Protein C Deficiency in a Family with Thromboembolism and Identified Gene Mutations

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Abstract

Protein C is the central component of the major anti-thrombotic regulatory system, and individuals with hereditary protein C deficiency tend to have an increased risk of thromboembolism. During the last several years, mutations causing protein C deficiency have been identified. In the present study, we report familial cases with three nucleotide substitutions: One is a missense mutation Arg169Trp, which was previously reported. The other two are C-154T promoter polymorphism (rs1799808 on dbSNP database), the function of which is unknown and Ser99Ser synonymous polymorphism (rs5936). All three mutations were found in a 24-year-old patient with pulmonary thromboembolism and his 54-year-old father who also had pulmonary thromboembolism. C-154T promoter polymorphism (rs1799808 on dbSNP database) and Ser99Ser synonymous polymorphism (rs5936) were found in the patient's mother.

Key words: protein C deficiency, missense mutation, pulmonary thromboembolism, promoter polymorphism, hotspot

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Introduction

Protein C is a vitamin K-dependent zymogen of serine protease that inhibits blood coagulation by the proteolytic inactivation of factors Va and VIIIa (1). Individuals affected with protein C deficiency are at risk for arterial or venous thrombosis (2). The diagnosis of protein C deficiency has been based on the laboratory assay of plasma protein C antigen and activity levels (3). Protein C deficiency has phenotypically been classified into two types. Type I deficiency, the most common, is characterized by the parallel reduction of protein C activity and antigen levels due to the reduced synthesis or stability of normally functioning molecules. In type II deficiency, protein C activity is reduced to a greater extent than the antigen due to the synthesis of an abnormal protein C molecule exhibiting a reduced specific activity (4).

Protein C deficiencies are inherited as an autosomal dominant trait. The protein C gene resides on chromosome 2 and comprises 9 exons (5). Researchers have found over 160 different mutations of the protein C gene that lead to the ab-

sence, or a defective form, of protein C (6). We found three nucleotide substitutions in the patient and his father. The result of protein C activity and antigen levels suggested that the patient and his father might have type II protein C deficiency, which is rare.

The patient's father had been given a diagnosis of pulmonary thromboembolism three years prior to the patient's admission. If the patient had had a chance to examine his protein C activity and antigen, lifestyle-related counseling might have been provided to him. Therefore, we report this case to enhance patient care and provide a review of the literature.

Case Report

A 24-year-old railway worker was admitted to our hospital because of dyspnea on exercise, which had gradually worsened in one month. He had experienced left leg pain three months prior to admission without any cause, such as a predominantly seated position for long time.

The patient had been drinking 0.5 liter of beer per day

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Figure 1. Computed tomography of the chest showing thrombosis of the right pulmonary artery (arrow).

Table 1. Laboratory Data of the Patient on Admission

Hematology		Na	142 mEq/l
WBC	13,200 / μ l	K	3.9 mEq/l
RBC	526X10 ⁴ / μ l	Cl	104 mEq/l
Hb	16.3 g/dl	Immunology	
Ht	49.9 %	CRP	0.3 mg/dl
PLT	45.6X10 ⁴ / μ l	ANA	(-)
Blood chemistry		Anticardiolipin antibody (-)	
TP	7.1 g/dl	Lupus anticoagulant	(-)
Alb	4.7 g/dl	Coagulation test	
BUN	13.4 mg/dl	PT	81 %
Creat	0.9 mg/dl	APTT	30 sec
T-Bil	0.7 mg/dl	Fibrinogen	252 mg/dl
AST	20 IU/l	AT- III	29.4 mg/dl
ALT	26 IU/l	FDP	7.1 μ g/ml
LDH	471 IU/l	D-dimer	4.8 μ g/ml

and smoking 20 cigarettes per day for over the past 4 years. His height was 177 cm and his weight was 56 kg. He was not febrile (36.8°C), his pulse rate was 87/min, and his blood pressure was 118/76 mmHg. There were no heart murmurs and his respiratory sounds were normal. His electrocardiogram showed terminal T inversions in leads II, III, aV_f and V₁₋₄. Computed tomography of the chest showed thrombosis of the right pulmonary artery (Fig. 1). Laboratory studies prior to heparin therapy demonstrated high levels of white blood cell counts (WBC, 13,200/ μ l), of fibrin/fibrinogen degradation products (FDP, 7.1 μ g/ml), and D-dimer (4.8 μ g/ml). His prothrombin time (PT) was 81% and activated partial thromboplastin time (APTT) was 30 seconds (normal range: 25.0-38.0 seconds). Only D-dimer was slightly elevated. Protein C antigen and activity were 68% (normal range: 70-150%) and 43% (normal range: 64-146%), and corresponding values for protein S were 72% (normal range: 65-135%) and 82% (normal range: 60-150%), respectively. Protein C activity and Protein S activity were measured by the APTT clotting time method. Protein C antigen was measured by latex photometric immuno-

Table 2. Laboratory Data of the Patient's Father on Admission

Hematology		Na	138 mEq/l
WBC	13,200 / μ l	K	3.6 mEq/l
RBC	380X10 ⁴ / μ l	Cl	104 mEq/l
Hb	12.4 g/dl	Immunology	
Ht	35.8 %	CRP	16.1 mg/dl
PLT	45.6X10 ⁴ / μ l	ANA	(-)
Blood chemistry		Anticardiolipin antibody (-)	
TP	5.2 g/dl	Coagulation test	
Alb	2.5 g/dl	PT	51 %
BUN	7.8 mg/dl	APTT	111 sec
Creat	0.7 mg/dl	Fibrinogen	641 mg/dl
T-Bil	0.7 mg/dl	AT- III	19.8 mg/dl
AST	59 IU/l	FDP	12.0 μ g/ml
ALT	69 IU/l	D-dimer	3.5 μ g/ml
LDH	489 IU/l		

assay. Protein S antigen was measured by enzyme immunoassay (7, 8). Lupus anticoagulant was negative (Tables 1, 3).

Initial intravenous administration of heparin (15,000 U/day) and urokinase (480,000 U/day for 2 days, then 120,000 U/day for 5 days) was followed by warfarin potassium combined with heparin. Then an oral anticoagulant (warfarin potassium) was continued to keep PT INR within the range from 2 to 3. Dyspnea on exercise improved. Computed tomography of the chest performed on day 32 showed no detectable thrombosis of the right pulmonary artery (data not shown).

The patient's father had been given a diagnosis of protein C deficiency accompanied by pulmonary thromboembolism at our hospital three years previously, who suffered sudden onset of dyspnea also without any cause. Protein C antigen and activity were 56% (normal range: 70-150%) and 28% (normal range: 64-146%), and corresponding values for protein S were 120% (normal range: 65-135%) and 120% (normal range: 60-150%), respectively (Tables 2, 3). His father also had received anticoagulant therapy.

The patient's father had 4 sisters (Fig. 2). None of them had any episode of pulmonary thromboembolism. The patient's sister had normal protein C and S antigen levels and activity (Table 3). The patient's mother also had normal protein C and S antigen levels and activity (Table 3).

Gene analysis was performed in the patient, his father, his mother and sister after written informed consent was obtained from all four subjects.

This genetic analysis was approved by our institutional ethical committee and genetic counseling was appropriately provided to the family. Isolation of genomic DNA from whole blood was followed by PCR/direct sequencing analysis of the protein C gene. DNA sequence was performed

Table 3. Phenotypic and Genotypic Data From the Patient and Family Members

Family member	PC activity (%) (NR 64–146 %)	PC antigen (%) (NR 70–150 %)	PS activity (%) (NR 60–150 %)	PS antigen (%) (NR 65–135 %)	PROC mutation		
					C6218T (Arg169Trp)	G3342T (Ser99Ser)	C-154T
II-5	28	56	120	120	+	+	+
II-6	118	144	71	112	—	+	+
III-1	77	92	N.T.	N.T.	—	—	—
III-2	43	68	82	72	+	+	+

NR: normal range, N.T.: not tested

Figure 2. The pedigree of the family. The patient is indicated by an arrow. † : deceased. ○ and □ : unaffected females and males. Half-solid symbols represent affected females and males with abnormal protein C gene and heterozygosity for protein C deficiency.

both 5'→3' and 3'→5' direction and we confirmed the entire matching of the DNA sequence. The sequence analysis of the patient's protein C gene and his father's protein C gene indicated a heterozygous transition of C to T at nucleotide 6,218 in exon7, which resulted in the substitution of the arginine for the tryptophan at amino acid residue 169 (Fig. 3). This mutation was not found in the patient's mother or the patient's sister (Fig. 3). Sequence analysis of the protein C gene indicated C-154T promoter polymorphism (rs1799808 on dbSNP database) and Ser99Ser synonymous polymorphism (rs5936) in the patient, his father and mother (Table 3).

Discussion

Griffin et al described familial thrombosis in association with protein C deficiency in 1981 (9). They emphasized the clinical importance of protein C in controlling the hemostatic system. Since that time, numerous cases of hereditary protein C deficiency have provided convincing evidence of the fact that individuals with protein C deficiency tend to have an increased risk of thromboembolism (10).

The prevalence of heterozygous protein C deficiency is estimated to be one in 200 to 300 of the general population (11). However, only a minor subgroup of these subjects develop thrombotic complications at a young age, whereas

most others remain asymptomatic throughout their lives (12). Estimates of the prevalence of clinically symptomatic deficiencies of protein C in the general population lie between 1 : 16,000 and 1 : 36,000 (13). This finding suggests that the severity of clinical manifestations may be related to some additional coagulation defects or genetic factors (14). In the present case, though we did not study all coagulation factors, only protein C deficiency was found as a risk factor for pulmonary thromboembolism of the patient and his father. For example, the ATIII level of the patient was normal and of his father was only 15% lower than normal range, so there was little possibility that their ATIII activity caused their pulmonary thromboembolism.

Thus, the spectrum of clinical manifestations caused by protein C deficiency is rather wide. Usually protein C deficiency is thought to be transmitted as autosomal dominant with significant variable penetrance, but individuals homozygous or compound heterozygous for protein C deficiency suffer from massive disseminated intravascular coagulation or fatal neonatal purpura fulminans (15, 16).

In heterozygous deficiency, the levels of plasma protein C are usually between 35-65% of normal level, whereas the majority of normal individuals have levels between 70-130% (10). The protein C deficiency members with genetic mutation in this study had protein C activities between 28-43% of the normal level, suggesting that the two affected individuals are in a heterozygous state with autosomal dominant inheritance of low penetrance with regard to thrombosis (17).

Both a quantitative and qualitative decrease in protein C were recognized, the former being type I deficiency and the latter, type II (18). The initial diagnosis of either form involves functional activity assay while differentiation between the two also requires an antigenic immunological assay (19). In this report, antigenic assays were also performed. Although variable, protein C activity of the patient was reduced to a greater extent than antigen. Protein C activity divided by antigen was 0.63. Protein C activity of his father was also reduced to a greater extent than antigen. Protein C activity divided by the antigen was 0.5. A ratio of protein C to antigen less than 0.7 suggests type II deficiency (4). Therefore, these data suggested that both the patient and his father might have type II deficiency. The patient's mother and sister had normal levels of both protein C activity and

Figure 3. DNA Sequences of the exon 7 of protein C. a: the patient, b: his father, c: his mother, d: his sister. The patient, his father, and his mother have C to T change at nucleotide number 6218 (arrow) of the protein c gene. This missense mutation causes that CGG coding for Arginine-169 in the heavy chain to be changed to TGG coding for tryptophan.

antigen.

In this paper, we identified the same C to T change at nucleotide number 6,218 of the protein C gene in the patient and his father. This missense mutation caused CGG coding for arginine-169 in the heavy chain to be changed to TGG coding for tryptophan. This mutation abolishes the site of thrombin cleavage that activates protein C (20). However, unaffected family members without protein C deficiency showed the same pattern as those of normal control subjects.

The identified missense mutation in this study was previously reported. Sakata et al examined 67 Japanese families with protein C deficiency including the results of other studies in 10 families (21). They identified 39 different gene defects, including 29 missense mutations, 2 nonsense mutations, 4 deletions and 4 splice site mutations (Table 4). Of 29 missense mutations, 21 were type I deficiency and only 8 were type II deficiency. The identified missense mutation in this study (Arg169Trp) was previously reported in 7 Japanese families and in 10 families worldwide (mutation of a CG to either TG or CA) (Tables 4, 5) (6, 21). Sakata et al (21) reported that all 7 Japanese families had type I deficiency. Reitsma et al (6) reported that PC-44-009 was probably type II and PC-43-011 was type II. PC-44-009 had a same missense mutation Arg169Trp as the present case. PC-43-011 had a missense mutation Arg169Gln. In the present case, the patient and his father may have type II deficiency, which is rare. 169Arg mutations are sometimes associated with type II deficiency but also with type I. The reason for the discrepancy is unknown (4). Since the C of the CG dinucleotide is frequently methylated, and subsequently deaminated, this event explains why CG dinucleotides are hotspots for mutation in the human DNA (22, 23).

We searched for possible causative mutations within at least 500 base pairs of promoter regions and all nine exons and exon-intron boundaries of the protein C gene and found three nucleotide substitutions: One was a missense mutation Arg169Trp, and the other two were C-154T promoter polymorphism (rs1799808 on dbSNP database) and Ser99Ser synonymous polymorphism (rs5936). All three mutations were found in the patient and his father. C-154T promoter polymorphism (rs1799808 on dbSNP database) and Ser99Ser synonymous polymorphism (rs5936) were found in the patient's mother. Five cis-elements were reported to regulate protein C expression (24). The region -88 to +45 was the main positive promoter, the region -418 to -298 was a weak positive promoter, and the region -144 to -88 was a weak negative promoter. Two different mutations of 5'-Untranslated region were identified in Japan, which were G-1458A and A-1469C (Table 4). Eight different mutations of 5'-Untranslated region were identified in Western countries: which were A-1533 G, T-1528A, T-1515C, C-1511T, G-1480A, C-93T, C-26T, and G-17C. The importance of the transition of C to T at nucleotide -154 in promoter region, which we identified is unclear. However, possible regulatory effects of promoter polymorphism on the protein C gene expression might affect the penetrance of individuals in heterozygous deficiency and might cause type II deficiency. If both parents had protein C deficiency, the child might tend to have symptomatic thrombosis, though the patients are asymptomatic (25). In the present case protein C activity seemed to recover in the patient compared to his father (Table 3), which might also suggest a possible role of C-154T mutation. The nucleotides of the protein C gene in this are numbered according to Foster et al (26).

Table 4. Genetic Defects of Protein C Deficiency Found in Japanese Subjects

Mutation	name of Proband	Total in Japanese	Worldwide
Missense mutations			
M-42T	Osaka 5	1	none
V-17M	Osaka 45	1	1 family
H-9Q	Osaka 47	1	none
R-1S* (II)	Osaka 10	1	none
R15G (II)	Yonago	1	none
E26K (II)	Mie	1	none
G83R*	Osaka 14	1	none
H107P	Osaka U2	1	none
F139V	Osaka 11, 19, 27, 29, 40	5	none
R143S	Osaka 38	1	none
R169W*	Osaka 1, 31, 42, 43, 46		
	Tochigi, Unnamed	7	10 families
R178Q*	Tokyo 1	1	6 families
H202Y	Osaka 37	1	1 family
G282S*	Osaka 29	1	4 families
R286H*	Osaka 18, 22	2	2 families
G292S*	Nagoya 2	1	4 families
V297M*	Osaka 17, 20, 24, 26, 30		
	41, 48, Nara, Fukuoka	9	6 families
T298M*	Osaka 44	1	5 families
P327L*	Osaka 6	1	2 families
C331R	Tokyo 1	1	none
E341G	Sakaiminato	1	none
G350R* (II)	Osaka 9	1	1 family
D359N* (II)	Osaka 7, 8, 37	3	2 families
M364I	Osaka 2, 21, 28, 35, 39		
	Kawasaki 2	6	none
F368S	Osaka 13	1	none
W380C (II)	Osaka 15	1	none
G385R (II)	Osaka 3	1	none
L387F (II)	Osaka 23	1	none
Y399H	Osaka 4	1	none
Nonsense mutations			
E92stop*	Osaka 12	1	2 families
R157stop*	Kyoto	1	5 families
Deletions and addition			
K150del	Osaka 36, 39, Kitakyushu	3	none
D255del	Osaka 16	1	none
G8857del	Osaka 25, Tokyo 2, Nagoya 1		
	Mie, Kawasaki 2, Tsuruoka	6	none
G7246add	Kawasaki 1	1	none
Splice site			
G3079A	Osaka 32, Osaka U1	2	none
G3218C	Osaka 49	1	none
5'-untranslated region			
G-1458A	Osaka 33	1	none
A-1469C	Osaka 34	1	none
Families		67	
Defects		39	

*: mutation of a CG to either TG or GA, (II): type II mutation

Table 5. Genetic Defects of Protein C Deficiency Found in Subjects Worldwide

Pedigree	Antigen (% of normal)	Activity (% of normal)	Nucleotide position and mutation	Amino acid change	Co segregation
PC-49-010	31	29	6218 C→T	169; R→W	5
PC-39-I-013	25	15	6218 C→T	169; R→W	4
PC-39-I-041	84	48	6218 C→T	169; R→W	
PC-81-Tochigi	19	<1	6218 C→T	169; R→W	
PC-81-Osaka 1	60	49	6218 C→T	169; R→W	2
PC-I-Unnamed	52	50	6218 C→T	169; R→W	2
PC-44-009	40	30	6218 C→T	169; R→W	3
PC-43-011	36	23	6219 G→A	169; R→Q	2
PC-31-LETS104		47	6219 G→A	169; R→Q	
PC-31-LETS623		59	6219 G→A	169; R→Q	

After treatment with heparin, followed by oral anticoagulant (warfarin potassium), deep vein angiography of the patient disclosed no thrombosis of the femoral veins of the right leg (data not shown). Also deep vein angiography of his father disclosed no thrombosis of the femoral veins of both legs (data not shown). Both of them had no episodes of spending many hours per day in a predominantly seated position (27), so the trigger of pulmonary thromboembolism was unclear.

In summary, we studied the molecular basis of protein C deficiency in the Japanese family of this patient with pulmonary thromboembolism. His father had been given a diagnosis of pulmonary thromboembolism. If the patient had had a

chance to examine his protein C activity and antigen, lifestyle-related counseling might have been provided to him. Therefore if there is a possibility of protein C deficiency in children, screening of the parents, careful monitoring of pregnancy and occasional antenatal diagnosis should be performed. Further studies on the genetic analysis of hereditary protein C deficiency are required in the Japanese population and we hope that this case will be a useful model for these studies.

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