

A Novel Silent Substitution (C8516T) in Exon 9 of the Human PROC Gene

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Protein C is a vitamin K dependent serine protease zymogen, which has a regulatory influence over the coagulation cascade via the inhibition of factors Va and VIIIa. Hereditary protein C deficiency is associated with an increased risk of thromboembolic disease. A multitude of families displaying protein C (PROC) gene defects have been reported, and a number of DNA sequence polymorphisms are known to occur in the PROC gene. We have identified a previously undescribed silent substitution (C8516T) by direct DNA sequencing in a Korean patient with thrombosis and protein C deficiency. In addition, a rare T allelic frequency (0.016) was determined in 123 patients with acquired or hereditary protein C deficiency.

Key Words: Protein C deficiency, silent mutation, PROC gene, thrombosis

INTRODUCTION

Protein C is the zymogen of a serine protease that fulfills an important anticoagulant role by inactivating activated factor V and factor VIII.¹ The human protein C (PROC) gene is located on chromosome 2q13-14, and contains nine exons separated by eight introns spread over 11 Kb of genomic DNA.² Hereditary protein C deficiency is an autosomally inherited disorder associated with a high risk of recurrent thromboembolic disease.³ More than 160 different mutations have been described for the PROC gene, and these are compiled on a regular basis in a database.⁴ In this study, we report a novel silent substitution of a DNA sequence in the human PROC gene in a patient with thrombotic episode, as well as allelic frequency of this silent mutation in Korean patients with protein C deficiency and thrombosis.

MATERIALS AND METHODS

Case

The patient was a 63-year-old Korean man admitted to the hospital because of dysarthria and diplopia for 6 days. The patient had been suffered from diabetes mellitus and immune thrombocytopenia for 4 years. A diagnosis of brain stem infarction and DM retinopathy was made. Plasma protein C activity was 38% (normal reference range: 70-130%) and free protein S antigen level was 98% (normal reference range: 70-130%). Liver function test and complete blood count were within normal ranges, though platelet count was low (63,000/ μ l).

Direct DNA sequencing

Genomic DNA was extracted from the patients peripheral blood. The exons and flanking intron regions of the PROC gene were amplified and directly sequenced as described previously.⁵ The nucleotides of the PROC gene are numbered according to Foster et al.²

Additionally, blood samples were drawn from 122 unrelated patients with thrombosis including acquired (n=117) and hereditary (n=5) protein C deficiency and direct sequencing analysis of exon 9 was performed to determine the allelic frequency in all of the study subjects.

Protein C assay

Protein C amidolytic activity was measured using a Stachrom kit (Stago, Asnieres, France) by STA coagulyzer (Stago, Asnieres, France).

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RESULTS

Direct sequencing in this case showed the presence of a novel silent substitution (C8516T, Ala267) in exon 9 of the *PROC* gene, which was heterozygous for the T allele (Fig. 1). Sequence variations were not present elsewhere in the *PROC* gene in this particular case.

Further DNA analysis of exon 9 in 28 thrombotic patients (including this case) and in 95 non-thrombotic patients indicated that the rare allele (T) frequency in the Korean population is 0.016, with an observed heterozygosity (C/T) and homozygosity (T/T) of 1.6% and 0.8%, respectively, while the other allele (C) frequency was 0.984. Protein C deficiencies in three unrelated patients with heterozygous (C/T) or homozygous (T/T) genotype were acquired rather than

inherited, as judged by clinical conditions and a genetic mutation study (Table 1).

DISCUSSION

The novel substitution of DNA sequence (C8516T) was identified by direct sequencing in a diabetic patient with brain stem infarction and acquired protein C deficiency. Cerebral thrombosis may be associated with both hereditary and acquired protein C deficient states.⁶ Hereditary protein C deficiency is not likely in this case because of a negative family history of thrombosis and the onset of thrombosis at an advanced age. In addition, no specific mutation was found in the *PROC* gene study. Protein C is consumed in intravascular coagulation states, vitamin K dependent, made in liver, and has a relatively short half-life in the circulation (7-9 h).⁶ Therefore, it is not surprising that decreased protein C levels are observed in many disease conditions including diabetes.

Twenty-seven patients with thrombosis were also studied for this novel silent substitution by direct sequencing of the amplified exon 9. However, none showed the same substitution (C8516T), indicating that this sequence variation is not common in the Korean population. This finding was supported by the fact that only one homozygous and one heterozygous patient were found among 95 protein C-deficient patients without thrombosis.

This mutation did not alter the amino acid composition (Ala267Ala) and is not likely to have a detrimental effect. However, the presence of this mutation in three protein C-deficient individuals suggests that this mutation could possibly

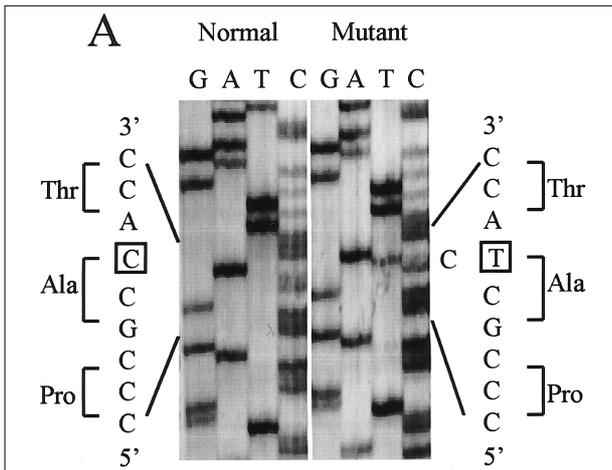


Fig. 1. DNA sequences in exon 9 of the *PROC* gene showing the novel silent mutation. The nucleotide affected by the mutation is boxed. DNA sequence (sense strand) of the heterozygous form. Bands at the same position in the C and T lanes indicate the heterozygosity at nucleotide position 8516.

Table 1. Clinical Data of Three Unrelated Patients Heterozygous or Homozygous for Substitution (C8516T) in the *PROC* Gene

Case	Sex/Age(yr)	Protein C	Thrombosis	Clinical conditions	Genotype (C8516T)
Proband	M/63	38%	Yes (Stroke)	Diabetes ITP	Heterozygous
D095	M/55	50%	No	Diabetes, ESRD	Homozygous
D109	M/84	46%	No	VB insufficiency, Hypertension	Heterozygous

ITP, idiopathic thrombocytopenic purpura; ESRD, end stage renal disease; VB, vertebrobasilar.

modulate the phenotypic expression of protein C.

A number of DNA sequence polymorphisms are known to occur in the *PROC* gene. These include an A/T polymorphism at nucleotide -1476 within the 5 non-coding region, C/T(-1654) and A/G(-1641) polymorphisms within the promoter region, G6376T in intron 7, and silent substitutions within codons Arg 87, Ser 99, Lys 156, Asp 214, and Asp 255(C8480T).^{4,7-9} Among these polymorphisms, we observed A/T polymorphisms at nucleotide -1476 within the 5 non-coding region in the Korean population indicating that the allelic frequencies were 0.85 (allele A) and 0.15 (allele T), which was similar in Asians (0.26 for allele T) and in the Chinese (0.133 for allele T) but different in American Caucasians (0.55 for allele T).^{8,10} In addition, we also have observed Ser 99, Asp 255 polymorphisms in the Korean population with some interethnic differences in allelic frequencies.

In this study, we report a case of a novel point variation for the worldwide database.

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