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Case Report

Inherited protein S deficiency due to a novel nonsense mutation in the *PROS1* gene in the patient with recurrent vascular access thrombosis: A case report

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A B S T R A C T

Vascular access thrombosis is one of the major causes of morbidity in patients maintained on chronic hemodialysis. Thrombophilia has been recognized as a risk factor of vascular access thrombosis. The authors report a case of inherited protein S deficiency associated with vascular access thrombotic events. DNA sequence analysis of the *PROS1* gene identified a novel heterozygous nonsense mutation in exon 10 by transition of AAG (lysine) to TAG (stop codon) at codon 473 (c.1417A > T, p.K473X). Results from the study suggest that the inherited protein S deficiency due to a *PROS1* gene mutation may cause vascular access thrombosis in hemodialysis patients.

Introduction

Vascular access thrombosis is relatively common in patients on hemodialysis and becomes a major cause of access failure. It is currently thought that most episodes of fistula thrombosis are consequences of underlying anatomic abnormalities such as venous neointimal hyperplasia or vascular stenosis formation [1]. However, various reports have noted a significant amount of spontaneous thrombosis occurring independently of any obvious anatomic cause, suggesting that an unexplained nonanatomic cause may be responsible for a large number of thromboses. Thrombophilias are inherited or acquired predisposition of thrombosis and have been proposed as a possible cause of the hemodialysis access thrombosis [2]. A large case-controlled study exhibited that the presence of thrombophilia increased the risk for dialysis access thrombosis. Furthermore, it was shown that each additional thrombophilic factor increased the likelihood of access thrombosis almost twofold [3].

In recent years, several polymorphisms or point mutations of proteins involved in the coagulation cascade have been examined regarding their potential influence on vascular access thrombosis [4]. However, little is known about the roles of inherited protein S (PS) deficiency and its genetic defect for vascular access thrombosis in maintenance hemodialysis. We present here a case of inherited PS deficiency with recurrent vascular access thrombosis.

Case report

A 58-year-old Korean man experienced more than three episodes of thrombotic occlusions of hemodialysis vascular

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access. The renal disease was first diagnosed in September 1981, as chronic kidney disease associated with severe vesicoureteral reflux. Kidney sonography showed bilateral hydronephrosis, and the initial serum creatinine level was 3.5 mg/dL. Voiding cystourethrography revealed severe vesicoureteral reflux, and urethroscopy confirmed congenital posterior urethral valve. The patient underwent suprapubic cystostomy, and after that, he had been hospitalized on numerous occasions with cystostomy-related complications. In September 1985, he progressed to end-stage renal disease and started hemodialysis with an arteriovenous shunt in his left wrist. In October 1988, he had an episode of arteriovenous fistula thrombosis, and a new Cimino shunt was placed in his right wrist. Five years later, another shunt thrombosis occurred, which was diagnosed by right upper extremity arteriography, and it showed no anatomic abnormality. At that time, another Cimino shunt was created in his left forearm. During the evaluation of the patient's second episode, his thrombophilic predisposition was disclosed. Less than 20% of serum PS activity was determined by clotting assays, whereas other coagulation studies were normal (Table 1). He had started to receive antiplatelet agents after this episode. However, another thrombotic event of vascular access occurred in April 2000, and thrombectomy of the old Cimino shunt was done. At that time, both upper angiographies were negative except for the presence of a thrombus at the arteriovenous shunt. Electrocardiography (ECG) showed a normal sinus rhythm. In August 2009, the patient presented with painful swelling in his right leg, and through evaluation with computed tomography, right popliteal artery thrombosis was diagnosed (Fig. 1). The ECG revealed new-onset atrial fibrillation with slow ventricular response. Transesophageal echocardiography was done to exclude the presence of atrial thrombi, and although the ventricular systolic function and cardiac valves were normal, an echogenic mobile mass at the appendage of the left atrium was found. Based on the transesophageal echocardiography findings and clinical presentation, the mass was concluded to be a thrombus. The patient underwent thrombectomy of the right popliteal artery and was treated with heparin and subsequently with oral anticoagulants under close supervision.

The family history revealed the thrombotic conditions over three generations. The patient's second eldest brother, a 66-year-old man, had spontaneous deep vein thrombosis of the right leg at age 43 years, and thrombectomy of the right femoral vein was done. His father, an 88-year-old man, had a stroke at age 85 years. His paternal uncle and one cousin had a history of strokes, and his paternal grandparents died from stroke. The pedigree of the family is shown in Fig. 2.

Genetic analysis of the family was done to obtain a molecular diagnosis, allowing screening for the family members. A total of 28 members from 4 generations were examined (Fig. 2). The *PROS1* gene was screened by direct DNA sequencing of polymerase chain reaction (PCR) products. After informed consent was obtained, the physician extracted genomic DNA from the patient's whole blood

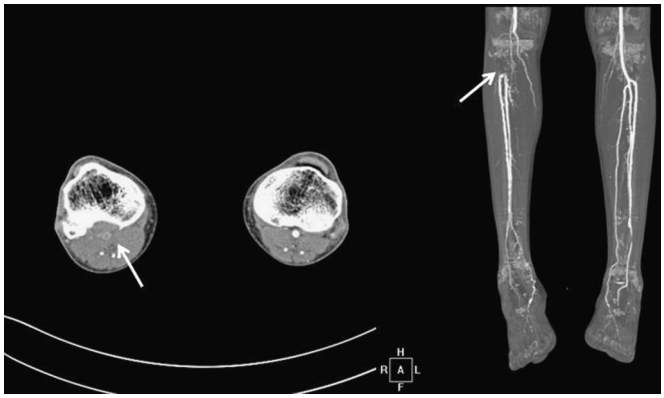

Figure 1. Axial and coronal images of CT angiography. CT angiography shows a total occlusion of the right popliteal artery (arrow).

Table 1. Hemostatic Studies in the Patient

Events	At first event	At second event	At third event	At fourth event	Normal values
The year after AV fistula formation	3 year	5 year	7 year	9 year	
Clinical feature	Vascular access thrombosis	Vascular access thrombosis	Vascular access thrombosis	Right popliteal artery occlusion by thrombus	
Treatment	A new Cimino shunt	Another Cimino shunt, antiplatelet start	Thrombectomy	Thrombectomy, anticoagulation start	
Laboratory parameters					
Hemoglobin, g/dL	10.6	11	11.3	11.9	13–17
Hct, %	30.7	32	33	36.6	39–52
Platelets, /nL	179	199	164	146	130–430
PT, INR	1.01	1.02	0.97	1.07	0.8–1.2
aPTT	26	33.6	34.8	36.9	29–45
Fibrinogen, mg/dL	357	311	544	276	170–350
AT III, %		93	86	85	80–120
D-dimers		1.49	1.05	1.04	<0.4
PS activity, %		13	17	19	70–140
PC activity, %		98	117	92	70–140
APL, IgG		2, negative			≤ 15 GPL
APL, IgM		1, negative			≤ 15 MPL
FANA		Negative			Negative

APL, antiphospholipid; AT III, antithrombin III; FANA, fluorescent antinuclear antibody; Hct, hematocrit; IgG, immunoglobulin G; IgM, immunoglobulin M; INR, international normalized ratio; PS, protein S; PT, prothrombin time.

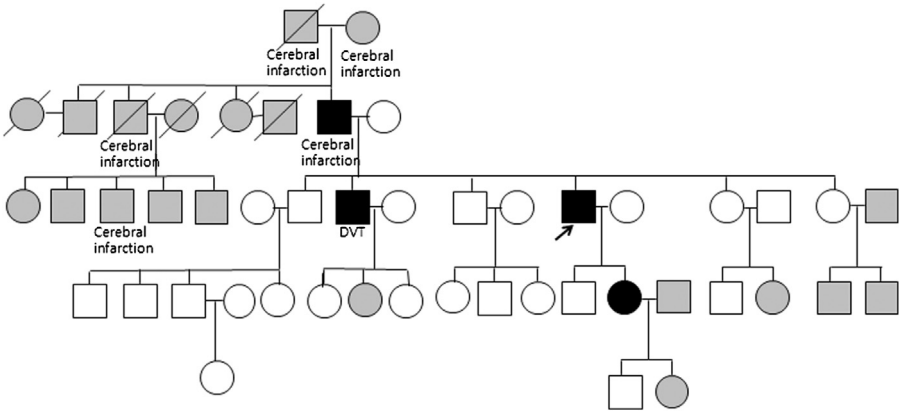

Figure 2. Pedigree of the family under investigation. Squares and circles indicate males and females, respectively, and the squares and circles that are crossed through indicate deceased family members. The arrow denotes the proposita. Black symbols indicate individuals who are affected by *PROS1* gene mutation (c.1417A > T, p.K473X), and gray symbols indicate those whose affection status is unknown.

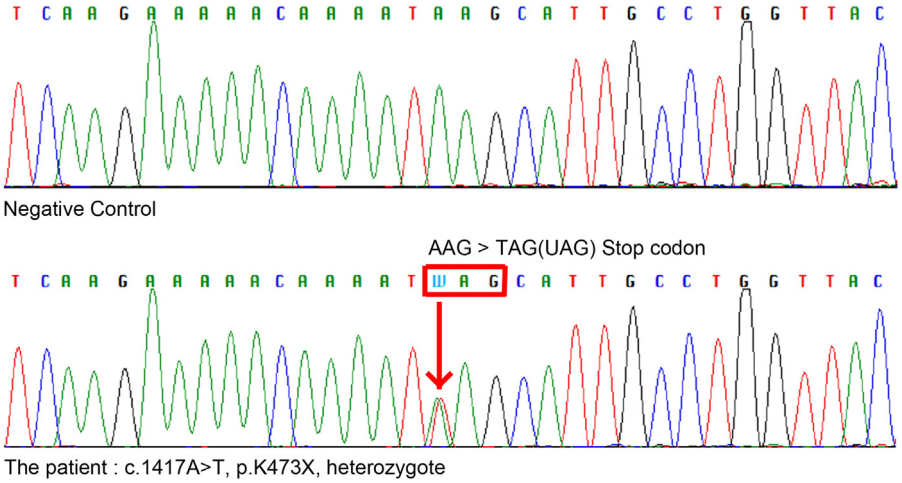

Figure 3. DNA sequence analysis. DNA sequence analysis of PROS1 exon 10 for the patient reveals a novel nonsense mutation.

using the Pure Gene DNA isolation kit. The exon and exon-intron boundary regions were amplified by PCR, and the PCR products of 5′ untranslational regions, coding regions of 15 exons, and flanking regions of *PROS1* gene were subjected to direct sequencing using ABI PRISM 3730 × 1 DNA analyzer. DNA sequencing revealed that the patient had a novel heterozygous nonsense mutation. In exon 10 of the *PROS1* gene, a transition AAG (lysine) to TAG (stop codon) at codon 473 by substitution of A to T at 1417 site (c.1417A > T, p.K473X) was detected (Fig. 3). The same mutation was found in three family members, including the patient's second eldest brother, father, and 28-year-old daughter, who had no clinical history of thrombosis (Fig. 2).

Discussion

Vascular access failure caused by thrombosis is a leading cause of morbidity and hospitalization in the hemodialysis population. Approximately 16–20% of all hospitalizations in these patients are related to vascular access dysfunction based on a survey done in the United States [5]. Inherited PS deficiency is present in 2–5% of patients with thrombosis and is considered a promoter of the hypercoagulable state in such patients [6]. It is inherited as an autosomal dominant trait, and heterozygous individuals frequently have recurrent thromboembolism. Aortic and coronary thrombosis, stroke, and renal vein thrombosis have also been reported in PS-deficient patients. In this case, we showed PS deficiency could also be associated with the thrombosis of vascular access. The patient suffered multiple thrombotic occlusions of the arteriovenous shunt without obvious anatomic cause. No other coagulation abnormalities except PS deficiency were found, suggesting that PS deficiency has a role in the thrombosis of vascular access.

This case draws attention to PS deficiency in patients on hemodialysis. There have been several studies investigating the relationship of PS deficiency with vascular access thrombosis, with a significant correlation between them

[7,8]. Of note, none of these studies used molecular genetic tests to confirm PS deficiency, and the patient discussed here represents a case of genetically confirmed PS deficiency underlying the vascular access thrombosis.

PS deficiency is the most difficult of all the hereditary thrombophilias to document with certainty. Free PS is probably the best screening test [9], although many use the functional PS assay, which has a larger coefficient of variation. PS levels in plasma are subject to biologic, physiologic, and pathologic influences [10]. Genetic analysis can be useful in the diagnosis [11].

PS is encoded by *PROS1*, which spans more than 80 kb of genomic DNA, and is composed of 15 exons and 14 introns. *PROS1* is located near the centromere on chromosome 3q11.2 [6]. So far, almost 200 different *PROS1* mutations resulting in a loss of function have been identified [12]. Although many mutations in PROS1 have been reported, we have identified another novel nonsense mutation. To our knowledge, this is the first description of a new mutation in the *PROS1* gene (c.1417A > T, p.K473X), which seems to have clinical relevance with recurrent vascular access thrombosis.

The optimal management of patients with thrombophilia-related vascular access thrombosis is still unclear. In this case, the patient received antiplatelet agents after the second thrombotic episode, and started anticoagulation after a new-onset atrial fibrillation was detected. An antithrombin agent might increase vascular access graft patency but is associated with the risk of serious hemorrhagic complications, especially in patients with end-stage renal disease. Because of the important role of platelets in thrombus formation, several antiplatelet agents such as aspirin and clopidogrel have been used to prevent perioperative and long-term thrombosis [2].

In conclusion, this case illustrates that genetic anomalies in the PS anticoagulation system could be an important risk factor of vascular access thrombosis. The inherited prothrombotic disorders might be considered as the differential diagnosis of recurrent vascular access thrombosis in patients with hemodialysis.

Conflict of interest

All authors have no conflict of interest. The result presented in this paper have not been published previously in whole or part, except in abstract format.

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