

## ORIGINAL ARTICLE

## Protein S deficiency: a clinical perspective

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**Summary.** Protein S (PS) is an extensively studied protein with an important function in the down-regulation of thrombin generation. Because of the presence of a pseudogene and two different forms of PS in plasma, a bound and a free form, it is one of the most difficult thrombophilias to study. A deficiency of PS predisposes subjects to (recurrent) venous thrombo-

embolism (VTE) and foetal loss. However, the conundrum of diagnosing PS deficiency has led to conflicting reports of PS as a risk factor for VTE. In this review, we aim to present a clinical perspective of PS deficiency.

**Keywords:** *PROS1*, protein S, protein S deficiency, review, thrombosis, venous thromboembolism

### Introduction

In 1976, the same year the first report on the identification of protein C (PC) was published, Di Scipio *et al.* [1] identified another new vitamin K-dependent plasma glycoprotein. They named the novel glycoprotein protein S (PS; MIM 176880) in reference to its isolation and characterization in Seattle. Four years later, Walker reported that PS possesses important anticoagulant properties. He observed that the rate of factor Va (FVa) inactivation by activated PC (APC) could be enhanced by the addition of plasma. The responsible cofactor was PS. In the presence of phospholipids, PS enhances the APC cleavage of FVa approximately 10-fold and consequently has an important function in the regulation of thrombin generation [2]. In contrast to PC, PS circulates in plasma in two forms. Approximately 60% is bound non-covalently to complement component C4b binding protein  $\beta$ -chain (C4BP), whereas the remaining 40% is free [3]. Until recently, it was thought that only free PS possesses APC cofactor activity [4]. In 1984, the first clinical report on PS deficiency as a risk factor for venous thromboembolism (VTE) was published. Schwarz

*et al.* [5] described a family with VTE caused by hereditary PS deficiency. Now, 30 years after the first publication, still new functions are attributed to PS.

### Methods

A PubMed search was conducted to identify the most relevant peer reviewed articles reporting on PS and PS deficiency. According to the manuscript outline of the Rare Coagulation Disorders Resource Room Project, only the 33 clinically most appropriate manuscripts were cited. Because of this reference limit, we chose to cite previous reviews on the pathophysiology, molecular basis, clinical presentation and diagnosis of PS deficiency. Also it should be noted that because of the scarceness of PS deficiency, some pronouncements are extrapolated from VTE and thrombophilia in general.

### Incidence of PS deficiency

The prevalence of hereditary PS deficiency in the general population remains largely unknown, probably because of its rarity and the difficulty of a correct diagnosis. However, a study in 3788 healthy Scottish blood donors showed a prevalence of hereditary PS deficiency ranging from 0.03% to 0.13% [6]. Molecular follow-up showed 1 subject with PS deficiency having a detrimental PS gene (*PROS1*) mutation and five subjects with PS deficiency carrying the PS Heerlen polymorphism [traditional PS nomenclature Ser460Pro; Human

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Genome Variation Society (HGVS) p.Ser501Pro], resulting in a prevalence ranging from 0.16% to 0.21% [7]. Although PS deficiency is uncommon in the general population, it is found in approximately 2% of unselected patients and 1–13% of thrombophilic patients with VTE respectively [8,9]. The broad range of the latter could be attributed to the different clinical criteria for selecting the thrombophilic patients in the different studies, the challenge of diagnosing PS deficiency, or transient PS deficiency. Furthermore, there is growing evidence that PS deficiency is more prevalent in the Japanese and Chinese populations. The estimated prevalence in the general Japanese population is 1–2% [10] and a *PROS1* mutation is found in 22% of patients with VTE, suspected of having thrombophilia [11], whereas up to 36% of Chinese patients with VTE in Vietnam showed decreased PS activity levels [12]. This discrepancy might be attributable to racial differences, different diagnostic tests for the detection of PS deficiency, or the high prevalence of a specific *PROS1* mutation confined to a geographical area, e.g. the PS Tokushima mutation (traditional PS nomenclature Lys155Glu; HGVS p.Lys196Glu) in the Japanese population.

### Pathophysiology

Protein S is mainly synthesized in hepatocytes, but also in megakaryocytes, osteoblasts, and endothelial, Leydig and vascular smooth muscle cells, and circulates in plasma at a concentration of 20–25 mg L<sup>-1</sup> (260–330 nM). A deficiency can be inherited or acquired because of vitamin K-antagonist therapy, oral contraceptives, pregnancy and various disorders, such as liver disease, nephritic syndrome, disseminated intravascular coagulation and chronic infections (e.g. HIV).

Protein S possesses both APC-dependent and independent anticoagulant properties and thus is an important guardian in controlling thrombin generation and fibrinolysis, although the contribution of these properties to the anticoagulant functions of PS is still unclear. Furthermore, PS is thought to play a role in other systems [3,13,14].

### APC-dependent anticoagulant functions

Protein S serves as a cofactor of APC in the proteolytic degradation of activated coagulation FV and FVIII, although intact FV is required for the latter [3,13,14]. It was believed that only free PS possesses APC cofactor activity. However, emerging new evidence demonstrates that the PS–C4BP com-

plex, although less effective than free PS, enhances the APC inactivation of FVa by boosting the APC cleavage of FVa at R<sup>306</sup>, while selectively impairing the APC cleavage at R<sup>506</sup> [15]. Besides its role in the down regulation of the thrombin generation, PS also enhances the profibrinolytic effects of APC, resulting in plasminogen activator inhibitor neutralization, as demonstrated in clot lysis assays [16].

### APC-independent anticoagulant functions

Protein S has been shown to inhibit directly the tenase and prothrombinase complexes. Also, in the early stages of clot formation, PS is involved in the regulation of fibrinolysis by inhibiting the initial thrombin formation independent of APC and thereby decreasing the rate of thrombin activatable fibrinolysis inhibitor activation [17]. Recently, control of the extrinsic pathway of coagulation has been demonstrated. PS serves as a cofactor of tissue factor pathway inhibitor (TFPI), resulting in inhibition of tissue factor activity by promoting the interaction between TFPI and FXa [18].

### Types of PS deficiency

Depending on the test results obtained from different PS assays, three subtypes of PS deficiency are recognized. Whereas types I and III (also known as type IIa) are quantitative defects, type II is a qualitative defect (also known as type IIb). The main features of PS and PS deficiency are summarized in Table 1.

### Genetic/molecular basis of disorder

Hereditary PS deficiency is an autosomal dominant disorder. The PS encoding gene *PROS1* or *PS-α*, and its transcriptionally inactive pseudogene *PROS2* or *PS-β*, are located near the centromere on chromosome 3 at 3q11.2 and 3p21-cen respectively. Both genes share approximately 97% similarity, but exon 1 is absent in the pseudogene. The *PROS1* gene spans roughly 80 kb of genomic DNA and contains 15 exons (Fig. 1) and mature PS consists of 635 amino acids. Only recently, the *PROS1* promoter has been characterized [19]. A minimal *PROS1* promoter 370 bp upstream from the translational initiation codon, containing four binding sites for transcription factors Sp1 and Sp3, is sufficient for maximal promoter activity in transient transfections [20].

So far, almost 200 different *PROS1* mutations resulting in loss of function have been identified (see

**Table 1.** Main features of protein S and protein S deficiency.

Gene name (location)	<i>PROS1</i> or <i>PS-α</i> (3q11.2)
PS	Vitamin K-dependent plasma glycoprotein; 60% bound to C4bBP-β chain, 40% free (together total PS)
Function PS	Cofactor for APC in the inactivation of activated coagulation FV and FVIII, resulting in reduced thrombin formation
Clinical characteristics of PS deficiency	VTE at young age (<30 years), unprovoked, at uncommon locations, recurrence, family history Foetal loss, especially late foetal loss
Management	First VTE: anticoagulant therapy for up to 2 years without other thrombophilia; life-long if other thrombophilia is present Recurrence: life-long anticoagulant therapy (primary) thromboprophylaxis: in all high risk conditions Oral contraceptives discouraged
Types of PS deficiency	Type I: ↓ APC cofactor activity, ↓ total PS, ↓ free PS Type II: ↓ APC cofactor activity, ↔ total PS, ↔ free PS Type III: ↓ APC cofactor activity, ↔ total PS, ↓ free PS
PS assays	Immunoassays for free and total PS, and clotting assays for PS activity
Prevalence of PS deficiency in the general population	0.16–0.21%
Prevalence of PS deficiency in patients with VTE	2%
Relative risk of VTE	5–11.5
Rule of 50	50% chance for men and women to be affected and 50% chance to pass it to their offspring 50% develop VTE by the age of 55 years 50% of VTE is unprovoked Total and free PS levels lower in women, but total PS levels in women rise to the levels of men by the age of 50 years A <i>PROS1</i> mutation is not detected in up to 50% of PS deficient subjects

PS, protein S; APC, activated protein C; VTE, venous thromboembolism; ↓, decreased; ↔, within normal range.



**Fig. 1.** *PROS1* messenger RNA (mRNA) (a) with corresponding protein S (PS) domains (b). (a) mRNA encoding protein S. Ex indicates exon; 3' UTR, 3' untranslated region. (b) Domain structure of mature PS. Colour coding corresponds to mRNA exons encoding each PS domain. GLA, γ-carboxyglutamic acid domain; AS, aromatic stack domain; TSR, thrombin sensitive region domain; EGF, epidermal growth factor-like domain; LGR, laminin G-type repeat.

Links to organizations). Genetic defects of *PROS1* causing PS deficiency types I, III or both are distributed throughout *PROS1*. A frequently encountered amino acid substitution is the PS Heerlen polymorphism, with a prevalence of 0.52% in the general population, resulting in type III PS deficiency. It is thought that heterozygous PS Heerlen carriers are not at increased risk of VTE [21]. *In vitro* experiments showed that the selective reduction of free PS in these carriers is not because of defective synthesis or secretion. Most likely, the type III PS deficient phenotype is a consequence of increased clearance of free PS Heerlen [22]. Conversely, mutations causing type II PS deficiency are usually located in the epidermal growth factor (EGF)-like domains (Fig. 1) and are missense mutations. The

most extensively studied mutation causing type II PS deficiency is PS Tokushima, located in EGF-2, with an estimated prevalence of 1.6% in the general Japanese population [10,11].

#### Genotype–phenotype associations

Genotype–phenotype associations have proven to be inconsistent in hereditary PS deficiency. Coexistence of types I and III PS deficiency in the same family has been observed frequently and has led to the speculation that both types are phenotypic variants of the same genetic disease caused by an increase in total PS levels with age. Studies screening for mutations of the *PROS1* gene in families with hereditary PS deficiency have resulted in *PROS1* mutation detection rates

ranging from 50% to 90% [14]. The low mutation detection rate has been attributed to relatively common large *PROS1* deletions [23], not detected by the standard screening methods, and genetic heterogeneity between PS deficiency types I and III [24,25]. Interestingly, a mutation is detected more frequently in families with type I compared with type III PS deficiency [24,25], and no linkage to the *PROS1* locus is found in the mutation negative type III PS deficient families [25]. Also, in families with a *PROS1* mutation, the mutation co-segregates more frequently with type I PS deficiency than with type III PS deficiency [25]. Therefore, it has been hypothesized that although PS deficiency type I is essentially a monogenic disease caused by *PROS1* allelic heterozygosity, type III is a more heterogeneous disorder [24,25].

### Clinical manifestations

In inherited thrombophilia, VTE is usually the result of a disparity of the delicate haemostatic balance between procoagulant and anticoagulant proteins, shifting the haemostatic balance in favour of thrombosis. Symptomatic subjects with hereditary PS deficiency typically present with (recurrent) deep vein thrombosis, pulmonary embolism or both. Less common manifestations are superficial, cerebral, visceral or axillary vein thrombosis. Approximately half the thromboembolic events are unprovoked, i.e. not preceded by transient risk factors for VTE, such as surgery, trauma, immobilization, air travel, pregnancy/puerperium or systemic hormonal contraception or replacement therapy. Depending on the phenotype, approximately half the PS-deficient subjects become symptomatic at 55 years of age [26]. It is not exactly known why some PS-deficient subjects develop VTE, whereas others remain unaffected. Part of it can probably be explained by concomitant thrombophilic defects and exposure to transient risk factors contributing to the risk of VTE [27]. Although suggested by several case reports, there is no convincing evidence that PS deficiency is a risk factor for arterial thrombosis.

It is well known that the use of third-generation oral contraceptives in particular increases the risk of VTE. A retrospective family cohort study in women with a deficiency of PS, PC or antithrombin showed an annual incidence of VTE of 3.5–12% during actual oral contraceptive exposure, depending on the number of concomitant thrombophilic defects [28]. This is up to 600-fold higher than the reported risk of VTE during oral contraceptive use in healthy women.

A meta-analysis showed that of pregnancy-related VTE, 21.9%, 33.7% and 47.6% occurred in the first, second and third trimesters respectively. Remarkably, 82.2% of deep vein thrombosis occurred in the left leg or bilateral, the latter accounting for <5% [29]. In women with inherited PS deficiency, pregnancy or puerperium is the provoking factor for VTE in up to 20%. Besides pregnancy-related VTE, women with PS deficiency are at increased risk of especially late fetal loss (odds ratio = 3.3) [9]. Conversely, there is evidence that thromboprophylaxis can effectively reduce the high rate of foetal loss [30].

### Levels associated with the risk of thrombosis

Data are conflicting concerning the risk of VTE because of PS deficiency. Although family-based studies show a consistent 5- to 11.5-fold higher life-time risk of VTE in affected family members when compared with their wild-type relatives, a population-based study failed to find an association between low PS levels and VTE [13]. The latter might be because of the conundrum of diagnosing inherited PS deficiency in a healthy population. This is supported by data from a large healthy blood donor population [6]. Only eight out of 56 initial PS deficient subjects consistently showed decreased PS levels, indicating that most of PS-deficient subjects in population-based studies might have transitory rather than inherited PS deficiency. Therefore, these transitory PS-deficient subjects are probably not at risk of VTE attributable to PS deficiency. Furthermore, recent studies suggest that the heterogeneous data on the risk of VTE can be explained by the risk conferred by different phenotypes [26] and genotypes [31]. Mutations resulting in large PS derangement particularly can cause severe secretion defects and functional impairments of PS, which are likewise reflected in the risk of thrombosis [31]. The absence of an association between the *PROS1* missense variant PS Heerlen and VTE is consistent with this consideration. Marked selective *increase* of free PS levels without a bleeding diathesis has been observed, suggesting that high levels of free PS do not predispose subjects to haemorrhage.

### Timing of presentation

Homozygous or compound heterozygous PS deficiency, though extremely rare, usually present neonatally with massive VTE or purpura fulminans [3,9]. Without treatment, this will most likely be lethal. However, in some infants, severe retinopathy

of prematurity can be the predominant initial symptom.

Heterozygous PS deficiency is an established risk factor for VTE, but with incomplete and variable penetrance. The timing of presentation is usually before 50 years of age and the life-time risk of VTE is similar in men and women. However, probably because of the use of oral contraceptives and pregnancy or puerperium, PS-deficient women seem to be at a greater risk of developing VTE early in life (<30 years) compared with PS-deficient men [28].

### Diagnosis

The diagnosis of hereditary PS deficiency is notoriously difficult. As a result of overlapping PS levels in carriers and non-carriers of mutations and variations in levels related to age, gender and acquired conditions, subjects can be misdiagnosed. To minimize false-positive test results, plasma samples should be taken after cessation of oestrogen or vitamin K-antagonist treatment. If an indication for prolonged anticoagulant therapy remains, low-molecular-weight heparin (LMWH) can be given for 2–4 weeks, depending on the type of vitamin K antagonist, followed by blood sampling. Positive test results should be confirmed by a second blood sample. In pregnant women, positive test results should be confirmed after the postpartum period. To prove inherited deficiency, testing of family members is recommended.

### Laboratory

Generally, there are two types of PS assays. Immunoassays for the determination of total and free PS levels and clotting assays to measure APC cofactor activity. A meeting of the International Society of Thrombosis and Haemostasis and the World Health Organization in 1996 resulted in the recommendation that determination of free PS may be more useful than total PS for the diagnosis of PS deficiency. However, free PS assays may suffer from poor reproducibility and demonstrate a time-, temperature- and dilution-dependent increase in free PS. Measurement of APC cofactor activity could in theory enable the detection of all three types of PS deficiency. Yet, APC cofactor activity determination results in a high rate of false-positive results because of the presence of APC resistance and high levels of prothrombin, FVIIIa and FVIIa [32]. In addition, it was recently reported that measuring PS activity is not suitable for identifying PS Tokushima carriers. Total PS assays, on the other hand, have proven to be

precise and accurate, but lack the capability to detect type II and type III PS deficiency. However, it has recently been suggested that the ratio of PC to total PS might be valuable to identify carriers of a *PROS1* mutation resulting in quantitative PS deficiency, i.e. type I and type III [25]. Similarly, the ratio of total PS and FII [33] or FX [5] has been proposed to detect PS deficiency in subjects receiving vitamin K-antagonist treatment, provided they have a stable level of anticoagulation.

### Molecular

The most frequently used approaches for the detection of *PROS1* mutations are polymerase chain reaction (PCR) amplification followed by single-strand conformation polymorphism or denaturing gradient gel electrophoresis analysis and DNA sequencing and particularly direct DNA sequencing [14]. Nevertheless, in many cases a *PROS1* mutation is not detected. To screen for large *PROS1* deletions and insertions, not detected by classic PCR-based approaches, the multiplex ligation-dependent probe amplification *PROS1* kit (see Links to organizations) could prove to be valuable. If an adequate number of family members are available, *PROS1* linkage analysis can be performed to implicate or exclude the *PROS1* region on the observed phenotype. Also, restriction fragment length polymorphism can be performed to detect aberrant messenger RNA (mRNA) products, although caution is recommended because many carriers of a *PROS1* mutation have been reported without detectable aberrant mRNA products.

### Management

The treatment of VTE in patients with PS deficiency only differs from those with VTE without thrombophilia in the duration of anticoagulation. Initial treatment consists of unfractionated heparin or LMWH overlapped with warfarin until an international normalized ratio of 2.0–3.0 is reached on two consecutive days. Heparin should be administered for at least 5 days to prevent skin necrosis, which is a rare adverse effect that occurs during early warfarin treatment in patients with PS and PC deficiency. Warfarin treatment should be considered for up to 2 years and even life-long in the presence of concomitant thrombophilic defects [9], whereas it is usually continued for 3–6 months after first VTE in patients without thrombophilia. To reduce the incidence of post-thrombotic syndrome (PTS), graduated compression stockings should be worn for at least

2 years. Thromboprophylaxis should be given to all PS-deficient subjects in high-risk situations, such as surgery, trauma, immobilization, air travel for more than 4 hours and pregnancy or puerperium. Warfarin should not be administered in the first trimester or after 36 weeks of gestation, because of the risk of both foetal bleeding and teratogenicity and maternal bleeding during delivery respectively. During these periods, LMWH is the drug of choice because of the higher risk of allergy, heparin-induced thrombocytopenia, and osteoporosis associated with prolonged unfractionated heparin therapy. Although warfarin crosses the placenta, it is not secreted in breast milk in clinically significant concentrations and it is safe to administer warfarin throughout lactation. Oral contraceptives and hormonal replacement therapy should be stoutly discouraged.

### Prognosis

Venous thromboembolism is associated with a high rate of morbidity (i.e. PTS and pulmonary hypertension) and mortality because of pulmonary embolism. However, there is no evidence that PS deficiency carries a poor prognosis when compared with subjects with VTE without thrombophilia. Nonetheless, PS-deficient subjects are at an increased risk of recurrence, which predisposes them to PTS and pulmonary hypertension. Prolonged warfarin treatment results in an increased risk of major haemorrhage; however, thrombophilic subjects might be less susceptible to major bleeding, while receiving warfarin treatment, as we observed in a large family cohort study (data not published yet).

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### Disclosures

The authors state that they have no interests which might be perceived as posing a conflict or bias.

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#### Links to organizations – professional, lay

Human Genome Variation Society (HGVS), <http://www.hgvs.org/>.

Protein S Deficiency: A Database of Mutations, <http://www.med.unc.edu/isth/>.

Human Gene Mutation Database (HGMD), <http://www.hgmd.cf.ac.uk/ac/>.

MRC-Holland MLPA PROS1-kit, <http://www.mrc-holland.com/pages/indexpag.html>.