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[#] and Heiko Rühl[#]
Institute of Experimental Hematology and Transfusion Medicine, University Hospital Bonn, Bonn, Germany
[*]SR and NS contributed equally as co-first authors
[#]BP and HR contributed equally as co-senior authors
Correspondence: HEIKO RÜHL - Heiko.Ruehl@ukbonn.de
doi:10.3324/haematol.2021.280573

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

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

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

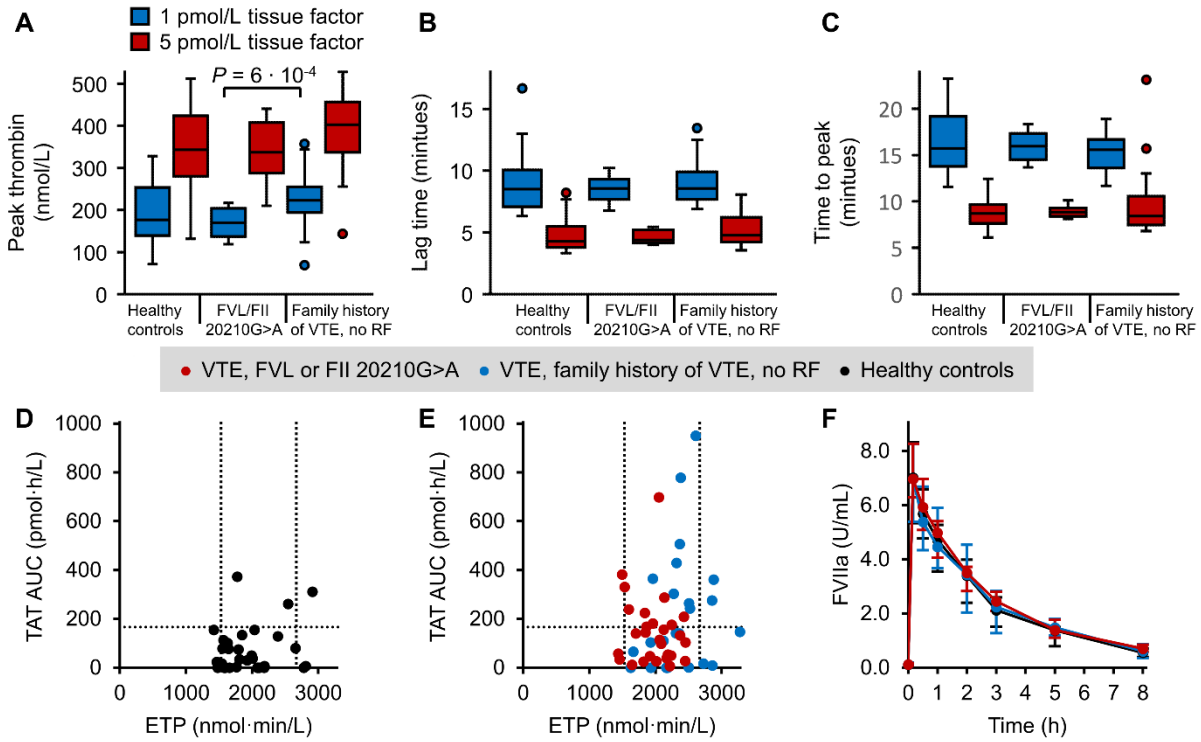

Figure S1. *In vitro* thrombin generation and kinetics of rFVIIa in plasma. *In vitro* thrombin generation was measured by the calibrated automated thrombogram (CAT) in healthy controls (n = 30) and in patients with venous thromboembolism (VTE) with factor V Leiden (FVL) or prothrombin (FII) 20210G>A 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 outlyers (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

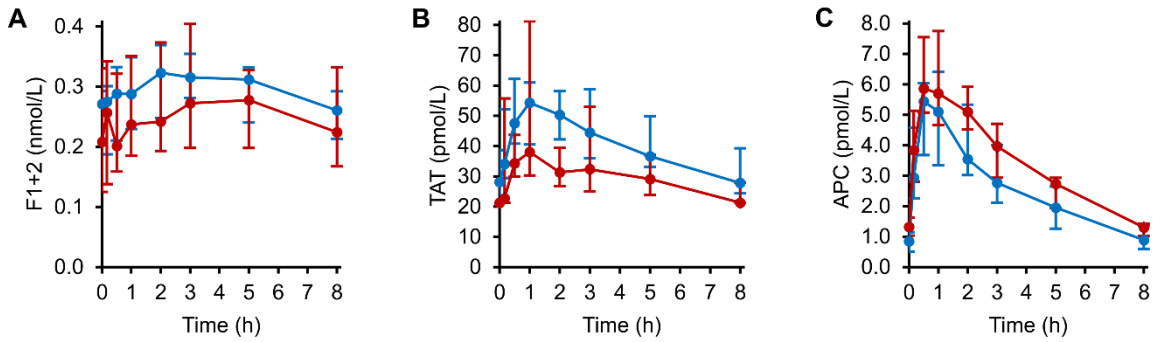

Figure S2. rFVIIa-induced thrombin/APC response in FVL and FII 20210G>A carriers. Plasma levels of (**A**) prothrombin activation fragment 1+2 (F1+2), (**B**) thrombin-antithrombin complex (TAT), and (**C**) activated protein C (APC)were measured before (t = 0) and after i.v. injection of 15 µg/kg recombinant activated factor VII (rFVIIa) in factor V Leiden carriers (FVL, n=14, red symbols) or FII 20210G>A carriers (n = 15, blue symbols).