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Late onset thrombosis in a case of severe protein S deficiency due to compound heterozygosity for PROS1 mutations

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Keywords

protein S; protein S deficiency; venous thrombosis

Dear sir,

Plasma protein S is an anticoagulant whose heterozygous genetic deficiency is associated with increased risk of venous thrombosis and juvenile stroke [1-3]. Several families were described with individuals who were homozygous or compound heterozygous for protein S defects and severely protein S-deficient. These families were identified from Thailand, Japan, China, and Turkey, and in two African-American children. Nine individuals presented as neonates with life-threatening thrombotic purpura fulminans, and required life-long treatment with a source of protein S and/or with anticoagulants (heparin or warfarin) [4-8]. Yet remarkably, several other severely protein S-deficient individuals did not experience a serious thrombotic event until ages of 10-20 years old, and one individual with 18% total protein S antigen was asymptomatic at age 23 years [2,9,10]. Thrombosis in these young adults was recurrent, including deep vein thrombosis, pulmonary embolism, mesenteric vein thrombosis, sagittal sinus thrombosis, and ankle ulcerations. Here we describe another severely deficient individual in an attempt to advance understanding of the variable phenotypes.

The proband, a 23 year old Filipino male in California, experienced multiple venous thrombosis episodes, beginning with lower limb thrombosis at age 10. Record of duration of anticoagulant treatment following that event was unavailable. At age 20, he underwent ileal resection and ileostomy as a result of mesenteric vein thrombosis. He was treated sequentially with heparin, warfarin, and then subcutaneous heparin. At age 21, he experienced a small left fronto-parietal subcortical hemorrhage following head trauma while on 2,000 units of heparin subcutaneously daily. He recovered without significant neurologic residua. Physical examination at age 23 was remarkable only for scars from his ileal surgery and for chronic bilateral punctuate erythematous lesions on the dorsum of each foot. Subcutaneous heparin (7,500 units every 12h) was resumed until a physician could be located near his home city to administer warfarin under close supervision.

The patient and his sister reported that several family members residing in the Philippines died of cardiovascular events, including their mother at age 50, a sister at age 35, and a brother at age 20. Two sisters, ages 31 and 29 years at the time of referral, had experienced recurrent venous thrombosis and were receiving warfarin therapy. Another sister, age 38 and a brother, age 25, residing in the Philippines, had no history of thromboembolic disease. A sister in California was asymptomatic at the time of the study (at age 37), but she later suffered recurrent venous thrombosis and is now receiving warfarin.

Before heparin treatment was resumed for the propositus, blood samples anticoagulated with trisodium citrate were taken from him, his sister in California, and this sister's five children. Informed consent was obtained. The patient's fibrinogen level was 177, hemoglobin/hematocrit 14.9/45.1, sedimentation rate 1, white blood count 7.4, platelet count 280,000, prothrombin time 12.1/11.6 s, APTT 27.9/26.1 s, factor X 115%, antithrombin 92%, and protein C 108%. The patient's sister had factor X and protein C levels of 99% and 95%, respectively.

Multiple protein S-related parameters were obtained (Table 1). The propositus (I-1) had barely detectable levels of free protein S or of anticoagulant cofactor activity for activated protein C, while his sister (I-2) and four of her five children had low levels of each of these. Total protein S was 10% of normal for the propositus, while his sister and two of her children had low total protein S, and two of her children had low-normal levels (normal range, 70-130%). Protein S anticoagulant activity in the absence of activated protein C (termed "PS-direct") was consistent with other protein S parameters. The APC/protein C activity ratio was twice the normal level only for the propositus, possibly indicating above normal levels of thrombin generation and protein C activation, consistent with a mildly elevated level of thrombin-antithrombin complexes (5.6 µg/L). The normalized APC resistance ratio was low-normal for subject II-1, for reasons that were not apparent.

To identify mutations in the *PROS1* gene, DNA was sequenced from all subjects except II-5, for all exons and exon/intron junctions and for part of the promoter region [11]. A novel mutation was identified in one allele of all subjects who had low free protein S, namely a deletion of 14 base pairs in intervening sequence L, -10 to -24 upstream of exon 13. This mutation probably impairs splicing of *PROS1* pre-mRNA, which usually requires a cis-acting element 10 to 30 bases upstream from the acceptor site. The mutation is in the putative branch point of intron L.

The propositus, but not the other subjects studied, carried a second novel mutation, namely a C→T mutation at -168 in the promoter region, in an Sp1 binding site. This mutation is in a promoter region that is highly conserved among species, and is within an indispensable Sp1 transcription regulation site located at nt -178 to -165 [12]. Presumably this compound heterozygosity for *PROS1* mutations caused the observed severe deficiency and recurrent thrombosis in the propositus.

The propositus in this study is one of only a few known individuals with severe protein S deficiency who remained free of thrombosis until age 10 or later and who survived into adulthood. He may carry a genetic polymorphism(s) that partly compensates for the increased thrombotic risk caused by low protein S levels.

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Table 1

Characteristics of the protein S-deficient family

	Sex/Age	Cofactor Activity ¹	% PS total ²	% PS free ³	% PS free ⁴	PS-direct ⁵	% C4BP ²	APC Resistance ⁶
I-1	M/23	< 7	10 ± 2	0.2 ± 0	2	1.12	74	1.06
I-2	F/37	28 ± 17	52 ± 10	5.4 ± 1	16	1.24	95	1.20
II-1	M/12	42 ± 8	67 ± 11	12 ± 2	27	NT ⁷	127	0.82
II-2	M/11	38 ± 8	70 ± 8	16 ± 9	27	1.30	82	1.10
II-3	F/5	128 ± 2	125 ± 19	> 78	109	1.73	117	1.01
II-4	M/3	32 ± 4	47 ± 4	13 ± 1	15	NT	64	NT
II-5	F/1.2	26 ± 8	70 ± 7	9 ± 2	18	NT	140	0.96

* Propositus

⁷ Not tested

¹ Staclot S kit, Diagnostica Stago.

² Elisa of plasma [13].

³ Elisa of polyethylene glycol supernatant of plasma [13].

⁴ Asserachrom free protein S antigen Elisa, Diagnostica Stago.

⁵ Ratio of factor Xa-1-stage clotting times determined without/with anti-protein S antibodies [13]; normal range, 1.3-1.9.

⁶ APC (activated protein C) resistance: normalized ratio of APTT determined in the presence/absence of APC for test plasma diluted in factor V-deficient plasma; normal range, 0.8-1.2 [14].