Homozygosity in the Single Nucleotide Polymorphism Ser128Arg in the E-Selectin Gene Associated With Recurrent Venous Thromboembolism

Bernd Jilma, MD; Florian M. Kovar, MD; Gregor Hron, MD; Georg Endler, MD; Claudia L. Marsik, MD; Sabine Eichinger, MD; Paul A. Kyrle, MD

Background: The single nucleotide polymorphism (SNP) Ser128Arg in the E-selectin gene is overrepresented in certain patient populations with atherosclerosis or restenosis. As this SNP enhances tissue factor–triggered coagulation in humans during systemic inflammation, we hypothesized that it may also predispose for the development of recurrent venous thromboembolism (VTE).

Methods: A total of 585 patients were prospectively observed after first VTE for recurrent, objectively documented, symptomatic VTE. Patients with secondary VTE, homozygous factor V Leiden, natural inhibitor deficiencies, lupus anticoagulant, or long-term anticoagulation therapy were excluded. The S128R SNP was genotyped by mutagenically separated polymerase chain reaction.

Results: A total of 102 patients (17%) were heterozygous, and 11 were homozygous (2%) for the Ser128Arg mutation. Ninety patients (15%) had recurrent VTE during follow-up. Homozygosity for the Ser128Arg SNP increased the cumulative likelihood, particularly for early recurrent VTE (log rank test, $P<.05$) and was an independent predictor of recurrent VTE (hazard ratio [HR], 4.1; 95% confidence interval [CI], 1.5-11.4) in a multivariate Cox regression model. In contrast, heterozygosity for the polymorphism was associated with an unaltered HR (HR, 1.1; 95% CI, 0.6-1.9) for recurrent VTE.

Conclusions: Homozygosity for the S128R E-selectin allele appears to increase the risk for recurrent VTE several fold. If these findings are confirmed, this may represent a novel risk factor for recurrent VTE. These results also expand our knowledge on the association of this SNP with thrombotic disorders.

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Our knowledge of risk factors for venous thromboembolism (VTE) and its recurrence has substantially increased in the last decade. However, identification of additional risk factors is of interest because in up to 20% of patients with recurrent VTE, no thrombotic risk factors can be found.

Recently, animal models of venous thrombosis have suggested that E-selectin may regulate thrombus formation and its fibrin content. The selectins mediate the rolling of leukocytes on endothelial cells. E-selectin is exclusively expressed on endothelial cells, mostly after their activation and E-selectin is proteolytically cleaved from the surface of endothelial cells on activation by different stimuli.

The common Ser128Arg polymorphism in the E-selectin gene has been found to be functional. It alters ligand affinity, enhances tethering of myeloid cells, and regulates leukocyte endothelial interaction in vitro. Clinically, the polymorphism has been associated with atherosclerosis, myocardial infarction, and restenosis after angioplasty. In addition, the S128R polymorphism in the E-selectin gene is associated with enhanced endotoxin–triggered, tissue factor–mediated coagulation in humans.

Thus, we hypothesized that it may also be associated with recurrent VTE.

METHODS

All patients are participants in the Austrian Study on Recurrent Venous Thromboembolism (AUREC), an ongoing, prospective, multicenter cohort study investigating risk factors for recurrent VTE. Between July 1992 and April 2004, 2574 patients older than 18 years and treated with vitamin K antagonists for at least 3 months after a symptomatic VTE were eligible for the study. Patients with a history of VTE; VTE provoked by trauma, surgery, or pregnancy; a deficiency of a natural coagulation factor; or a demonstrating tendency to anticoagulation therapy were excluded from the study. The study protocol was approved by the medical ethics committee of each participating center.
tion inhibitor; the lupus anticoagulant; a known malignancy; requirement of long-term antithrombotic treatment; or missing laboratory values were excluded (n = 1989). All patients had been treated with standard heparin at doses designed to keep the activated partial thromboplastin time 1.5 to 2.0 times that of the control value or with subcutaneous low-molecular-weight heparin at therapeutic doses followed by at least 3 months of vitamin K antagonists. Patients were included after discontinuation of vitamin K–antagonist treatment and observed at 3-month intervals for the first year and every 6 months thereafter. They were provided with detailed written information on the symptoms of VTE and were instructed to report to 1 of the thrombosis centers in case of symptoms. All women were strongly discouraged from using contraceptive pills or hormone therapy regardless of whether they had a history of an association between the use of these hormones and the initial VTE. At each visit, a data form was completed regarding the patient’s medical history.

DIAGNOSIS OF VTE

The diagnosis of deep venous thrombosis was established by venography or color-coded duplex sonography (in the case of proximal deep venous thrombosis). If venography was used, one of the following direct or indirect criteria had to be fulfilled: a constant filling defect present on 2 views; an abrupt discontinuation of the contrast-filled vessel at a constant point in the vein; and failure of the entire deep vein system to fill without an external compressing process, with or without venous flow through collateral veins. Diagnostic criteria for color-coded duplex sonography were the following: visualization of an intraluminal thrombus in a deep vein, lack of or incomplete compressibility, and lack of flow spontaneously and after distal manipulation. The diagnosis of pulmonary embolism was made by ventilation-perfusion lung scanning according to the criteria of the Prospective Investigation of Pulmonary Embolism Diagnosis.10 Patients with both deep venous thrombosis and pulmonary embolism were categorized as having pulmonary embolism.

OUTCOME MEASURES

The end point of the study was recurrence of symptomatic VTE confirmed by venography, ventilation-perfusion lung scanning, or both, according to the detailed criteria. The diagnosis was established by an adjudication committee consisting of independent clinicians and radiologists who were aware of the patient’s sex but unaware of the presence or absence of thrombotic risk factors. Recurrent deep venous thrombosis was diagnosed if the patient had a thrombus in another deep vein in the leg involved in the previous event, a thrombus in the other leg, or a thrombus in the same venous system involved in the previous event with a proximal extension of the thrombus if the upper limit of the original thrombus had been visible or the presence of a constant filling defect surrounded by contrast medium if it had not.

LABORATORY ANALYSIS

Venous blood was obtained after the patient had fasted overnight, placed in 1:10 volume of 0.11 mM trisodium citrate, and centrifuged for 20 minutes at 2000g. The plasma was stored at −80°C. Routine laboratory methods were used to identify antithrombin, protein C, and protein S. Screening for factor V Leiden and factor II G20210A was carried out on genomic DNA as described previously.19,20 Factor VIII and factor IX were measured by 1-step clotting assays with the use of factor VIII– or factor IX–deficient plasma (Immuno Baxter, Vienna, Austria) and a Sysmex CA 6000 (TOA Medical Electronics Co, Kobe, Japan) fully automated coagulation analyzer. The presence of the lupus anticoagulant was established on the basis of the criteria of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis.21 The technicians were unaware of the patients’ characteristics at all times.

Presence of the Ser128Arg polymorphism was determined by polymerase chain reaction analysis, exactly as described in earlier studies.14,22

STATISTICAL ANALYSIS

Categorical data were compared between groups with contingency table analyses (χ² test), and continuous data (presented as mean±SD unless otherwise indicated) were compared with Kruskall-Wallis tests. All P values were 2-tailed, and P <.05 was considered statistically significant. Survival time methods were used to analyze the time to recurrent VTE among patients with a subsequent episode (uncensored observations) or the duration of follow-up among patients without recurrence (censored observations).

The Kaplan-Meier method was used to estimate the probability of recurrence. Survival data on patients who left the study because of a requirement for potentially long-term antithrombotic treatment, a diagnosis of cancer, or pregnancy, or who were lost to follow-up or died were censored at the time of withdrawal. To test for homogeneity among the various groups of patients, we used the log-rank test. Univariate and multivariate Cox proportional hazards models were used to analyze the association of Ser128Arg polymorphism with the risk of recurrent VTE. Analyses were adjusted for sex, age, the presence or absence of symptomatic pulmonary embolism at the time of a first thrombotic event, and the presence or absence of factor V Leiden, factor II G20210A, and elevated levels of factors VIII and IX (dichotomized at the 90th percentile [225 IU/dL] and at the 75th percentile [135 IU/dL] of the patient population, respectively). All computations were performed with SPSS software, version 12.01 (SPSS Inc, Chicago, Ill).

RESULTS

STUDY POPULATION

Patients had no relevant comorbidities. This was owing to their relatively young age and exclusion criteria such as malignancy and cardiovascular disease. Patients were enrolled after the discontinuation of oral anticoagulants, and their mean±SD time undergoing anticoagulation therapy was 8±11 months. Patients were observed for a mean 48 months. DNA material for genotyping of E-selectin was available from 585 patients, mean±SD age 48±16 years. Of 585 patients, 102 (17%) were heterozygous and 11 (2%) homozygous for the Ser128Arg mutation. The genotype distribution was within the Hardy-Weinberg equilibrium as was expected from previous findings in patients and healthy volunteers from Austria.14,15,22

Heterozygous carriers of the Ser128Arg allele had a lower frequency of pulmonary embolism (with or without manifest deep venous thrombosis) than patients homozygous for the frequent or rare allele (33% vs 45% vs 46%, respectively; P = .04) (Table 1). Otherwise basal
values of other parameters and known risk factors were not different between carriers of the mutant allele and patients with the frequent allele (Table 1).

ASSOCIATION OF E-SELECTIN POLYMORPHISM WITH RECURRENT VTE

Ninety (15%) of the patients had recurrent VTE during follow-up. As depicted in the Figure, heterozygous carriers of the Ser128Arg polymorphism had event-free survival curves similar to subjects with the wild-type. In contrast, the probability of recurrence of VTE was higher in individuals homozygous for the Ser128Arg polymorphism (log rank test, \( P = .04 \) vs wild-type; \( P = .03 \) vs heterozygous carriers). Interestingly, all episodes of recurrent VTE in the homozygous carriers occurred within 18 months after discontinuation of anticoagulation therapy.

The hazard ratio (HR) was 2.83 (95% confidence interval [CI], 1.03-7.76) for homozygosity and 1.1 (95% CI, 0.6-1.9) for heterozygosity for the Ser128Arg polymorphism in a crude Cox regression model in which only the time component, but no other adjusters, were taken into account. A multivariate Cox regression analysis confirmed the previously identified risk factors for VTE; that is, pulmonary embolism, male sex, and high factor VIII and factor IX levels were predictive of recurrent VTE (Table 2). It also demonstrated that homozygosity for the Ser128Arg polymorphism is an independent predictor of recurrent VTE with a 4.1-fold increase (95% CI, 1.5-11.4) in the relative risk (\( P = .01 \)). In contrast, heterozygosity for the polymorphism was associated with an unaltered HR for recurrent VTE (HR, 1.1; 95% CI, 0.6-1.9).

There were no significant differences in other risk factors (mutations of prothrombin and factor V; plasma levels of factor VIII, factor IX, factor XI, and thrombin activatable fibrinolysis inhibitor; age; sex; or months of therapy) between E-selectin carriers of the S128R allele and patients homozygous for the frequent allele.

### Table 1. Baseline Characteristics of Patients Stratified According to the E-Selectin S128R Polymorphism*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequent E-Selectin Allele (n = 472)</th>
<th>Heterozygous for Ser128Arg Allele (n = 102)</th>
<th>Homozygous for Ser128Arg Allele (n = 11)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localization VTE</td>
<td></td>
<td></td>
<td></td>
<td>.04</td>
</tr>
<tr>
<td>Proximal DVT</td>
<td>258</td>
<td>70</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>PE ± DVT</td>
<td>214</td>
<td>32</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Female sex</td>
<td>251</td>
<td>58</td>
<td>5</td>
<td>.68</td>
</tr>
<tr>
<td>Factor V Leiden carrier</td>
<td>123</td>
<td>35</td>
<td>3</td>
<td>.27</td>
</tr>
<tr>
<td>Factor II G20210A carrier</td>
<td>32</td>
<td>6</td>
<td>1</td>
<td>.89</td>
</tr>
<tr>
<td>Factor VIII, IU/dL</td>
<td>163 (133-195)</td>
<td>156 (127-187)</td>
<td>173 (160-208)</td>
<td>.31</td>
</tr>
<tr>
<td>Factor IX, IU/dL</td>
<td>116 (100-133)</td>
<td>116 (97-133)</td>
<td>119 (100-138)</td>
<td>.93</td>
</tr>
<tr>
<td>D dimer, ng/mL</td>
<td>317 (220-486)</td>
<td>300 (197-496)</td>
<td>379 (258-529)</td>
<td>.83</td>
</tr>
<tr>
<td>Age, y</td>
<td>48 ± 16</td>
<td>49 ± 17</td>
<td>49 ± 12</td>
<td>.86</td>
</tr>
<tr>
<td>Re-event free survival, mo</td>
<td>49 ± 42</td>
<td>44 ± 37</td>
<td>44 ± 51</td>
<td>.47</td>
</tr>
<tr>
<td>Duration of anticoagulation, mo</td>
<td>9 ± 12</td>
<td>7 ± 4</td>
<td>7 ± 1</td>
<td>.80</td>
</tr>
</tbody>
</table>

*Unless otherwise noted, data are reported as number of patients, median (interquartile range), or mean ± SD.

### Table 2. Risk Factors for Recurrent Venous Thromboembolism in a Multivariate Cox Regression Model

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Hazard Ratio (95% CI)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male vs female</td>
<td>3.0 (1.8-4.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Factor VIII, ≥225 IU/mL vs &lt;225 IU/mL</td>
<td>2.5 (1.3-4.9)</td>
<td>.01</td>
</tr>
<tr>
<td>Factor IX, ≥135 IU/mL vs &lt;135 IU/mL</td>
<td>1.6 (1.0-2.5)</td>
<td>.04</td>
</tr>
<tr>
<td>PE vs DVT</td>
<td>1.7 (1.1-2.6)</td>
<td>.02</td>
</tr>
<tr>
<td>Homozygous for Ser128Arg E-selectin polymorphism vs wild-type</td>
<td>4.1 (1.5-11.4)</td>
<td>.01</td>
</tr>
<tr>
<td>Heterozygous for Ser128Arg E-selectin polymorphism vs wild type</td>
<td>1.1 (0.6-1.9)</td>
<td>.83</td>
</tr>
<tr>
<td>Prothrombin mutation vs wild type</td>
<td>1.9 (0.9-3.9)</td>
<td>.07</td>
</tr>
<tr>
<td>Factor V Leiden mutation vs wild type</td>
<td>1.1 (0.7-1.7)</td>
<td>.80</td>
</tr>
</tbody>
</table>

Abbreviations: DVT, deep venous thrombosis; PE, pulmonary embolism.
Recently, E-selectin has been identified as an important regulator of thrombus formation and fibrin content in a mouse venous thrombosis model. The thrombus weight in E-selectin wild-type mice was 3-fold higher than in the E-selectin knockout mice. In addition, endotoxin-induced, tissue factor-mediated coagulation is enhanced in humans carrying the S128R E-selectin allele. Thus, we hypothesized that this polymorphism may also be associated with increased recurrence rates of VTE after discontinuation of anticoagulation therapy in patients with a previous episode of VTE.

Our data support the hypothesis that patients homozygous for the S128R E-selectin allele have an increased risk for early recurrence of VTE; all episodes of recurrent VTE occurred within 1.5 years (Figure). This early recurrence of VTE is interesting and was also observed for patients with plasma levels of factor VIII higher than the 90th percentile. However, patients homozygous for the S128R E-selectin allele did not have increased factor VIII levels. In contrast, other risk factors including coagulation factor IX and thrombin-activatable fibrinolysis inhibitor conferred an increased risk also later during the observation period.

In contrast to homozygosity, heterozygosity for the S128R polymorphism was not associated with an increased risk for recurrent VTE (Figure). A power calculation indicates that we had 80% power (α = 0.05) to detect a 53% higher rate of VTE in heterozygous individuals vs patients with the frequent allele at 18 months. Of interest, heterozygous carriers of the Ser128Arg allele had markedly enhanced thrombin generation in our human model where tissue factor-mediated coagulation is induced by endotoxin infusion. In the present study, D dimer levels were only insignificantly (20%) higher in carriers of the mutant allele. Conceivably, the phenotypic penetration of the examined mutation is dependent on environmental factors, e.g., inflammation. In the case of patients with VTE, a homozygous genotype may be required.

While the prevalence of pulmonary embolism was slightly lower in carriers of the S128R allele, this could be due to chance. Alternatively, and more unlikely, this could reflect a survivor phenomenon, and carriers of the S128R allele may have died from pulmonary embolism before inclusion in the study.

The prevalence of the well-established genetic risk factors for VTE (but not recurrent VTE), heterozygous factor V Leiden mutation, and the G20210A prothrombin mutation was 3- to 5-fold higher in our patients with VTE than the prevalence found in an otherwise healthy white population. This is not surprising and is owing to the inclusion criteria of the study population. However, the frequency of the S128R mutation in our cohort of patients with VTE was not different from healthy young volunteers or other patient groups in Austria. This provides indirect evidence that the S128R allele may not be associated with an increased risk for the first VTE episode.

Monocytes can interact with E-selectin, and the S128R single nucleotide polymorphism (SNP)-associated alterations in E-selectin enhance the interaction with myeloid cells. One may speculate that this observed in vitro interaction could be the operative mechanism of action in vivo, how the S128R SNP enhances coagulation and increases the risk of recurrent VTE. However, further investigations are necessary to clarify how the S128R polymorphism in the E-selectin gene enhances coagulation.

Nonetheless, the present study further corroborates that the S128R allele is associated with procoagulant effects. This may be of potential clinical relevance also for other diseases: it not only may contribute to the association of the S128R SNP to atherosclerosis and restenosis but also may have consequences for many thrombotic vascular disorders. This should be subject of further scrutinized studies.

Among the obvious limitations of our study is that the observed effect could also be caused by less-characterized polymorphisms, which could be in linkage disequilibrium with the examined E-selectin polymorphism. Also, we had no plasma from these patients left to examine the relationship between the polymorphism and the plasma levels of the cardiovascular risk marker soluble E-selectin. Finally, although our cohort of patients is probably one of the largest published, the number of patients homozygous for the examined SNP is limited. Thus, these data need be confirmed by follow-up of more patients over the next years and/or by independent research groups to eventually establish the S128R E-selectin SNP as a risk factor for recurrent VTE.

In conclusion, homozygosity for the S128R E-selectin allele appears to increase the risk for recurrent VTE by several fold. If these findings are confirmed, this may represent a novel risk factor for recurrent VTE. These results also expand our knowledge of the association of this SNP with thrombotic disorders.

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Correspondence: Bernd Jilma, MD, Department of Clinical Pharmacology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Wien, Austria (Bernd.Jilma@meduniwien.ac.at).

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REFERENCES


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