*Disorders of Hemostasis*

## Identification of a novel *PROS1* c.1113T→GG frameshift mutation in a family with mixed type I/type III protein S deficiency

**We report a family with type I and type III protein S (PS) deficiency, which showed to be phenotypic variants of the same genetic disease. Direct sequencing analysis of the *PROS1* gene was performed to establish the genotype. The ratio of protein C antigen and total PS antigen levels (protein C/S ratio) was used to classify subjects at risk of venous thromboembolism. All PS deficient subjects had increased protein C/S ratios as well as a novel *PROS1* c.1113T→GG frameshift mutation.**

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Hereditary protein S (PS) deficiency predisposes to venous thromboembolism and is probably the most difficult thrombophilic disorder to diagnose. As a result of overlapping PS levels in carriers and non-carriers of PS gene mutations,[1] and variations of levels related to age,[2] sex,[3] hormonal state[3] and acquired conditions,[4] subjects can easily be misclassified by measuring PS levels. While total PS assays have proven to be precise and accurate, free PS and activated protein C cofactor activity assays may suffer from poor reproducibility and a high rate of false-positive results, respectively.[5] Further, it has been suggested that type I and type III PS deficiency within one family are phenotypic variants of the same genetic disease, due to an increase of total PS levels with age.[6] As both protein C (PC) and total PS levels increase with age, we speculated that the ratio of PC and total PS levels (protein C/S ratio) might be valuable to classify carriers of PS gene mutations. Previously we confirmed this in a cohort of PC deficient families. The ratio proved to be more sensitive at identifying carriers of PC gene mutations than were PC plasma levels alone.[7]

Here we describe a family with mixed type I/ type III PS deficiency. The protein C/S ratio was used to identify subjects at risk of venous thromboembolism due to PS deficiency. DNA direct sequencing analysis was performed to establish the genotype after informed consent had been obtained. Normal ranges of protein C/S ratios are 0.77-1.36 in women, not using oral contraceptives or pregnant; 0.94-1.94 in women on oral contraceptives; and 0.69-1.40 in men.[7] The 15 exons of the PS gene, *PROS1*, together with part of the flanking introns, were amplified by polymerase chain reaction (PCR). The primers used for both the PCR and sequencing have been previously described.[8]

Characteristics and results are summarized in Table 1. Of 21 tested relatives, ten had type I PS deficiency and three had type III deficiency. Total PS levels in type I deficient relatives ranged from 38% to 63%, in type III deficient relatives from 68% to 72%, and in non-deficient relatives from 93% to 150%. Venous thromboembolism had occurred in four of the ten type I deficient relatives and in all three type III deficient relatives (overall 54%), but in none of eight non-deficient relatives. After sequence analysis of all 15 exons of *PROS1*, including the intron/exon boundaries, a novel c.1113T→GG frameshift mutation in exon 10, codon 282, was identified (Figure 1a). This mutation introduces a premature stop codon at c.1127-1129 (TGA), resulting in a truncated PS molecule. This mutation was demonstrated in all type I and type III PS deficient relatives, but in none of the non-deficient relatives. All type I and type III PS deficient relatives had increased protein C/S ratios, the means

**Table 1.** Family characteristics.

| *ID* | *Sex* | *Age (yr)* | *PS deficiency* | *VTE episodes, n* | *Age at 1st VTE (yr)* | *TPS (%)* | *p* | *FPS (%)* | *p* | *PC (%)* | *Protein C/S ratio* | *p* | *PROS1 mutation* | *Concomitant thrombophilic defects* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| II:4 | M | 75 | type I | 0 | - | 58 (↓) | | 10 | | 93 | 1.60 (↑) | | yes | - |
| II:7 | F | 71 | type I | 2 | 26 | 63 (↓) | | 16 | | 140 | 2.22 (↑) | | yes | FVIII (↑), HH |
| II:9 | F | 69 | type I | 2 | 52 | 57 (↓) | | 14 | | 113 | 1.98 (↑) | | yes | APC-resistance |
| III:1 | F | 48 | type I | 1 | 29 | 38 (↓)* | | 13 * | | 102 * | 2.68 (↑)* | | yes | APC-resistance |
| III:2 | M | 46 | type I | 1 | 44 | 47 (↓) | | 2 | | 79 | 1.68 (↑) | | yes | FVIII (↑) |
| III:5 | F | 49 | type I | 0 | - | 62 (↓) | | 10 | | 98 | 1.58 (↑) | | yes | - |
| III:7 | M | 47 | type I | 0 | - | 60 (↓) | | 18 | | 125 | 2.08 (↑) | | yes | APC-resistance |
| III:9 | F | 44 | type I | 0 | - | 61 (↓) | | 10 | | 96 | 1.57 (↑) | | yes | - |
| IV:6 | M | 23 | type I | 0 | - | 55 (↓) | | 18 | | 97 | 1.76 (↑) | | yes | - |
| IV:7 | M | 16 | type I | 0 | - | 53 (↓) | | 13 | | 75 | 1.42 (↑) | | yes | - |
| Mean | - | 49 | type I | - | 38 | 55 (↓) | 0.01$ | 12 | 0.93$ | 102 | 1.86 (↑) | 0.24$ | yes | - |
| II:3 | M | 81 | type III | 1 | 42 | 68 (↔) | | 15 | | 96 | 1.41 (↑) | | yes | FVIII (↑), FIX (↑), HH |
| III:14 | M | 34 | type III | 2 | 34 | 72 (↔) | | 10 | | 117 | 1.63 (↑) | | yes | - |
| IV:3 | M | 29 | type III | 1 | 29 | 71 (↔) | | 13 | | 114 | 1.61 (↑) | | yes | - |
| Mean | - | 48 | type III | - | 35 | 70 (↔) | 0.01# | 13 | 0.01# | 109 | 1.55 (↑) | 0.01# | yes | - |
| III:3 | F | 43 | no | 0 | - | 112 (↔)* | | 98* | | 139* | 1.24 (↔)* | | no | FVIII (↑) |
| III:10 | M | 41 | no | 0 | - | 131 (↔) | | 83 | | 110 | 0.84 (↔) | | no | FVIII (↑) |
| III:11 | F | 35 | no | 0 | - | 93 (↔) | | 70 | | 92 | 0.99 (↔) | | no | - |
| III:12 | M | 44 | no | 0 | - | 121 (↔) | | 113 | | 120 | 0.99 (↔) | | no | FIX (↑), FXI (↑) |
| III:13 | M | 42 | no | 0 | - | 116 (↔) | | 110 | | 109 | 0.94 (↔) | | no | FIX (↑) |
| III:15 | F | 36 | no | 0 | - | 93 (↔) | | 85 | | 95 | 1.02 (↔) | | no | - |
| III:16 | M | 42 | no | 0 | - | 139 (↔) | | 85 | | 103 | 0.74 (↔) | | no | - |
| IV:4 | M | 26 | no | 0 | - | 150 (↔) | | 95 | | 130 | 0.87 (↔) | | no | - |
| Mean | - | 39 | no | - | - | 119 (↔) | | 92 | | 112 | 0.95 (↔) | | no | - |

*PS indicates protein S; TPS, total protein S antigen; FPS, free protein S antigen; PC, protein C antigen; HH, hyperhomocysteinemia; Protein C/S ratio, protein C antigen / total protein S antigen; VTE, venous thromboembolism; ID, pedigree identification code; ↔, within normal range; ↑, increased; ↓, decreased; $compared to type III PS deficient relatives; #compared to non-deficient relatives; *on oral contraceptive.*

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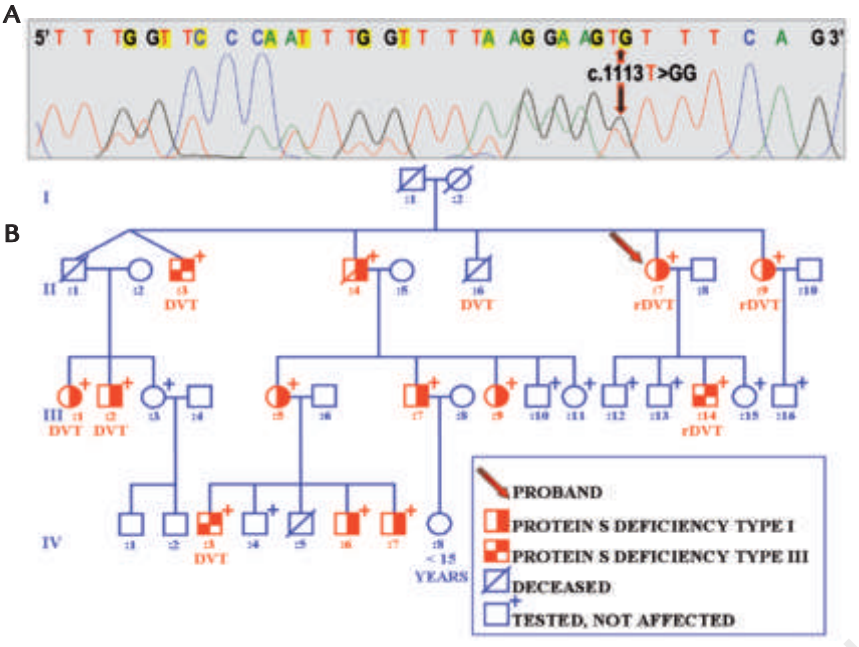


**Figure 1. A. PROS1 c.T1113>GG frameshift mutation, exon 10, codon 282. Note: Reverse reading in reverse and complement. Due to the mutation's close proximity to the forward primer it was not possible to obtain a good illustration of the forward reading. Highlighted bases with associated peaks are caused by the mutation. B. Pedigree of a mixed type I/ type III protein S deficient family. DVT indicates deep vein thrombosis; rDVT, recurrent deep vein thrombosis.**

being 1.86 and 1.55, respectively ($p$=0.24). In contrast, the ratios in all non-deficient relatives were within normal ranges (mean 0.95; $p$=0.01 compared to type III PS deficient relatives).

As we recently reported, type I PS deficiency is a strong risk factor for venous thromboembolism, unlike type III PS deficiency, unless the latter is diagnosed in relatives from a type I PS deficient family.[9] Free PS levels alone lack the ability to differentiate between the two types of PS deficiency. If we had only measured total PS levels, type III PS deficient relatives who apparently were at risk of venous thromboembolism, would have been overlooked. Since both PC and total PS levels increase with age, the protein C/S ratio should not be affected by age. However, as the mean age of type I PS deficient relatives was 49 years, compared to 48 years in type III PS deficient relatives, the difference in phenotypes could not be explained by age alone. Considering increased protein C/S ratios in these relatives, phenotypic type III deficient relatives were actually type I deficient, i.e. with respect to their PC levels they had relatively decreased total PS levels. This finding was confirmed by both type I and type III deficient relatives having the same genotype. The sensitivity and specificity of the protein C/S ratio for identifying relatives with a *PROS1* gene mutation were both 100%. It should be noted that in this family, the same applied for free PS levels. Of course, our finding needs to be validated more extensively, preferably in a larger number of families with an identified mutation, before it could be used in clinical practice. Nevertheless, this ratio might be of interest for clinical use, considering that both PC and total PS levels are commonly measured in screening for thrombophilic defects and its simplicity.

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*Key words: protein S deficiency, thrombosis, hereditary thrombophilia, PROS1, mutation.*

*Appendix: electronic database information: accession number and URL for the sequence variation of the c.1113T→GG mutation at GenBank is as follows: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=nucleotide. Accession number: DQ382334.*

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