasma sEPCR levels and with an increased risk of venous thrombosis in men. This candidate inherited risk factor for thrombosis warrants studies in other populations.

Acknowledgments
We thank Diagnostica Stago Laboratories for kindly providing the sEPCR Asserachrom kits. We are grateful to Philippe Coudoul (Genetics Department, Hôpital Européen Georges Pompidou) for his skilful help with sequencing. We also thank the nursing staff of the Clinical Investigation Center 9201-Inserm AP-HP of Hôpital Européen Georges Pompidou, and Alvine Bissery for statistical help.

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A haplotype of the *EPCR* gene is associated with increased plasma levels of sEPCR and is a candidate risk factor for thrombosis

Beatrice Saposnik, Jean-Luc Reny, Pascale Gaussem, Joseph Emmerich, Martine Aiach and Sophie Gandrille