Impaired in vivo activated protein C response rates indicate a thrombophilic phenotype in inherited thrombophilia

by Sara Reda, Nadine Schwarz, Jens Müller, Johannes Oldenburg, Bernd Pötzsch and Heiko Rühl

Received: December 21, 2021.
Accepted: January 31, 2022.

Citation: Sara Reda, Nadine Schwarz, Jens Müller, Johannes Oldenburg, Bernd Pötzsch and Heiko Rühl. Impaired in vivo activated protein C response rates indicate a thrombophilic phenotype in inherited thrombophilia.

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
Impaired *in vivo* activated protein C response rates indicate a thrombophilic phenotype in inherited thrombophilia

Sara Reda*, Nadine Schwarz*, Jens Müller†, Johannes Oldenburg†, Bernd Pötzsch†#, Heiko Rühl*#

†Institute of Experimental Hematology and Transfusion Medicine, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany

*SR and NS contributed equally as co-first authors

#BP and HR contributed equally as co-senior authors.

**Running head:** Thrombin/APC response in familial thrombosis

**Correspondence:** Heiko.Ruehl@ukbonn.de

**Data sharing statement:** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All authors have complete and on-going access to the study data.

**Word count:** 1449. Figures and tables: 3. Supplementary files: 1

**Acknowledgements:** This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 419450023. HR is recipient of a fellowship from the Stiftung Hämotherapie-Forschung (Hemotherapy Research Foundation).

**Contributions:** HR, JM, and BP designed the experiments. SR, NS, and HR collected the data. SR and HR analysed the data. SR, NS, JM, JO, BP, and HR drafted and edited the manuscript. SR and NS contributed equally to this work. BP and HR are co-senior authors.
Disclosures: BP and JM have a patent DE102007063902B3 including the aptamer HS02-52G binding to APC. An assay for the quantification of APC levels in human plasma, based on this aptamer, has been licensed to ImmBioMed, Pfungstadt, Germany. The other authors have no conflict of interest.
Venous thromboembolism (VTE) is a multifactorial disease. Hereditary risk factors include the common mutations factor V Leiden (FVL) and prothrombin (FII) 20210G>A, with a prevalence of 3 – 15 % among whites, as well as deficiencies of the coagulation inhibitors antithrombin (AT), protein C (PC), and protein S.\(^1\) In the recent past, novel risk loci have been found by genome-wide association studies.\(^2,3\) However, their consideration in addition to the classical thrombophilic defects results in an estimated heritability of VTE of only 15 %, in contrast to 40 – 60 % heritability observed in family-based studies.\(^4\)

To identify further unknown genetic thrombophilic defects, consideration of the laboratory phenotype of increased thrombin formation in addition to the clinical phenotype of VTE has been proposed, based on the observation of elevated \textit{in vitro} thrombin generation parameters in families with unexplained thrombophilia and in carriers of genetic variations in hemostasis-related genes other than FVL and FII 20210G>A.\(^5\)

It remains unclear, however, if increased \textit{in vitro} thrombin formation rates indeed reflect increased \textit{in vivo} thrombin formation. To investigate this, we comparatively analyzed \textit{in vitro} and \textit{in vivo} thrombin formation in a cohort of healthy individuals and in thrombophilic patients. \textit{In vivo} coagulation activation was induced by low-dose recombinant activated factor VII (rFVIIa). Subsequent hemostasis biomarker-monitoring included measurement of activated PC (APC) as a measure of the endothelial-dependent anticoagulant response. Recently, using this stimulated hemostasis activity pattern evaluation (SHAPE) approach, we were able to show increased \textit{in vivo} thrombin generation rates and a comparable APC response in FVL and FII 20210G>A carriers.\(^6,7\) Moreover, we found that APC response rates correlated with the thrombotic risk in FVL carriers.\(^7\)

The study population consisted of 30 healthy individuals and 51 patients with a history of VTE, thereof 28 FVL or FII 20210G>A carriers (FVL/FII 20210G>A cohort), and 23 unrelated subjects with unexplained familial VTE (FH cohort). A diagram of patient recruitment and selection criteria is shown in Figure 1, along with a description of study procedures. Blood samples were drawn before and during 8
hours after administration of 15 µg/kg rFVIIa. No adverse events were observed. APC was measured
using an oligonucleotide-based enzyme capture assay (OECA)\(^8\). The thrombin biomarkers prothrombin
activation fragment 1-2 (F1+2), thrombin-antithrombin complex (TAT), and other hemostasis
parameters were determined using commercially available assays. \textit{In vitro} thrombin generation was
assessed before rFVIIa administration, using the calibrated automated thrombogram (CAT) assay
(Thrombinoscope, Maastricht, NL). \textbf{Table 1} lists demographic features and measurement results of
hemostasis parameters in the three cohorts at baseline, and rFVIIa-induced changes of F1+2, TAT, and
APC over time, expressed as area under the curve (AUC). Hemostasis parameters at baseline were
comparable in FVL and FII 20210G>A carriers (\textit{Online Supplementary Table S1}).

\textit{In vitro} thrombin formation kinetics were higher in the FH cohort than in FVL/FII 20210G>A carriers and
healthy controls, indicated by an elevated endogenous thrombin potential (ETP) (\textit{Figure 2A}).
Additionally, peak thrombin concentration was increased compared with FVL/FII 20210G>A carriers,
whereas lag time and time-to-peak did not differ significantly (\textit{Online Supplementary Figure S1A -}
\textit{S1C}). The difference in the ETP was more pronounced at 1 pmol/L tissue factor (TF) concentration.
This could be explained by higher FXI levels in the FH cohort, which have been shown to affect \textit{in vitro}
thrombin generation at a greater extent at lower TF concentrations.\(^9\) In the resting state, plasma levels
of F1-2 were slightly increased in the FVL/FII 20210G>A cohort, giving additional evidence of increased
thrombin formation.

After infusion of rFVIIa, plasma levels of F1+2 (\textit{Figure 2B}) and TAT (\textit{Figure 2C}) increased significantly
in all three cohorts (peak versus baseline values, Wilcoxon signed-rank test \(P < 0.05\) after Bonferroni
correction). F1+2 increased in every participant, indicating that rFVIIa activates the clotting cascade,
resulting in thrombin formation. Every FVL/FII 20210G>A carrier showed an increase of F1+2 and TAT,
whereas four subjects in the FH group and seven healthy controls showed an isolated increase of F1+2.
This absence of a TAT increase could indicate a comparably lower thrombin formation rate. The most
probable explanation of this discrepancy is the longer F1-2 half-life of approximately 2 hours in comparison to the TAT half-life of 44 minutes,\textsuperscript{10} making F1-2 a more sensitive thrombin generation marker. The \textit{in vivo} thrombin generation parameters F1-2 AUC and TAT AUC correlated with each other in healthy controls and patients with a history of VTE (Figure 2D). However, as is representatively shown for ETP and TAT AUC (Online Supplementary Figure S1D and S1E), they did not correlate with \textit{in vitro} thrombin generation, suggesting that different factors determine and interfere with the outcome in both distinct and complex methodological approaches. In addition, compared with FVL and FII 20210G>A carriers, a more heterogenous risk profile can be expected in the FH cohort.

If the endothelium is intact, the thrombin formation capacity is effectively controlled by APC formation. The extent to which thrombin formation induces an increase in APC might therefore indicate the functionality of the APC-generating pathway in an individual patient and, moreover, modulate the thrombotic potential of increased thrombin formation rates. To investigate the reactivity of the PC-system to thrombin formation we measured plasma levels of APC. After infusion of rFVIIa, APC increased significantly in all cohorts (Wilcoxon signed-rank test, \( P < 0.05 \) after Bonferroni correction). Changes in APC (and thrombin biomarkers) did not differ in FVL and FII 20210G>A carriers (Online Supplementary Figure S2). In contrast to thrombin formation rates the APC response was significantly lower in the FH cohort than in the FVL/FII 20210G>A cohort and did not differ to healthy controls (Figure 2E). As the APC response is a direct marker of the APC formation capacity of the endothelium, the disproportionately low APC response in relation to the thrombin formation rate indicates an impaired endothelial APC generating activity in the FH cohort. This relative APC deficiency after coagulation activation would consecutively result in increased thrombin formation. Several data support this conclusion: 1) Previously, reciprocal and opposite changes of indirect thrombin and PC activation markers were observed in patients with abnormalities of the PC pathway in a basal state.\textsuperscript{11} 2) In a previous study, asymptomatic FVL carriers showed a higher APC response in the SHAPE approach than those with prior VTE.\textsuperscript{7} 3) In the present study, thrombin and APC formation rates (TAT AUC and
APC AUC) correlated with each other in both FVL/FII 20210G>A carriers and patients with unexplained familial thrombosis, but not in healthy controls (Figure 2F). With seven subjects (25%) in the FVL/FII 20210G>A cohort and six subjects (26%) in the FH cohort, both TAT AUC and APC AUC lay above the 90th percentiles of the healthy controls in similar rates of patients. However, only two individuals (9%) in the FH cohort showed a disproportionately high APC formation rate, as evidenced by an APC AUC (slightly) above and TAT AUC within the 90th percentiles of the healthy controls. In the FVL/FII 20210G>A cohort such a pattern was observed more often (29%), and more distinctively (Figure 2F).

Thrombomodulin (TM) and endothelial PC receptor (EPCR) are two main factors that determine the APC formation capacity of the endothelium and variants in both genes have been suggested as thrombotic risk factors. To assess interindividual variations in TM and EPCR, we measured plasma levels of soluble EPCR and TM but did not find significant differences between cohorts.

Potential sources of bias or imprecision include the size of the study population, the precision of rFVIIa dosing and times of blood draw, and laboratory analysis. To account for these issues, sample size, rFVIIa dosage and blood sampling times were chosen in orientation to previous pharmacokinetic studies on rFVIIa, yielding expected pharmacokinetic results (Online Supplementary Figure S1F). The OEC A for APC measurement has been extensively assessed. Except for sECPR and sTM the other assays were covered by accreditation with the national accreditation body and were performed according to ISO standards. Moreover, the age and sex distribution, and the body mass index were similar in the different subgroups, ruling out a potential confounding effect of these variables. Finally, one might argue that instead of assessing a genetic hypercoagulable state in patients with unexplained familial thrombosis, an effect of the previous VTE may have been measured, as we did not include asymptomatic family members.

In conclusion, the data indicate that a dysbalanced APC response characterized by increased thrombin formation rates and simultaneously decreased APC formation rates contributes to the increased
thrombotic risk of patients with familial thrombosis. Further studies are now warranted to elucidate the pathophysiological and genetic basis of the described phenotype. Moreover, the data show that the SHAPE procedure is a useful tool to measure the functionality of the PC pathway, which is helpful to investigate prothrombotic mechanisms in patients with thrombophilia without an established risk factor.

REFERENCES


## Table 1. Baseline characteristics and rFVIIa-induced biomarker changes

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls, ( n = 30 )</th>
<th>VTE, FVL or FII 20210G&gt;A, ( n = 28^* )</th>
<th>( P )</th>
<th>VTE, family history of VTE, no RF, ( n = 23 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>35 (21;60)</td>
<td>41 (18.60)</td>
<td>-</td>
<td>38 (20:53)</td>
<td>-</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>12 / 18</td>
<td>12 / 16</td>
<td>-</td>
<td>9 / 14</td>
<td>-</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23 (18;27)</td>
<td>24 (18;27)</td>
<td>-</td>
<td>24 (19;27)</td>
<td>-</td>
</tr>
<tr>
<td>DVT / PE / both, n</td>
<td>-</td>
<td>15 / 3 / 10</td>
<td>-</td>
<td>10 / 6 / 7</td>
<td>-</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>252 (221;284)</td>
<td>262 (250;309)</td>
<td>-</td>
<td>267 (256;331)</td>
<td>-</td>
</tr>
<tr>
<td>FII, %</td>
<td>103 (98;116)</td>
<td>124 (115;135)</td>
<td>( 10^{-5} )</td>
<td>114 (103;120)</td>
<td>-</td>
</tr>
<tr>
<td>Factor XI, %</td>
<td>102 (90;115)</td>
<td>101 (95;107)</td>
<td>-</td>
<td>115 (100;127)</td>
<td>0.044</td>
</tr>
<tr>
<td>Antithrombin, %</td>
<td>107 (100;111)</td>
<td>98 (93;104)</td>
<td>-</td>
<td>100 (98:106)</td>
<td>-</td>
</tr>
<tr>
<td>sTM, ng/mL</td>
<td>1.62 (1.30;2.15)</td>
<td>1.61 (1.47;2.19)</td>
<td>-</td>
<td>1.62 (1.19;1.86)</td>
<td>-</td>
</tr>
<tr>
<td>sEPCR, ng/mL</td>
<td>45.6 (26.0;81.6)</td>
<td>57.0 (35.4;91.0)</td>
<td>-</td>
<td>72.5 (46.4;108.0)</td>
<td>-</td>
</tr>
<tr>
<td>PC, %</td>
<td>106 (97;118)</td>
<td>112 (103;122)</td>
<td>-</td>
<td>105 (97;116)</td>
<td>-</td>
</tr>
<tr>
<td>F1+2, nmol/L</td>
<td>0.16 (0.12;0.21)</td>
<td>0.25 (0.17;0.30)</td>
<td>( 0.002 )</td>
<td>0.15 (0.12;0.20)</td>
<td>-</td>
</tr>
<tr>
<td>AUC, nmol·h/L</td>
<td>0.29 (0.16;0.45)</td>
<td>0.34 (0.23;0.49)</td>
<td>-</td>
<td>0.42 (0.19;0.73)</td>
<td>-</td>
</tr>
<tr>
<td>TAT, ng/mL</td>
<td>&lt;21.2 (&lt;21.3; &lt;21.3)</td>
<td>&lt;21.3 (&lt;21.3;29.7)</td>
<td>-</td>
<td>&lt;21.3 (&lt;21.3;24.7)</td>
<td>-</td>
</tr>
<tr>
<td>AUC, pmol·h/L</td>
<td>35.9 (0.81;109.5)</td>
<td>123.9 (45.1;188.2)</td>
<td>( 0.008 )</td>
<td>141.6 (12.8;332.3)</td>
<td>( 0.021 )</td>
</tr>
<tr>
<td>APC, pmol/L</td>
<td>0.68 (0.40;1.11)</td>
<td>1.13 (0.75;1.43)</td>
<td>( 0.022 )</td>
<td>0.79 (0.39;1.11)</td>
<td>-</td>
</tr>
<tr>
<td>AUC, pmol·h/L</td>
<td>6.55 (5.22;8.82)</td>
<td>15.1 (10.7;22.7)</td>
<td>( &lt;10^{-4} )</td>
<td>9.46 (5.50;14.55)</td>
<td>-</td>
</tr>
</tbody>
</table>

Age and body mass index (BMI) are shown as mean (range), all other variables as median (interquartile range). The area under the curve (AUC) quantifies changes of prothrombin activation fragment F1+2 (F1+2), thrombin-antithrombin complex (TAT), and activated protein C (APC) over 8 hours after i.v. injection of recombinant activated factor VII (rFVIIa). \( P \) describes significant (< 0.05) differences to healthy controls. \( P \) was calculated using the unpaired Student t-test (prothrombin, FII; protein C, PC) or the Mann-Whitney test (all other parameters) and corrected for multiple testing using the Bonferroni method. DVT, deep vein thrombosis; FVL, factor V Leiden; PE, pulmonary embolism; sEPCR, soluble endothelial PC receptor; sTM, soluble thrombomodulin; VTE, venous thromboembolism. *14 heterozygous FII 20210G>A carriers, 1 homozygous and 13 heterozygous FVL carriers, thereof 2 with HR2 haplotype.
**FIGURE LEGENDS**

**Figure 1. Eligibility criteria and study procedures.** Healthy individuals were recruited from blood donors. Patients with a history of venous thromboembolism (VTE) were recruited from the thrombophilia outpatient clinic of our hospital. The study proposal was approved by the Ethics committee of the Medical Faculty of the University Bonn (reference number 016/16). Written informed consent was received prior to participation. All finally included study participants (n = 81) received morning administration of 15 µg/kg recombinant activated factor VII (rFVIIa) as single i.v. bolus injection after overnight fast. Blood samples were drawn immediately before and 10 minutes, 30 minutes, 1, 2, 3, 5, and 8 hours after administration, each from a new venipuncture. After discarding the first 2 mL, blood was drawn into citrate tubes (10.5 mmol/L, Sarstedt, Nümbrecht, DE). Citrate tubes were supplemented with aprotinin (10 µmol/L) and bivalirudin (250 µg/mL) for APC measurement. Plasma samples were obtained by centrifugation (2,600 x g, 10 minutes) within 30 minutes and stored at less than -70°C until assayed. All finally included study participants completed rFVIIa administration and follow-up blood sampling. All collected samples were analyzed. *Surgery, trauma, immobilization, pregnancy, and puerperium. # Transaminases, γ-glutamyl transferase, urea, creatinine in serum. **Decreased plasma levels of antithrombin, protein C, protein S, anti-cardiolipin and anti-β2 glycoprotein I IgG and IgM, functional lupus anticoagulants (activated partial thromboplastin time, dilute Russell viper venom time), and factor V Leiden (FVL) and prothrombin (FII) 20210G>A mutation (except for inclusion into the FVL/FII 20210G>A cohort).

**Figure 2. In vitro thrombin generation and in vivo thrombin-APC response to rFVIIa.** In vitro thrombin generation was measured by the calibrated automated thrombogram (CAT, Thrombinoscope, Maastricht, NL) in healthy controls (n = 30) and in patients with venous thromboembolism (VTE) with factor V Leiden (FVL) or prothrombin (FII) 20210G>A mutation (n = 28), or a family history of VTE without an established risk factor (RF, n = 23). Plasma levels of prothrombin activation fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), and activated protein C (APC) were measured in the same population before (t = 0) and after i.v. injection of 15 µg/kg recombinant activated factor VII (rFVIIa). (A) Endogenous thrombin potential (ETP) measured by CAT, presented as median and interquartile range (IQR, boxes), 1.5 fold IQR (whiskers), and outliers (circles). P values < 0.05 (Mann-Whitney test) are shown. (B) F1+2 and (C) TAT in plasma (median, IQR). (D) Area under the F1+2 generation curve (F1+2 AUC) in comparison to TAT AUC. Dotted lines indicate 90th percentiles of F1+2 AUC and TAT AUC, and 10th percentile of F1+2 AUC in healthy controls. (E) APC in plasma (median, IQR). (F) TAT AUC in comparison to APC AUC. Dotted lines indicate 90th percentiles of TAT AUC and APC AUC, and 10th percentile of APC AUC in healthy controls. The red area highlights the absence of a
thrombin-related increase of APC in patients with unexplained familial thrombophilia (blue symbols). r, Pearson’s correlation coefficient.
Subjects screened for eligibility (n = 94)
- Healthy individuals (n = 42)
- Patients with a history of VTE (n = 52)

**Inclusion criteria**
- Body mass index 18 – 27 kg/m²
- Age 18 – 60 years
- For patients with a history of VTE:
  - VTE at least 6 months ago
  - VTE not in high-risk situation*
  - FVL / FII20210G>A mutation OR
  - VTE in at least one first degree relative
- Eligible but withdrew consent (n = 6)

**Exclusion criteria**
- Antithrombotic medication within two weeks
- Arterial cardiovascular or malignant disease
- Acute or chronic infections
- Pregnancy or breast feeding
- Abnormal hepatic and renal function tests#
- Abnormal thrombophilia screening**
- Ineligible due to laboratory abnormalities (n = 7)

Included in the study (n = 81)

- Healthy individuals (n = 30)
- FVL or FII 20210G>A carriers with a history of VTE (n = 28)
- Personal and family history of VTE without established risk factor (n = 23)

Administration of rFVIIa completed, samples collected and analyzed (n = 81)
Supplementary data to

Impaired *in vivo* activated protein C response rates indicate a thrombophilic phenotype in inherited thrombophilia

SUPPLEMENTARY TABLES

Table S1. Comparison of baseline hemostasis parameters between FVL and FII 20210G>A carriers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FVL, n = 14</th>
<th>FII 20210G&gt;A, n = 14</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, g/L</td>
<td>250 (244;267)</td>
<td>279 (260;345)</td>
<td>0.005</td>
</tr>
<tr>
<td>FII, %</td>
<td>122 (108;135)</td>
<td>125 (121;134)</td>
<td>-</td>
</tr>
<tr>
<td>Factor XI, %</td>
<td>100 (95;101)</td>
<td>105 (96;114)</td>
<td>-</td>
</tr>
<tr>
<td>Antithrombin, %</td>
<td>97 (92;102)</td>
<td>99 (94;108)</td>
<td>-</td>
</tr>
<tr>
<td>sTM, ng/mL</td>
<td>1.49 (1.43;1.80)</td>
<td>1.83 (1.60;2.32)</td>
<td>-</td>
</tr>
<tr>
<td>sEPCR, ng/mL</td>
<td>35.2 (15.4;57.9)</td>
<td>76.1 (36.8;94.8)</td>
<td>-</td>
</tr>
<tr>
<td>Protein C, %</td>
<td>112 (110;120)</td>
<td>110 (98;126)</td>
<td>-</td>
</tr>
<tr>
<td>Thrombin, pmol/L</td>
<td>&lt;0.46 (&lt;0.46;0.64)</td>
<td>&lt;0.46 (&lt;0.46;0.87)</td>
<td>-</td>
</tr>
<tr>
<td>F1+2, nmol/L</td>
<td>0.21 (0.13;0.33)</td>
<td>0.27 (0.21;0.29)</td>
<td>-</td>
</tr>
<tr>
<td>TAT, ng/mL</td>
<td>&lt;21.3 (&lt;21.3;&lt;21.3)</td>
<td>28.1 (&lt;21.3;38.7)</td>
<td>-</td>
</tr>
<tr>
<td>APC, pmol/L</td>
<td>1.32 (1.04;1.63)</td>
<td>0.85 (0.50;1.15)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

*P* describes significant (< 0.05) differences between factor V Leiden (FVL) and prothrombin (FII) 20210G>A carriers with a history of venous thromboembolism and was calculated using the unpaired Student t-test (FII, protein C) or the Mann-Whitney test (all other parameters). APC, activated protein C; F1+2, prothrombin activation fragment 1+2; sEPCR, soluble endothelial PC receptor; sTM, soluble thrombomodulin; TAT, thrombin-antithrombin complex.
**SUPPLEMENTARY FIGURES**

**Figure S1. In vitro thrombin generation and kinetics of rFVIIa in plasma.** In vitro thrombin generation was measured by the calibrated automated thrombogram (CAT) in healthy controls (n = 30) and in patients with venous thromboembolism (VTE) with factor V Leiden (FVL) or prothrombin (FII) 20210G>A mutation (n = 28), or a family history of VTE without an established risk factor (RF, n = 23). Plasma levels of thrombin-antithrombin complex (TAT) were measured in the same population before (t = 0) and after i.v. injection of 15 µg/kg recombinant activated factor VII (rFVIIa). (A) Peak thrombin concentration, (B) lag time and (C) time to peak measured by CAT, presented as median and interquartile range (IQR, boxes), 1.5 fold IQR (whiskers), and outliers (circles). P values < 0.05 (Mann-Whitney test) are shown. (D) Endogenous thrombin potential (ETP, 1 pmol/L tissue factor) in comparison to the area under the curve (AUC) of TAT formation in healthy controls and (E) patients with VTE. Dotted lines indicate 90th percentiles of ETP and TAT AUC, and 10th percentile of ETP in healthy controls. (F) Activated factor VII (FVIIa) in plasma (median, IQR).
Figure S2. rFVIIa-induced thrombin/ APC response in FVL and FII 20210G>A carriers. Plasma levels of (A) prothrombin activation fragment 1+2 (F1+2), (B) thrombin-antithrombin complex (TAT), and (C) activated protein C (APC) were measured before (t = 0) and after i.v. injection of 15 μg/kg recombinant activated factor VII (rFVIIa) in factor V Leiden carriers (FVL, n=14, red symbols) or FII 20210G>A carriers (n = 15, blue symbols).
Supplementary data to

Impaired *in vivo* activated protein C response rates indicate a thrombophilic phenotype in inherited thrombophilia

SUPPLEMENTARY TABLES

Table S1. Comparison of baseline hemostasis parameters between FVL and FII 20210G>A carriers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FVL, n = 14</th>
<th>FII 20210G&gt;A, n = 14</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, g/L</td>
<td>250 (244;267)</td>
<td>279 (260;345)</td>
<td>0.005</td>
</tr>
<tr>
<td>FII, %</td>
<td>122 (108;135)</td>
<td>125 (121;134)</td>
<td>-</td>
</tr>
<tr>
<td>Factor XI, %</td>
<td>100 (95;101)</td>
<td>105 (96,114)</td>
<td>-</td>
</tr>
<tr>
<td>Antithrombin, %</td>
<td>97 (92;102)</td>
<td>99 (94;108)</td>
<td>-</td>
</tr>
<tr>
<td>sTM, ng/mL</td>
<td>1.49 (1.43;1.80)</td>
<td>1.83 (1.60;2.32)</td>
<td>-</td>
</tr>
<tr>
<td>sEPCR, ng/mL</td>
<td>35.2 (15.4;57.9)</td>
<td>76.1 (36.8;94.8)</td>
<td>-</td>
</tr>
<tr>
<td>Protein C, %</td>
<td>112 (110;120)</td>
<td>110 (98;126)</td>
<td>-</td>
</tr>
<tr>
<td>Thrombin, pmol/L</td>
<td>&lt;0.46 (&lt;0.46;0.64)</td>
<td>&lt;0.46 (&lt;0.46;0.87)</td>
<td>-</td>
</tr>
<tr>
<td>F1+2, nmol/L</td>
<td>0.21 (0.13;0.33)</td>
<td>0.27 (0.21;0.29)</td>
<td>-</td>
</tr>
<tr>
<td>TAT, ng/mL</td>
<td>&lt;21.3 (&lt;21.3;&lt;21.3)</td>
<td>28.1 (&lt;21.3;38.7)</td>
<td>-</td>
</tr>
<tr>
<td>APC, pmol/L</td>
<td>1.32 (1.04;1.63)</td>
<td>0.85 (0.50;1.15)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

*P* describes significant (< 0.05) differences between factor V Leiden (FVL) and prothrombin (FII) 20210G>A carriers with a history of venous thromboembolism and was calculated using the unpaired Student t-test (FII, protein C) or the Mann-Whitney test (all other parameters). APC, activated protein C; F1+2, prothrombin activation fragment 1+2; sEPCR, soluble endothelial PC receptor; sTM, soluble thrombomodulin; TAT, thrombin-antithrombin complex.
**SUPPLEMENTARY FIGURES**

**Figure S1. In vitro thrombin generation and kinetics of rFVIIa in plasma.** *In vitro* thrombin generation was measured by the calibrated automated thrombogram (CAT) in healthy controls (*n* = 30) and in patients with venous thromboembolism (VTE) with factor V Leiden (FVL) or prothrombin (FII) 20210G>A mutation (*n* = 28), or a family history of VTE without an established risk factor (RF, *n* = 23). Plasma levels of thrombin-antithrombin complex (TAT) were measured in the same population before (*t* = 0) and after i.v. injection of 15 µg/kg recombinant activated factor VII (rFVIIa). (A) Peak thrombin concentration, (B) lag time and (C) time to peak measured by CAT, presented as median and interquartile range (IQR, boxes), 1.5 fold IQR (whiskers), and outliers (circles). *P* values < 0.05 (Mann-Whitney test) are shown. (D) Endogenous thrombin potential (ETP, 1 pmol/L tissue factor) in comparison to the area under the curve (AUC) of TAT formation in healthy controls and (E) patients with VTE. Dotted lines indicate 90th percentiles of ETP and TAT AUC, and 10th percentile of ETP in healthy controls. (F) Activated factor VII (FVIIa) in plasma (median, IQR).
Figure S2. rFVIIa-induced thrombin/APC response in FVL and FII 20210G>A carriers. Plasma levels of (A) prothrombin activation fragment 1+2 (F1+2), (B) thrombin-antithrombin complex (TAT), and (C) activated protein C (APC) were measured before (t = 0) and after i.v. injection of 15 μg/kg recombinant activated factor VII (rFVIIa) in factor V Leiden carriers (FVL, n=14, red symbols) or FII 20210G>A carriers (n = 15, blue symbols).