A Rare Splicing Mutation in the PROS1 Gene of a Korean Patient with Type I Hereditary Protein S Deficiency

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Abstract. Hereditary protein S (PS) deficiency (Gene ID: 5627; MIM # 176880) is a notable risk factor for recurrent venous thrombosis, inherited as an autosomal-dominant trait, either homozygous or heterozygous. It may be caused by point mutations in the gene (PROS1) encoding PS, which contains 15 exons on the chromosome 3q11.2. Only a few point mutations associated with the PROS1 gene in patients with hereditary PS deficiency have been reported. A 60-year-old woman was admitted for deep vein thrombosis (DVT) of the right lower extremity. Upon coagulation examination, both the free PS antigen level and the total PS antigen level were decreased, so the DNA-PCR products of all 15 exons, including the exon-intron boundaries of the PROS1 gene, were directly sequenced. A substitution from guanine to adenine at position +5 of the donor splice site of intron 10 (c.1155+5G>A) was identified. Further familial study was performed, and the patient’s older sister was revealed to have the same mutation; she was already taking warfarin due to diagnosed pulmonary thromboembolism. Here we report a G to A transition at position +5 of intron 10 from the splice donor site as a rare case of a patient with type I hereditary PS deficiency in Korea.

Key words: hereditary protein S deficiency, PROS1 gene, splice mutation

Introduction

Protein S (PS), as a vitamin K-dependant plasma glycoprotein, is an important anticoagulant [1]. In human plasma, about 60% of total PS is complexed to C4b-binding protein, while the remaining free PS (40%) acts as a cofactor of activated protein C (APC) in the proteolytic inactivation of coagulation factor (F)Va and FVIIIa [2,3]. It can also perform APC-independent anticoagulant functions, including inhibition of the prothrombinase and tenase complexes [4]. Hereditary PS deficiency (GeneID: 5627; MIM # 176880) is an important risk factor for recurrent venous thrombosis, inherited as an autosomal-dominant trait with variable clinical expression, either homozygous or heterozygous [5]. It had been identified by point mutations in the gene (PROS1) encoding PS, which contains 15 exons on the chromosome 3q11.2 [6]. Some studies on the anticoagulant function of PS indicated that PS deficiency is strongly related to venous thromboembolism in patients with familial history [7,8]. A few point mutations including nonsense, frameshift, splice mutations, and others leading to PS deficiency have already been discovered [3]. Among all mutations associated with PS deficiency, splice mutations account for only about 10% of the cases on the PROS1 database from the HGMD (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/) and International Society on Thrombosis and Haemostasis (http://www.medi.unc.edu/isth/) [2]. Moreover, a splice mutation with substitution from guanine (G) to adenine (A) at position +5 of the splice donor site of intron 10 (c.1155+5G>A) in the PROS1 gene has rarely been reported [9]. Here we report a G to A transition at position +5 of intron

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10 from the splice donor site as a rare case of a patient with type I hereditary PS deficiency in Korea.

Case Report

A 60-year-old woman who had been taking medication for hypertension for four years was admitted to our hospital due to painful swelling of the right lower extremity after a morning walk. She had occasionally experienced the pain during a period of several years. According to her familial history, some of her sisters and brothers had felt the same symptoms in the upper or lower extremities. Two of her three brothers had already suffered from sudden death as a result of acute myocardial infarction at around 60 years of age. Initial diagnostic ultrasonography of the painful extremity showed deep vein thrombosis (DVT) involving the right superficial femoral and popliteal veins. On her visit to our hospital as an outpatient, coagulation tests without anticoagulation revealed a decreased free PS antigen level at 31% (LIATEST Free protein S, Diagnostica Stago, Asnieres, France; reference range, 50-150%) and decreased PS activity of 17% (STACLOT Protein S, Diagnostica Stago; 55-123%). The total PS antigen level on a separate blood sample was 0.5 mg/dl (Human Protein S NL Nanorid, Binding Site, Birmingham, UK; 0.9-2.1 mg/dl). Other hypercoagulability tests (antithrombin activity, protein C (PC) activity, lupus anticoagulant, alpha2-antiplasmin, plasminogen, and factor V Leiden) were within reference interval, suggesting type I hereditary PS deficiency. After obtaining informed consent, blood samples were collected from the patient and her sisters (Figure 1).

Materials and Methods

PCR and sequence analysis. The genomic DNA was isolated from the leukocytes of the venous blood sample using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). All coding exons and the flanking introns of the PROS1 gene were amplified using genomic DNA with primers as previously described [10,11] and a thermal cycler (Model 9700; Applied Biosystems, Foster City, CA, USA). Direct DNA sequencing was performed with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in conjunction with an ABI Prism 3100 automated genetic analyzer (Applied Biosystems).

Results

Four members of the patient’s family, herself included, were examined in this study (Figure 1). Direct sequencing analysis revealed a substitution from guanine to adenine at position +5 of the splice donor site of the intron 10 (c.1155+5G>A) in the patient’s PROS1 gene. Among her three family members with molecular approach available, the same point mutation was identified in only one sister (Figure 2). She also had a decrease in free and total PS antigen level, which is classified as type I PS deficiency. However, the family members who had no point mutation were within normal intervals on the coagulation tests.

Discussion

Hereditary PS deficiency with mutations of the PROS1 gene is a notable risk factor for venous thromboembolism [12]. Therefore, in the case of patients suffering from major coagulation defects with a familial history of recurrent thromboembolic events, tests related to hereditary PS deficiency should be performed [13]. Diagnosis of hereditary PS deficiency is dependent on laboratory tests, including immunoassay of PS antigen levels of total and free forms, functional activity of PS (clotting assays to determinate APC cofactor activity) and molecular approaches [3]. Based on these results, PS deficiency is categorized into three subtypes. Types I and III PS deficiency are characterized by quantitative deficiencies of free PS antigen. While a decrease of total PS antigen is represented in type I, it remains at a normal level in type III. Type II, or qualitative PS deficiency feature normal levels of PS antigen, but because of dysfunctional PS, functional PS activity related to APC cofactor is reduced [2]. The clinical difference between types I and II has been disputed [14,15].
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The risk of thromboembolism conferred by a certain trait generally depends on the reference values used and its prevalence in the general population. Moreover, since the PS concentration in plasma is influenced by genetic and environmental factors such as sex, age, hormonal state, etc., it is difficult to determine the risk in the case of PS deficiency [16].

According to some reports, the prevalence of PS deficiency has been calculated to be less than 0.5% in the European population and about 1.5% in the Japanese population [17,18]. Recently, cases with mutation-related PS deficiency have been reported with increasing frequency [1,3,5,19-21]. Splice mutations make up only around 10% of mutations identified in the PROS1 gene, accounting for less than missense, nonsense, and frameshift mutations [2]. The substitution from G to A at position +5 of the splice donor site of intron 10 (c.1155+5G>A) in the PROS1 gene, while the others (II-6 and II-8) did not have the mutation. The location of the splice mutation is indicated by the arrow.

Figure 2. Identification of the PROS1 gene mutation. Direct sequencing of the proband and her older sister (II-4) demonstrated a splice mutation with substitution from G to A at position +5 of the splice donor site of intron 10 (c.1155+5G>A) in the PROS1 gene, while the others (II-6 and II-8) did not have the mutation. The location of the splice mutation is indicated by the arrow.

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Patients with phenotypic PS deficiency may develop thrombosis during adulthood, with an approximately 50% probability of remaining free of thrombosis at age 45 [25]. In this study, the proband and her older sister developed thrombosis at age 60. The incidence of venous thromboembolism (VTE) in asymptomatic PS-deficient relatives of symptomatic probands was decreased by prophylactic use of oral anticoagulants [26]. The consensus of the authors is that the PROS1 genetic testing of at-risk asymptomatic individuals younger than
In summary, we described a Korean patient with hereditary PS deficiency that was revealed to be heterozygous for rare G to A transition at position +5 of intron 10 from the splice donor site. This report further supports the significance of splice mutations in hereditary PS deficiency. The meticulous identification of splice mutations would be necessary to precisely assess the risk of thrombosis in individual patients and families, and also to better delineate the spectrum of genetic defects related to PS deficiency.

References
