REVIEW ARTICLE

Venous thromboembolism associated with protein S deficiency due to Arg451* mutation in *PROS1* gene: a case report and a literature review.

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# Equal contribution

Short title: Association of venous thromboembolism with Arg451* mutation

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Abstract

Objective. Protein S (PS) is a vitamin K-dependent glycoprotein which plays an important role in the regulation of blood coagulation. PS deficiency has been found in 1.5% to 7% of thrombophilic patients. We report here the first Polish case with PS deficiency caused by the p.Arg451X in the *PROS1* gene detected in a 21-year- old man with trauma-induced

venous thromboembolism. To our knowledge, we provided the review of all available data on this mutation (a total of 56 cases).

Methods. The proband, his mother and his sister were screened for thrombophilia. To elucidate genetic background of PS deficiency, all *PROS1* gene was subjected to direct sequencing. The free PS levels were 35% in the proband, 21% in the proband's mother and 28% in the proband's sister. PS total levels were 37.1%, 47.5% and 55.1% , respectively. Type I PS deficiency was diagnosed. In all patients, genetic analysis revealed the presence of heterozygous nonsense mutation (c.1351C>T; p.Arg451*) located in exon 12 of *PROS1* gene. This mutation interrupts the reading frame by premature termination codon at position 451 and may lead to the production of truncated protein.

Conclusions. The present case combined with the review of the literature suggests that p.Arg451* in the *PROS1* gene leads to clinically evident thrombosis mainly following trauma, surgery or serious comorbidities especially malignancy.

Keywords: *PROS1* gene; Arg451* mutation; venous thromboembolism

Introduction

Protein S (PS) is a vitamin K-dependent glycoprotein that acts as a natural anticoagulant. It is synthesized by hepatocytes, endothelial cells, megakaryocytes, testis Leydig cells and in the brain (Wypasek and Undas 2013). Plasma PS occurs in two forms. Sixty percent is noncovalently bound to C4b binding protein (C4bBP), a regulatory protein of the classic complement pathway, while the remaining 40% represents an unbound or ‘‘free” fraction ready to interact with the activated protein C (APC). PS and APC form the APC complex, that is responsible for the proteolytic deactivation of activated factors (F) V (Va) and VIII (VIIIa). Binding functional free protein S with C4bBP abolishes its anticoagulant activity (Dahlback and Stenflo 1981, Walker 1981). PS was also identified as a cofactor of

tissue factor pathway inhibitor (TFPI) which stimulates inhibition of the FXa by TFPI via the APC-independent mechanism (Hackeng et al. 2006).

It has been long recognized that reduced PS activity is a risk factor for venous thromboembolism (VTE) (Comp and Esmon 1984, Schwarz et al. 1984). PS deficiency was found in 1.5% to 7% of thrombophilic patients (Mulder et al. 2011, Makris 2000). Based on quantitative and qualitative tests, PS deficiency is classified as type I (low total and low free antigen plus reduced PS activity), type II (normal total and normal free antigen plus reduced PS activity) and type III (normal total antigen, reduced free antigen and reduced PS activity) (Bertina 1990). Type I and type III deficiencies account for 95% of cases of PS deficiency.

PS is encoded by *PROS1* gene which is approximately 80 kb long and consists of 15 exons on chromosome 3, locus 3p11.1-3q11.2 (Ploos van Amstel et al. 1987, Watkins et al. 1988, Long et al. 1988). Sequencing of *PROS1* gene intensified studies of genotype-phenotype correlation and search for mutations explaining inherited causes of PS deficiency. According to Human Gene Mutation Database (HGMD), 344 *PROS1* gene mutations have been described. Most of these abnormalities (61%) are missense and nonsense mutations with a ratio of 5:1 (Stenson et al. 2014).

The aim of our study is to summarize for the first time the current literature on Arg451* mutation in the exon 12 of the *PROS1* gene and thrombotic manifestations. Additionally, we report here the first Polish case of PS deficiency with p.Arg451* mutation diagnosed in a young Polish man with trauma-induced VTE.

Case history

A 21-year old man (height 186 cm, weight 110 kg, non-smoker) developed proximal deep vein thrombosis (DVT) involving the right superficial femoral and popliteal veins

followed by pulmonary embolism (PE) in August 2013. Two months earlier he experienced the right ankle joint injury and the leg was immobilized without thromboprohylaxis and one month later pain with slight oedema developed. Trauma-induced VTE was diagnosed, however thrombophilia screened was planned. He was treated with enoxaparin followed by rivaroxaban 20 mg daily. Seven weeks later the patient was subjected to stent implantation to the femoral vein to reduce symptoms of residual vein obstruction. While on acenocoumarol with stable anticoagulation (time in the therapeutic range 80%) no recurrence or serious bleeding was observed during a 22-month follow-up. The proband's pedigree (IV-11) is given in Fig. 1. Proband's personal medical history reveal no prior thromboembolic events, however, his family history for VTE was remarkable. Lower limb varices without documented DVT in proband's mother (III-11) and her two sisters (III-7 and III-9), idiopathic DVT in her third sister (III-5) and DVT with leg ulceration in her brother (III-2) have been documented. The proband's grandmother (II-9) and grandfather (II-8) on the mother's side (III-11) experienced lower limb varices and leg ulceration, respectively. An 18-year proband's son developed idiopathic DVT (IV-1). The proband's great grandmother (I-2) on the grandfather's side (II-8) also developed leg ulceration. The proband's sister (IV-12) remained asymptomatic.

Methods

The proband, his mother (III-11) and his sister (IV-12) were screened for thrombophilia including the assessment of the F5 G1691A (FV Leiden, rs6025) and F2 G20210A (prothrombin) polymorphisms, antithrombin, protein C, free and total protein S, and factor VIII , along with markers of antiphospholipid syndrome, including lupus anticoagulant, anticardiolipin antibodies and anti-β2-glycoprotein I (β2GP-I) antibodies, both in IgG and IgM classes. Other family members were unavailable for genetic analysis.

Plasma homocysteine (tHcy) was determined in EDTA plasma by the high-performance liquid chromatography (HPLC). Hyperhomocysteinemia was defined as tHcy of 15 μmol/l or more after an overnight fast.

Lupus anticoagulant (LA) was assessed using clot-based assays. Anticardiolipin and anti-β2GP-I antibodies were determined by immunoenzymatic assays (INOVA Diagnostics, San Diego, USA). Reference ranges for IgG were up to 15 GPL and 8 SGU, respectively, and for IgM up to 17 MPL and 10 SMU, respectively.

Antithrombin activity was determined by a chromogenic assay (INNOVANCE™ ATIII, Siemens Healthcare Diagnostic, Marburg, Germany); reference range was from 83% to 118%. Free protein S was determined by the immunoturbidimetric method (Innovance Free PS Ag immunoassay; Siemens Healthcare Diagnostics, Erlangen, Germany). The reference ranges for the levels of free PS antigen were 67-139% for male and 60%-114% for female. Total PS levels were assessed by an immunoenzymatic assay (Asserachrom Kit; Diagnostica Stago, Asnieres, France). The reference intervals were 60%-140% for male and 75%-140% for female. Protein C was determined by a chromogenic assay (Berichrom® Protein C, Siemens Healthcare Diagnostic, Marburg, Germany); reference range was from 70% to 140%.

Plasma factor VIII activity was determined by the coagulometric assay (Instrumentation Laboratory) and levels of 150% or more were considered elevated.

The genomic DNA was extracted from whole blood or a buffy coat using the NucleoSpin® Blood DNA Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol and stored at -80°C until analysis. F5 G1691A mutation was determined by the Real-Time PCR with the use of TaqMan Genotyping Assays in 7900 Fast Real-Time PCT System (Life Technologies Co. Carlsbad CA, USA). F2 G20210A mutation was determined by the RFLP polymorphism analysis with the use of HindIII restrictase (Fermentas Hanover, MD USA).

To elucidate genetic background of PS deficiency, all *PROS1* exons, exon-intron boundaries and 700 bp of the promoter region were subjected to direct sequencing (Alhenc-Gelas et al. 2007).

The Jagiellonian University Ethical Committee approved the study, and all the participants provided their written informed consent.

Results

The free PS levels were 35% in the proband, 21% in the proband's mother and 28% in the proband's sister. PS total levels were 37.1%, 47.5% and 55.1% , respectively. Results were obtained on two separate occasions. Type I PS deficiency was diagnosed. Only the proband was symptomatic. Deficiencies in protein C and antithrombin were excluded. Factor VIII activity, antiphospholipid antibodies and total homocysteine were within normal limits and lupus anticoagulant was negative. FV Leiden and F2 gene G20210A mutation were absent.

In the proband (IV-11), his mother (III-11) and his sister (IV-12) genetic analysis revealed the presence of heterozygous nonsense mutation c.1351C>T, p.Arg451* located in exon 12 of *PROS1* gene. This mutation interrupts the reading frame by premature termination codon at position 451 and may lead to the production of truncated protein. It is well known that mutations resulting in a premature termination mostly undergo nonsense-mediated mRNA decay (NMD) preventing synthesis of the truncated protein.

Discussion

To the best of our knowledge, this is the first report of PS deficiency related to the nonsense mutation p.Arg451* in *PROS1* gene in a Polish family.

The p.Arg451* mutation, prior known as Arg410Ter, was reported for the first time in 1995 (Mustafa et al. 1995) in three out of five members of an Austrian family with PS deficiency type I. Two of them experienced recurrent DVT with/or without pulmonary embolism (PE) at

the age of 23 and 57 years. There was no data on predisposing factors in relation to the recurrence of thrombotic episodes.

Possessing of Arg451* variant was the cause of PS deficiency in a Thai girl with purpura fulminans (Pung-Amritt et al. 1999). Her case has been reported in 1990 but nine years later the mutations caused PS deficiency in this patients have been identified. It turned out that the patient carried two null mutations in heterozygous state: one allele contained Arg451* variant and the other allele contained a novel sequence variation, an A-insertion in an A5-tract that results in a frameshift and a stop codon occurrence. At that time patient was treated successfully with the combination of oral warfarin and fresh frozen plasma transfusion. Co-segregation of PS deficiency with the two genetic defects has been also observed in patient's family. The patient's mother was heterozygous for the A-insertion while her father and her brother were heterozygous for Arg451* mutation (Pung-Amritt et al. 1999).

Further, Arg451* mutation in *PROS1* gene was reported in five French (Borgel et al. 1996) and three Danish (Andersen et al. 1996) families - altogether in 18 individuals. In each of the five out of 118 investigated French families there was one symptomatic individual; in two pedigrees it was the only individual available for testing. In one pedigree the symptomatic individual had PS deficiency type I but was unavailable for DNA testing while her two asymptomatic relatives with PS deficiency type I tested positively for p.Arg451* mutation. In the fourth family two relatives with PS deficiency type I tested positively for the mutation, including one subject with VTE and the other asymptomatic. In the fifth family three relatives had PS deficiency type I, all of them tested positively for the p.Arg451* mutation including only one symptomatic patient; the fourth relative was asymptomatic and unavailable for testing. Typically, all symptomatic individuals suffered from recurrent or single episodes of DVT and/or PE and were rather young (the youngest was 14 years old and the oldest 44).

Among the sixteen Danish thrombophilic families with PS deficiency, p.Arg451* mutation was shared by 9 individuals from three families (Andersen et al. 1996). Analysis of PS mRNA from platelets showed that this mutation caused the PS mRNA reduction and in consequence exerted its deleterious effects on gene expression at the transcriptional level (Andersen et al. 1996). It has been proved that the Danish patients have a common ancestor who had no familiar relation to the Austrian family (described above) (Andersen et al. 1999). A possible French founder, has not been confirmed so far.

In turn, the p.Arg451* mutation has been found in one out of nine Spanish proband presenting coexistence of type I and III- deficient phenotypes (Espinosa-Parrilla et al 2000). Five out of 11 of his family members carried the mutation but only two were suffering from VTE (Espinosa-Parrilla et al 2000).

Then, the Protein S Italian Team (PROSIT) study has shown that among 79 PS deficient families from regional centers throughout Italy, four symptomatic probands and 11 family members, where 6 of them were symptomatic, carried the p.Arg451* mutation (Biguzzi et al. 2005). In this study the expression experiments and functional analysis of p.Arg451* variant has been also performed. The p.Arg451* variant introduced into an expression vector has been transfected into fibroblast-like cell line. This experiment has shown that PS was not secreted upon transient transfection with p.Arg451* which confirmed the hypothesis that this variant causes quantitative PS deficiency (Biguzzi et al. 2005). Interestingly, in Japanese population of thrombophilic patients where the frequency of *PROS1* mutations is 5-10 times higher than in Caucasian patients, *PROS1* gene mutations has been detected in 19 of 39 DVT patients (Kinoshita et al. 2005). One out of those 19 patients possessed Arg451* variant and developed thrombosis at the age of 16. His mother and sister who carried Arg451* variant remained asymptomatic (Kinoshita et al. 2005).

Recently, the presence of p.Arg451* variant has been evaluated in German patients (Duebgen et al. 2012). Of 135 PS- deficiency-suspected adults, 49 from 35 families had mutations in *PROS1* gene and three patients from two families carried p.Arg451* variant which was associated with DVT/PE and family history of DVT (Duebgen et al. 2012). In a multicenter study of PS deficiency in children with venous VTE, the p.Arg451* mutation was found in 2 German families (Klostermeier et al. 2014). The probands in one family were 10.5- and 17-year-old brothers who experienced the episodes of cerebral sinus vein thrombosis triggered by acute lymphoblastic anemia and DVT triggered by surgery and immobilization, respectively. In the other family the probands were 16- year-old sister and her 18-year-old brother, both were suffering from DVT. In that siblings, DVT was provoked by oral contraceptives and smoking or surgery and immobilization, respectively (Klostermeier et al. 2014).

To summarize the above, to the best of our knowledge, the p.Arg451* mutation in *PROS1* gene has been found to cause PS deficiency in 56 individuals originating from different regions who displayed a similar clinical presentation. We may conclude that our family affected by the mutation p.Arg451* in *PROS1* gene fits well to the clinical picture of cases described above. The proband developed DVT triggered by ankle sprain and immobilization. The proband, his sister and his mother were diagnosed with type I PS deficiency, but only the proband case was symptomatic. Family history on the patient's mother side was positive for DVT.

It seems reasonable to suppose that in the case of PS deficiency type I caused by heterozygous p.Arg451* mutation in *PROS1* gene, fairly predictable genotype-phenotype correlation may exist: 1. site of thrombosis-predominantly DVT of the lower extremities frequently complicated by PE; 2. episode is triggered by a transient VTE risk factor (injury or surgery and immobilization, oral contraceptives, smoking); 3. it can happen in otherwise

healthy adolescents and young adults; 4. family history is positive for VTE predominantly on the side of one of the proband parent; 5. asymptomatic individuals with PS deficiency outnumber symptomatic ones at the time of diagnosis; 6. it is exclusively the type I PS deficiency.

The clinical manifestation for the p.Arg451* mutation in *PROS1* gene could be considered typical of all nonsense mutations, since in this type of mutation mRNA product of mutated allele is not detectable. It probably undergoes a quick NMD and there is no protein product (i.e. truncated protein in this case) of the mutated gene (Mustafa et al. 1995, Borgel et al. 1996, Andersen et al. 1999). So all synthesized protein S is a product of the other allele (presumably "normal") albeit of inadequate amount for the need (e.g. in injury followed by immobilization). It also explains the type of PS deficiency.

A major limitation of this study is fact that the family members of the proband's mother's side were unavailable for mutation screening. However, the family history was collected from probands mother while she had an appointment in the Center of Coagulation Disorders. Moreover, no data concerning the occurrence of p.Arg451* mutation in a greater number of Polish patients are available.

In conclusion, our paper based on a new case report summarizes for the first time the current literature on Arg451* mutation in PROS1 gene and thrombotic manifestations. All carriers of this mutation, like other associated with PS-deficiency are at risk of VTE and should be advised for prophylaxis after high-risk situations like e.g. trauma. It should be also stressed that due to positive family history of VTE, genetic counselling in such families should be implemented and appropriate thromboprophylaxis in high-risk states should be considered in asymptomatic carriers.

The present analysis leads to an important conclusion that this abnormality is phenotypically evident mainly in the presence of transient thrombosis risk factors, especially

trauma, surgery and serious comorbidities like malignancy. Genetic analysis to elucidate the mutation underlying thrombophilia allows to identify either new abnormalities or to provide additional information on the clinical course and prognosis if the detected mutation is known. Our current case supported by the detailed analysis of the literature illustrates well the importance of genetic diagnosis by providing more accurate information specifically on this mutation based on all the available data.

Figure 1. Four-generation pedigree of the patient with p.Arg451* mutation in *PROS1* gene. The affected subjects are marked with grey and the proband is indicated with an arrow. PS-deficient patients are marked with black, only those patients had measured the PS levels and were subjected to direct sequencing. The diagonal line through a symbol means a person is deceased. DVT denotes deep vein thrombosis.

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Conflict of interest

No conflict of interest to be declared.

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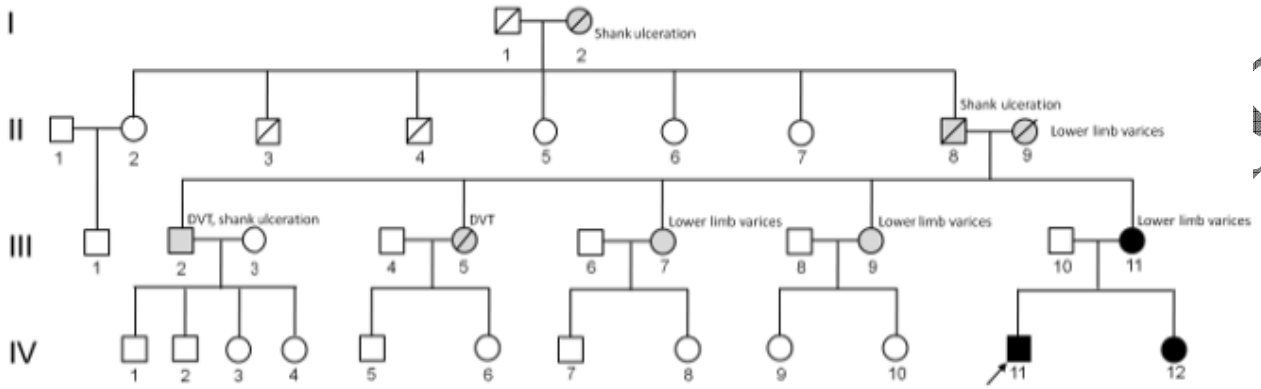
Figure 1. Four-generation pedigree of the patient with p.Arg451* mutation in *PROS1* gene.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