

## Similar hypercoagulable state and thrombosis risk in type I and type III protein S-deficient individuals from families with mixed type I/III protein S deficiency

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### Supplementary Design and Methods

#### Demographic and clinical characteristics of the study population

Two hundred and forty-two individuals belonging to 30 families with PS deficiency (28 with pure type I deficiency or mixed type I/III deficiency and 2 with pure type III deficiency) were investigated. Of the 30 probands, 18 presented with type I deficiency and 12 with type III deficiency (age at inclusion  $46.8 \pm 11.9$  versus  $52.5 \pm 15.1$  years,  $P = \text{n.s.}$ ). The age at the first thrombotic event was  $30 \pm 10.4$  years (median, 28.5 years) for probands with type I deficiency and  $36.6 \pm 15.5$  years (median, 33.5 years) for probands with type III deficiency ( $P = \text{n.s.}$ ). Out of 212 family members, 112 had normal PS levels and 100 were PS-deficient (55 type I and 45 type III). The age of family members at the time of inclusion was  $46.9 \pm 20.2$  years for normal individuals,  $48.5 \pm 19.8$  years for type I deficient individuals ( $P = \text{n.s.}$ ) and  $56.9 \pm 15.9$  years for type III deficient individuals ( $P = 0.037$  versus normal individuals). At the time of inclusion, thrombotic events in family members had occurred in 17/55 (30.9%) type I deficient subjects, 19/45 (42.2%) type III deficient subjects and in 7/112 (6.2%) normal subjects. The mean age at the first thrombotic event was  $40.6 \pm 16.7$  years (median, 42 years) in type I deficient individuals,  $42.6 \pm 16.7$  years (median, 45 years) in type III deficient individuals and  $47 \pm 23.4$  years (median, 41 years) in normal individuals ( $P = \text{n.s.}$ ).

#### Determinants of the endogenous thrombin potential measured in the absence and presence of activated protein C

Age ( $B = 4.4$  nM.min/year,  $P = 0.001$ ) and prothrombin level ( $B = 129.3$  nM.min/10%,  $P < 0.001$ ) were both found to be strong determinants of the ETP<sub>-APC</sub>, which accounts for the higher ETP<sub>-APC</sub> in type III deficient individuals. Similarly, age ( $B = 6.4$  nM.min/year,  $P < 0.001$ ) and prothrombin ( $B = 89.4$  nM.min/10%,  $P < 0.001$ ) were positive determinants of the ETP<sub>+APC</sub>, whereas free PS ( $B = -58.6$  nM.min/10%,  $P = 0.028$ ) and TFPI ( $B = -16.5$  nM.min/10%,  $P = 0.009$ ) were negative determinants. This explains the absence of a difference in ETP<sub>+APC</sub> between type I and type III deficient individuals. In fact, type III deficient individuals would be expected to have a lower ETP<sub>+APC</sub> than type I deficient individuals because of their higher free PS and TFPI levels, but their concomitant higher age and prothrombin level tend to abolish this difference, making the ETP<sub>+APC</sub> similar in the two groups.

#### Determinants of thrombin peak measured in the absence and presence of antibodies against protein S or tissue factor pathway inhibitor

Peak height in the absence of antibodies was dependent not only on free PS ( $B = -13.4$  nM/10%,  $P = 0.021$ ) and TFPI ( $B = -5.7$  nM/10%,  $P < 0.001$ ), but also on age ( $B = 0.8$  nM/year,  $P = 0.035$ ) and prothrombin ( $B = 8.3$  nM/10%,  $P = 0.023$ ), thereby accounting for the equally elevated peaks in type I and type III deficient individuals. The higher peak<sub>+aPS</sub> of type I deficient individuals was attributable their lower TFPI levels ( $B = -6.8$  nM/10%,  $P = 0.002$ ), whereas the higher age ( $B = 1.1$  nM/year,  $P < 0.001$ ) and prothrombin level ( $B = 17.5$  nM/10%,  $P < 0.001$ ) of type III deficient individuals were responsible for their elevated peak<sub>+aTFPI</sub>.

**Online Supplementary Table S1. Thrombin generation parameters (median and interquartile range) at 6.8 pM TF±APC.**

	N.*	Lag time (min)	-APC Peak (nM)	ETP (nM.min)	Lag time (min)	+APC Peak (nM)	ETP (nM.min)	nAPCs <sub>r</sub>
Normal	49	2.55 2.25-3.03	295 246-337	1065 890-1183	2.69 2.50-3.22	50.4 33.0-79.8	211 143-347	1.84 1.33-2.59
PS-deficient	75	2.36 2.15-2.47	330 308-357	1114 992-1227	2.50 2.25-2.69	207 161-253	784 650-902	6.14 4.90-6.82
p (U)		<0.001	0.003	0.207	0.001	<0.001	<0.001	<0.001
Type I	56	2.36 2.15-2.47	322 303-341	1053 963-1150	2.54 2.25-2.88	213 170-249	778 673-877	6.34 5.16-6.96
Type III	19	2.25 2.02-2.55	359 321-387	1241 1141-1314	2.35 2.25-2.58	191 161-270	795 623-979	5.51 3.65-6.30
p (U)		0.678	0.005	<0.001	0.394	0.697	0.784	0.057

\* FV Leiden carriers were excluded. ETP, endogenous thrombin potential. U, Mann-Whitney-Wilcoxon test.

**Online Supplementary Table S2. Thrombin generation parameters (median and interquartile range) at 1.36 pM TF ± αPS or αTFPI**

	N.*	No antibodies			+αPS			+αTFPI			PS-ratio	TFPI-ratio
		Lag time (min)	Peak (nM)	ETP (nM.min)	Lag time (min)	Peak (nM)	ETP (nM.min)	Lag time (min)	Peak (nM)	ETP (nM.min)		
Normal	49	3.82 3.05-5.12	42.5 31.5-81.7	528 398-654	3.08 2.77-3.66	112 77.0-167	582 510-694	2.73 2.59-3.15	189 158-215	589 529-662	0.47 0.31-0.67	0.23 0.18-0.39
PS-deficient	75	2.98 2.70-3.33	81.3 59.4-115	576 521-662	2.77 2.51-2.98	121 95.1-150	549 514-619	2.63 2.33-2.94	193 177-220	605 521-670	0.73 0.57-0.85	0.42 0.31-0.57
p (U)		<0.001	<0.001	0.019	<0.001	0.541	0.297	0.018	0.347	0.884	<0.001	<0.001
Type I	56	2.98 2.70-3.36	81.9 58.7-118	568 512-658	2.77 2.51-3.00	121 105-147	538 493-602	2.63 2.45-2.94	189 174-210	586 515-627	0.75 0.58-0.87	0.45 0.33-0.60
Type III	19	3.20 2.63-3.27	81.3 59.4-110	621 571-742	2.77 2.51-2.81	111 80.7-178	576 541-821	2.50 2.33-2.63	215 193-255	664 635-810	0.67 0.54-0.78	0.34 0.29-0.48
p (U)		0.826	0.559	0.050	0.859	0.683	0.007	0.127	0.010	<0.001	0.192	0.034

\* FV Leiden carriers were excluded. ETP, endogenous thrombin potential. U, Mann-Whitney-Wilcoxon test.