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PROS1 mutation in a Dutch patient suffering from thrombosis

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Introduction

PROS1 encodes protein S (PS), a vitamin K-dependent plasma protein that serves as cofactor for activated protein C and inhibits blood clotting. Mutations in *PROS1* may lead to PS deficiency and are often associated with increased risk of thrombosis.
Casus: a thrombophilia patient presented at the Jeroen Bosch Hospital with a family history of deep venous thrombosis and pulmonary embolism. General thrombophilia tests indicated type III PS deficiency. To elucidate the cause of the deficiency we sequenced *PROS1*. After identification of a *Val467Gly* mutation, an RFLP test was designed to screen family members of the patient and a group of 155 control individuals.

Materials and methods

Total and free PS antigen and PS activity: Total and free PS antigen and PS activity were assessed using plasma assays (Diagnostica Stago, Asnieres, France).
RNA isolation, cDNA synthesis and analysis: Platelet Rich Plasma (PRP) was prepared from 2x10 ml EDTA blood by centrifugation at 125 g for 10'. Supernatant was further processed. Platelets were collected by centrifugation at 1800 g for 15'. RNA was extracted and DNAse I treated using the Qiagen RNA blood kit (Qiagen Benelux B.V., Venlo, The Netherlands) according to manufactures instructions and eluted in 50 µl H_2O. Protein S cDNA was synthesized from 10 µl RNA-solution using gene specific primers (2) and Superscript II kit (Invitrogen, Breda, The Netherlands). Two overlapping fragments covering the entire coding sequence were amplified and sequenced (2). CD-525 and CD-2129 contained typing errors and were rectified: CD-525: 5' GAT GGA AAA GCT TCT TTT AC (Genbank GI: 36578) and CD-2129: 5' CCA AAC TGA TGG ACA TGA GTG AGC TCT (Genbank GI:36578). The PCR was performed in 25 µl containing 20 mM Tris-HCl pH=8.4, 50 mM KCl, 200 µM dNTP's, 0.5 µM primers, 1.5 mM $MgCl_2$ (fragment 1) or 3 mM $MgCl_2$ (fragment 2), cDNA (3µl) and 1 Unit of Platinum Taq Polymerase (invitrogen). Amplification conditions were: 94°, 10:00; 36 cycles of 94° 1:00; 50°C, 1:00; 72°C, 2:00, followed by a final extension step at 72°C for 7 minutes.

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PCR products were analysed on a 1.5% agarose gel in 1x TBE, cut out and purified using the Qiaquick gel Extraction kit (Qiagen). Purified products were sequenced commercially (Baseclear Labservices, Leiden, The Netherlands).
DNA isolation and analysis: Genomic DNA was isolated from 200 µl EDTA blood using the Qiagen DNA blood kit (Qiagen) according to manufactures instructions and diluted to a final concentration of 10 ng/ul. Exon 13 of the PROS1 gene was amplified using gene specific primers (2). The PCR was performed as described above with 3 mM $MgCl_2$ and 50 ng DNA template. Amplification conditions were: 94°, 10:00; 38 cycles of 94° 1:00; 48°C, 0:45; 72°C, 1:00, followed by a final extension step at 72°C for 7 minutes. PCR products were analysed on a 1.5% agarose gel in 1X TBE and sequenced commercially (Baseclear Labservices).
RFLP Val467Gly: Exon 13 of the *PROS1* gene was amplified as described above. PCR products were digested with 1 Unit *Nsp*I for 2 hours at 37°C and analysed on a 1.5% agarose gel in 1X TBE. The 280 bp PCR product was cut in 196 bp and 84 bp bands in the absence of *Val467Gly*, and remained intact in the presence of the *Val467Gly* mutation.

Results and discussion

The proband's total PS (antigen) was slightly lowered (76%), while his PS activity was severely lowered (26%; Family member 8 in Table 1). This type III PS

Table 1. Family member number (FM nr), thrombosis history, genotype and protein S parameters of the proband (FM nr 8) and his brothers and sisters. All *Val467Gly* heterozygous individuals have low protein S activity. *Spontaneous thrombosis events. ND: not done.

FM nr	Thrombosis event	Genotype *Val467Gl*	Total protein S (antigen) %	Free protein S (antigen) %	Protein S activity %
1	+	Wild type	96	100	96
2	+	Heterozygous	70	25	33
3	–	Wild type	87	106	ND
4	–	Heterozygous	54	23	34
5	+*	Heterozygous	65	21	24
6	–	Wild type	87	99	ND
7	+*	Heterozygous	74	27	36
8	+*	Heterozygous	76	ND	26
9	–	Wild type	96	93	95

deficiency prompted us to sequence *PROS1*. Both cDNA -prepared from mRNA obtained from thrombocytes- and genomic DNA were sequenced. We took this approach because non-coding mutations may remain undetected when analysing cDNA, while the analysis of genomic DNA is complicated by the presence of the pseudogene *PROS2* and the large size of *PROS1* (>100 kb). wOnly a single mutation (*Val467Gly*; Figure 1A) was found in the entire coding region of *PROS1* cDNA. The sequence results obtained from genomic DNA (exon 13) confirmed the heterozygous presence of this mutation (Figure 1A). The intensity of the sequence signal of A (wild type) and C (mutant) in cDNA (Figure 1A) was roughly equivalent, indicating similar transcription from both alleles. Of the 8 brothers and sisters of the proband, 4 carried the *Val467Gly* mutation. All the *Val467Gly* carriers had a type I or type III PS deficiency (Table 1), 4 of them with a history of deep venous thrombosis or pulmonary embolism (Figure 1B and 1C). In a control group of 155 individuals the *Val467Gly* mutation was absent.

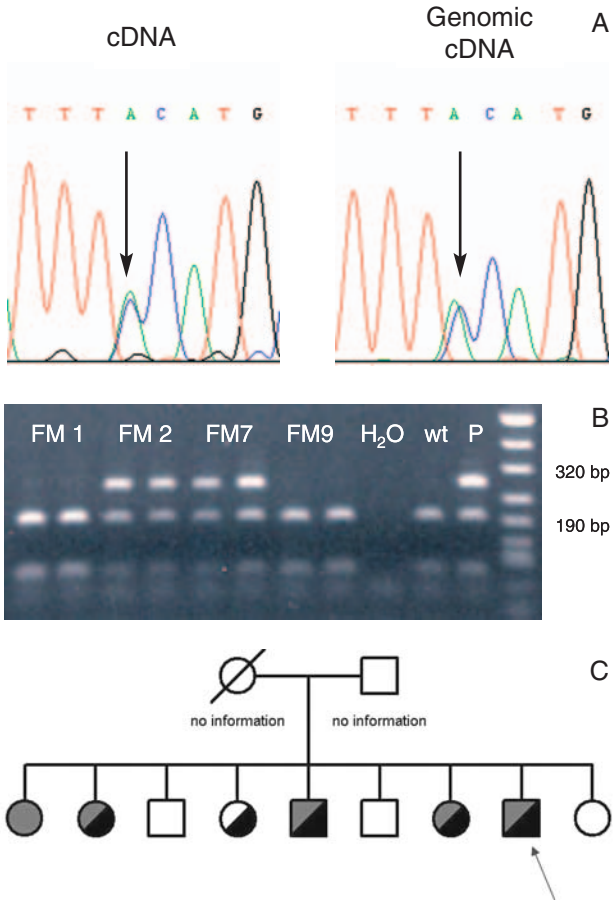

Figure 1. (A) Antisense sequence of cDNA and genomic DNA, exon 13. Arrows indicate the *Val467Gly* mutation. (B) RFLP analysis for the *Val467Gly* mutation. Various family members (FM) in duplo, a H_2O control, a wild type control (wt), the proband (P) and a molecular weight marker. (C) Pedigree of the proband and his family. Black areas indicate heterozygosity for the *Val467Gly* mutation. Gray areas indicate a history of deep venous thrombosis or pulmonary embolism. Arrow denotes the proband. (With cordial thanks to J.A. Ropela.)

We have identified a *Val467Gly* mutation in the *PROS1* gene in a family with PS deficiency and a history of deep venous thrombosis and pulmonary embolism. The same mutation has been described before in two seemingly unrelated patients with PS deficiency (1). Both in our pedigree, and in the one pedigree that was analyzed by Gomez et al. (1), the mutation inherited with the PS deficiency.

The affected amino acid is located in a beta strand in the interior of the PS molecule. Substitution of this valine by the smaller glycine may create a small cavity that might cause misfolding of the molecule. This could affect PS activity and/or binding to C4BP.

Because of the above, and the absence of the *Val467Gly* mutation in 155 control individuals, we believe that in the family described here, the *Val467Gly* mutation may well be responsible for the PS deficiency.

References

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