Original Article

Gene diagnosis of seven patients with hereditary protein S deficiencies

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Abstract: Background: Hereditary protein S (PS) and protein C (PC) deficiencies are caused by gene mutations. The present research aimed to determine gene mutation sites in patients with thrombosis due to protein S deficiencies combined or not combined with protein C deficiencies. Methods: Patients with protein S deficiencies were enrolled. DNA was extracted from peripheral blood samples from these patients. All exons and their flanks of protein S genes (PROS) were amplified by polymerase chain reaction (PCR). After purification, the PCR products were sequenced directly and blasted to normal sequences, seeking out gene mutation sites. Polymorphism analysis was conducted by detecting the certain mutation in 50 normal people. Results: Seven patients were enrolled with thrombosis. They were diagnosed of protein S deficiencies combined or not combined with deficiencies of protein C. Polymorphism site G68395T on exon4 was identified in all seven patients. Non-sense mutation C68430T on exon4 was identified in patients No. 2 and No. 5. Missense mutation C86066T on exon10 was identified in patient No. 2. Missense mutation G82512C on exon9 was identified in patient No. 3. Searching PubMed, C86066T had been reported previously in a Hong Kong study, while C68430T and G82512C on PROS were the first ones reported worldwide. Conclusion: Polymorphism site G68395T and missense mutation C68430T, C86066T, and G82512C on PROS were identified and may be related to deficiencies of protein S. C68430T and G82512C were reported worldwide.

Keywords: Thrombosis, protein S, protein C, gene mutation, polymorphism

Introduction

Venous thrombosis may be caused by a variety of factors, including pregnancies, tumors, surgeries, and immobilization. It may also be related to hereditary factors, such as protein C and protein S deficiencies, antithrombin deficiencies, and Factor V Leiden and G20210A mutations in prothrombin [1-3]. Patients forming vein thrombosis due to hereditary factors have a tendency of young age manifestation and higher recurrence rates than those suffering from acquired factors [4]. In the Caucasian population, FV Leiden and G20210 prothrombin mutations show a high frequent occurrence [5-7], while there has been limited related discoveries of such mutations in Asian populations. Deficiencies of protein C and S consist of the main etiology of hereditary thrombophilia in Asia populations [7-9]. Most studies on protein C and S have been restricted to Japanese and Korean populations [9-13]. Chinese studies on this category are particularly sparse [8, 14, 15, 22]. Protein C and S are both vitamin K dependent proteins, playing a key role for anticoagulation in the circulatory system. Deficiencies of proteins C and S due to gene mutations lead to moderate to severe thrombosis, causing disabilities and critically affecting life quality [16]. It is extremely urgent to identify mutation sites and types which may offer references to early intervention and guidance for future gene-targeted therapy.

Protein C is a Vitamin K dependent plasma protein produced by the liver. Human protein C gene (PROC) is localized in chromosome No. 2 and covers genomic DNA for 11 kb length. It is...
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Protein C transfers into activated Protein C (APC) under regulation of thrombin or thrombin-thrombomodulin complex [17]. Together with its cofactor Protein S, APC inactivates FVa and FVIII, increases fibrinolytic activity, having anticoagulant effects. Inactivation of FVa and FVIIla decreases and hemolytic capacity of blood circulation reduces when protein C is deficient, resulting in excessive formation of fibrin and thrombosis [15]. A protein C deficiency pedigree was first reported by Griffin et al. in 1981 [18]. Until now, more than 300 kinds of PROC mutations have been discovered. However, only a few of them involve Asian populations. This is a paradox since Asian populations have a higher incidence in this scenario [9-15].

Protein S is a Vitamin K dependent single chain glycoprotein that was first discovered in plasma by DiScipio et al. in 1977 [19]. Approximately sixty percent of protein S is complexed to C4b-binding protein, while the remaining forty percent of protein S plays as a cofactor of APC in the anticoagulant process in APC-dependent pathways. It can perform APC-independent pathways by inhibition of the prothrombinase and tenase complexes [20]. Human protein S gene (PROS) spans a length of 80 kb and comprises of fifteen exons. PROS with transcriptional activity is localized in Chromosome 3p11.1-11.2 [12]. Protein S is mainly synthesized in the liver and other tissues, such as endothelial cells, monocytes, megakaryocytes, and osteoblasts, with a small amount of synthesis [22]. The half-life of protein S in vivo is approximately 42 hours. Same as deficiencies of protein C, deficiencies of protein S also have a high correlation with formation of thrombosis [23]. Reports related to hereditary protein S mutations among Asians are rare, especially in Chinese populations [8].

The aim of this study was to identify hot gene mutations among patients enrolled, enriching the gene mutation spectrum of Chinese populations. In addition, this study attempted to determine the gene diagnosis of patients with protein S deficiencies combined or not combined with protein C deficiencies.

Methods

Patients

Patient No. 1 protein S deficiency combined with protein C deficiency: Patient No. 1, a female, 40 years old, was admitted to the hospital due to ‘left lower limb swelling for one week’. PC activity (PC:C) was 48% (PC:C normal range is detected by machine in the hospital is 60%-140%), PS activity (PS:C) was 26.3% (PS:C normal range is 59%-118%), and AT activity (AT:C) was 75.6% (AT:C normal range is 103.2%-113.8%). Color Doppler ultrasonography showed formation of deep vein thrombosis in the left lower limb. The patient had no special history, never manifesting with thrombosis in the past. She denied the history of a close relative marriage. Other family members never showed severe thrombotic events. They refused to come to the hospital for screening. The patient was diagnosed with deep vein thrombosis in the left lower limb. After inferior vena cava filter implantation and thrombolytic measures, together with anticoagulant therapy, the patient improved and went home.

Patient No. 2 Protein S deficiency combined with protein C deficiency: Patient No. 2, a female, 32 years old, was admitted to the hospital due to ‘progressive swelling of double lower limbs’ for one month, aggravated with skin rupturing for 2 days. PT and APTT were not condensate (had already received anticoagulant therapy before admitted). D-dimer was 6.350 mg/L and hemoglobin was 62 g/L. PC:C was 27%, PS:C was 22.9%, and AT:C was 86.7%. Color Doppler ultrasonography showed formation of deep vein thrombosis in both lower limbs, as well as stenosis in upper and lower limb arteries. The patient had no special history, never manifesting with thrombosis in the past. She denied the history of a close relative marriage. Other family member information was lacking. The patient was diagnosed with deep vein thrombosis in both lower limbs, as well as stenosis in upper and lower limb arteries. After anticoagulant and anti-infection therapy, the patient died of respiratory failure, septic shock, and disseminated intravascular coagulation (DIC).

Patient No. 3 Protein S deficiency combined with protein C deficiency: Patient No. 3, a
female, 25 years old, was admitted to the hospital due to swelling and pain for three weeks. PC:C was 56%, PS:C was 52.3%, and AT:C was 78.3%. Color Doppler ultrasonography showed formation of vein thrombosis in the right iliac and femoral vein. The patient had no special history, never manifesting with thrombosis in the past. She denied a history of close relative marriage. Other family members never showed severe thrombotic events and they refused to come to the hospital for screening. The patient was diagnosed with vein thrombosis in the right iliac and femoral vein. After filter implantation, thrombolytic measurements, and anticoagulant therapy, the patient improved and went home.

Patient No. 4 Protein S deficiency: Patient No. 4, a female, 49 years old, was admitted to the hospital due to menopause for 41+4 weeks, without production trillion. D-dimer: 1.92 mg/L. PC:C was 83.3%, PS:C was 38.8%, and AT:C was 89.9%. Color Doppler ultrasonography showed formation of vein mini thrombosis in the left lower limb. The patient had no special history. She received thrombolytic therapy in the left lower extremities due to deep vein thrombosis at the age of sixteen. No recurrence had been discovered by regular examinations. She denied a history of close relative marriage. Other family members never showed severe thrombotic events and they refused to come to the hospital for screening. She was diagnosed with a pregnancy for 41+1 weeks and left lower limb thrombosis. The patient refused vaginal trial production. After a cesarean section and anticoagulant therapy, the patient was discharged.

Patient No. 5 Protein S deficiency: Patient No. 5, a female, 34 years old, was admitted to the hospital due to swelling and pain in left lower limb for one week. PC:C was 81.0%, PS:C was 35.2%, and AT:C was 83.5%. Color Doppler ultrasonography showed formation of thrombosis in the left iliac vein. The patient had no special history, never manifesting with thrombosis in the past. She denied a history of close relative marriage. Other family members never showed severe thrombotic events and they refused to come to the hospital for screening. The patient was diagnosed with deep vein thrombosis at the age of sixteen. No recurrence had been discovered by regular examinations. She denied a history of close relative marriage. Other family members never showed severe thrombotic events and they refused to come to the hospital for screening. The patient was discharged.

Patient No. 6 Protein S deficiency: Patient No. 6, a male, 25 years old, was admitted to the hospital due to coughing with hemoptysis for one day. PC:C was 76.0%, PS:C was 43.7%, and AT:C was 89.8%. Computed Tomography Angiography (CTA) showed superior mesenteric artery thrombus formation. The patient had no special history, never manifesting with thrombosis in the past. He denied a history of close relative marriage. Other family members never showed severe thrombotic events and they refused to come to the hospital for screening. The patient was diagnosed with thrombosis in the superior mesenteric artery. After superior mesenteric angiography, filter implantation, and anticoagulant therapy, the patient discharged was with improvement.

Patient No. 7 Protein S deficiency: Patient No. 7, a male, 25 years old, was admitted to the hospital due to sudden onset of pain in upper abdomen for 36 hours. PC:C was 76.0%, PS:C was 43.6%, and AT:C was 91.0%. CTA showed a left pulmonary embolism. Color Doppler ultrasonography showed formation of deep vein thrombosis in the left lower limb. The patient had no special history, never manifesting with thrombosis in the past. He denied a history of close relative marriage. Other family members never showed severe thrombotic events and they refused to come to the hospital for screening. The patient was diagnosed with a left pulmonary embolism and left lower limb deep vein thrombosis. The patient refused to undergo anticoagulant therapy and went home due to economic problems.

Except for Patient No. 2, APTT, PT, and TT of all patients were at the normal range, with D-dimer increasing to varying degrees. The normal range of three anticoagulant proteins are as follows: PC:C 60-140%, PS:C 59-118%, and AT:C 103.2-113.8%. Results of anticoagulant protein activities of the seven patients are shown in Table 1.

Methods

Gene analysis

After obtaining informed consent from all patients and family members, peripheral blood samples were collected. Extract DNA genome with E.Z.N.A. SE Blood DNA Kit (Solarbio Bioscience & Technology Co.LTD. Shanghai, China) was used. DNA exons and their flanks were amplified by the PCR method. PCR amplifica-
tion primers were designed by Primer 5.0 (Primer, Canada) [14]. After purification, the dideoxy chain termination method was introduced to sequence PCR products on an ABI 3130 sequencer (Meiji Bioscience & Technology Co.LTD, Shanghai, China). Sequencing results were compared with the normal sequences exhibited in NCBI GeneBank published in PubMed by Chromas and Lasergene software (Technelysium, Australia). The serial number of Protein S gene sequence and encoding information in GeneBank was NG_009813.1, NP_000304.2. In a previous study, present researchers also sequenced PROC, comparing it to normal sequences in PubMed. The serial number of Protein C gene sequence and encoding information in GeneBank was NG_016323.1, NP_000303.1.

The aim was to discover gene mutation sites by comparing sequences of probands with the normal sequence, confirming it by reverse sequencing.

**Gene polymorphism site analysis**

After obtaining informed consent of all people enrolled, this study extracted DNA from peripheral blood samples of fifty healthy people. DNA was amplified by the PCR method to confirm whether the sites found were gene mutations or polymorphisms.

**Results**

**Protein S gene analysis**

In patient No. 1, this study only detected a polymorphism site G68395T, which could cause 90th amino acid substitution from Arginine to Leucine.

In patient No. 2, polymorphism site G68395T was found on exon4. This study also found heterozygous mutation site C68430T causing a nonsense mutation, leading to subsequent protein coding terminated after 101th amino acid. This nonsense mutation was considered the extremely serious mutation type that can cause severe altering in protein function. This is coincidental with the fact that patient No. 2 suffered with life threatening thrombosis, developing DIC and sepsis shock, eventually leading to death. To supplement, a heterozygous mutation site C86066T was also found on exon10 of protein S in this patient, causing 355th amino acid substitution from Arginine to Cysteine. Sequencing results of these two mutation sites are exhibited in Figures 1, 2, respectively.

In patient No. 3, polymorphism site G68395T causing Arg90Leu also appeared. Heterozygous mutation G82512C was detected on exon9, leading 321th amino acid altering from Serine to Threonine. Sequencing results of this newly discovered mutation site are illustrated in Figure 3.

In patient No. 4, only polymorphism site G68395T was found on exon4 of protein S gene.

In patient No. 5, this study found polymorphism site G68395T on exon4 of protein S gene and heterozygous mutation site C68430T, causing Gln101Ter nonsense mutations. Sequencing results are exhibited in Figure 4.

In patients No. 6 and No. 7, only polymorphism site G68395T was found on exon4 of protein S gene.

Polymorphism site G68395T was found among fifty healthy normal people. This site was first identified in a protein S deficiency pedigree in a previous research [24]. The polymorphism site is shown in Figure 5.

Gene mutations on PROS, discovered above, among these seven patients, and other synonymous mutation sites are summarized in Table 2.

In patient No. 2, polymorphism site G68395T was found on exon4. This study also found heterozygous mutation site C68430T causing a nonsense mutation, leading to subsequent protein coding terminated after 101th amino acid. This nonsense mutation was considered the extremely serious mutation type that can cause severe altering in protein function. This is coincidental with the fact that patient No. 2 suffered with life threatening thrombosis, developing DIC and sepsis shock, eventually leading to death. To supplement, a heterozygous mutation site C86066T was also found on exon10 of protein S in this patient, causing 355th amino acid substitution from Arginine to Cysteine. Sequencing results of these two mutation sites are exhibited in Figures 1, 2, respectively.

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In patients No. 6 and No. 7, only polymorphism site G68395T was found on exon4 of protein S gene.

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Gene mutations on PROS, discovered above, among these seven patients, and other synonymous mutation sites are summarized in Table 2.

Mutation G68395T is polymorphism site of PROS. Heterozygous mutation C68430T could terminate protein coding process, while C86066T and G82512C had the effect on substituting amino acids to alter protein space conformation. In searching through articles pub-
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Figure 1. Heterozygous mutation C68430T on exon4 of protein S gene in Patient No. 2 (A: mutation type, B: wild type). The arrow points to the mutation site. (A) is the sequencing results of exon4 on PROS in patient No. 2; (B) is the wild type sequencing results.

Figure 2. Heterozygous mutation C86066T on exon10 of protein S gene in Patient No. 2 (A: mutation type, B: wild type). The arrow points to the mutation site. (A) represents the sequencing results of exon10 on PROS in patient No. 2; (B) represents the wild type sequencing results.

In a previous study, polymorphism sites C4867T and A50-45T, along with missense mutation A6578T, A15240G, and deletion mutation AGA12702-12704, 12705-12707del, were discovered on PROC in patients combined with protein C deficiencies. This result was published in Chinese Journal of Hematology, written in the Chinese version, and will be elaborated in detail in the discussion section [26].

Discussion

Incidence rates of protein C and protein S deficiencies, worldwide, among normal people are 0.2-0.4% and 0.16-0.21%, respectively. Rates among patients with deep vein thrombosis range between 2.3-3.3% [27]. Prevalence of protein C and S deficiencies among Chinese populations is approximately 2.26% and 1.06% [28]. In contrast with the fact that main risk factors of hereditary thrombophilia in Caucasian populations are FV Leiden and prothrombin G20210A, deficiencies of three natural anticoagulant factors, including protein C, protein S, and antithrombin, are the main factors of thrombophilia in Asian populations. Prevalence of anticoagulant protein deficiencies in Chinese populations is obviously higher than that of Caucasian populations [1-3, 15]. Unfortunately, there are few studies concerning hereditary anticoagulant protein deficiencies in Chinese populations. The exact prevalence of it among Chinese patients with deep vein thrombosis is unknown [28]. In this study, six of the seven patients...
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were aged from the twenties to the forties, which is in accord with the report that patients with venous thrombus embolisms (VTE) due to gene mutations are mainly before the age of fifty [29]. Younger age onset of the disease brings a heavy burden, not only patients themselves but also the whole family and society.

A Chinese study showed that most hereditary anticoagulant protein deficiencies are due to PROS mutations [15], ignited present interest in elucidating whether this is the case. Mature PROS has three glycosylation sites (Asn458, 468, 489) and seven functional domains: a γ-carboxyglutamic acid domain (Gla), which is Vitamin K-dependent. There is a thrombin sensitive region (88-116aa), in abbreviation of TSR domain. The four consecutive epidermal growth factor (EGF)-like domains are 119-155aa, 157-200aa, 201-241aa, and 243-283aa. SHBG-like domain is a C-terminal sex hormone binding globulin-like domain containing two laminin protein G domains (312-455aa, 509-647aa), SHBG-like domain involves the complete formation of protein S structure [10]. Exon1 of Protein S gene encodes signal peptides. Exon2 encodes raw peptides and Gla domain. Exon3 encodes an aromatic rich amino acid stacking domain. Exon4 encodes TSR domain. Exon5-8 encode the four EGF-like domains. Exon9-15 encode carboxyl-ending portion [10].

Polymorphism site G68395T and heterozygous mutation C68430T on exon4 leads to Arg90Leu and Gln101Ter, respectively. Arg90 is localized in the thrombin sensitive region (88-116a). Arginine is a posi-

Figure 3. Heterozygous mutation G82512C on exon9 on PS gene in Patient No. 3 (A: mutation type, B: wild type). The arrow points to the mutation site. (A) represents the sequencing results of exon9 on PROS in patient No. 3; (B) shows the wild type sequencing results.

Figure 4. Heterozygous mutation C68430T on exon4 of protein S gene in Patient No. 5. The arrow points to the mutation site.

Figure 5. Polymorphism site G68395T on exon4 of protein S gene. The arrow points to the mutation site.
Table 2. Summary of mutations discovered on PROS among the seven patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>PS:C</th>
<th>Location</th>
<th>Mutation Site</th>
<th>Polymorphism Site</th>
<th>AA</th>
<th>Mutation Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.3</td>
<td>E4</td>
<td>G68395T/Het</td>
<td>Yes</td>
<td>Arg90Leu</td>
<td>M</td>
</tr>
<tr>
<td>2</td>
<td>22.9</td>
<td>E4</td>
<td>G68395T/Het</td>
<td>Yes</td>
<td>Arg90Leu</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E4</td>
<td>C68430T/Het</td>
<td>No</td>
<td>Gln101Ter</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E10</td>
<td>C86066T/Het</td>
<td>No</td>
<td>Arg355Cys</td>
<td>M</td>
</tr>
<tr>
<td>3</td>
<td>57.3</td>
<td>E4</td>
<td>G68395T/Het</td>
<td>Yes</td>
<td>Arg90Leu</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E9</td>
<td>G82512C/Het</td>
<td>No</td>
<td>Ser321Thr</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E15</td>
<td>A10481G/Het</td>
<td>No</td>
<td>Pro667Pro</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>38.8</td>
<td>E4</td>
<td>G68395T/Het</td>
<td>Yes</td>
<td>Arg90Leu</td>
<td>M</td>
</tr>
<tr>
<td>5</td>
<td>35.2</td>
<td>E4</td>
<td>G68395T/Het</td>
<td>Yes</td>
<td>Arg90Leu</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E4</td>
<td>C68430T/Het</td>
<td>No</td>
<td>Gln101Ter</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E4</td>
<td>T68387G/Het</td>
<td>No</td>
<td>Val87Val</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>43.7</td>
<td>E14</td>
<td>G68395T/Het</td>
<td>Yes</td>
<td>Arg90Leu</td>
<td>M</td>
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<td>7</td>
<td>43.6</td>
<td>E4</td>
<td>T68387G/Het</td>
<td>No</td>
<td>Val87Val</td>
<td>S</td>
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<tr>
<td></td>
<td></td>
<td>E4</td>
<td>G68395T/Het</td>
<td>Yes</td>
<td>Arg90Leu</td>
<td>M</td>
</tr>
</tbody>
</table>


Genetically charged hydrophilic amino acid with polarity. Leucine is a hydrophobic amino acid. Arg90Leu would inevitably lead to changes of partial space conformation, bringing about decreasing of protein S activity. Heterozygous mutation C68430T, leading to nonsense mutation, is also localized in this thrombin sensitive domain. Peptide chain synthesis is terminated prematurely by this nonsense mutation, seriously affecting the function and activity of the protein. This mutation has been considered the primary etiology of anticoagulant protein deficiency. Heterozygous mutation G68395T was frequently discovered among this population sample in this study and appeared in the sample of healthy normal people. It was boldly assumed that this was highly relevant to ethnicity and this polymorphism site was relevant to the higher prevalence of protein S deficiency in Chinese populations than in Caucasian populations. Missense mutation Arg355Cys was not a polymorphism site. This mutation had been published by a Hong Kong study [25]. In the study, a cerebral infarction propondb in a protein S deficiency pedigree was involved. In sequencing protein S gene, Arg355Cys caused by C86066T on exon10 was discovered, as with the current study. In addition, according to the theory of existence of founder effect, samples collected in the current study were all from Nanjing city. Whether these mutations were caused by the identical ancestors of the seven patients remains unknown.

Of the seven patients with hereditary protein S deficiencies, three of them combined with deficiencies of protein C. A previous study detected mutations on PROC of these protein C deficiency patients. This data had been written in a Chinese version and published in a Chinese journal [26]. The mutations found on PROC are listed in Table 3. PROC consists of nine exons. Eight of them encode amino acids. Exon1 on promoter has no effects of translating. Exon2 encodes signal peptides and exon3 encodes propeptides and carboxyl glutamic acid domain. Exon4 is mainly associated with the combination site. Exon5 and Exon6 encode EGF-like domain. Exon7 encodes connection peptides (Lys156-Arg157) and activation peptides. Exon8 encode activation center His211. Exon9 encode activation center Asp257 and Ser360 [30]. In case No. 1, A6578T mutation on exon2 of protein C gene led to Thr18Ser and can alter protein activity by influencing signal peptides. This is considered the most significant factor not discovered. Polymorphism site G68395T was frequently discovered among this population sample in this study and appeared in the sample of healthy normal people. It was boldly assumed that this was highly relevant to ethnicity and this polymorphism site was relevant to the higher prevalence of protein S deficiency in Chinese populations than in Caucasian populations. Missense mutation Arg355Cys was not a polymorphism site. This mutation had been published by a Hong Kong study [25]. In the study, a cerebral infarction propondb in a protein S deficiency pedigree was involved. In sequencing protein S gene, Arg355Cys caused by C86066T on exon10 was discovered, as with the current study. In addition, according to the theory of existence of founder effect, samples collected in the current study were all from Nanjing city. Whether these mutations were caused by the identical ancestors of the seven patients remains unknown.
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Table 3. Gene mutations on PROC found in three patients [26]

<table>
<thead>
<tr>
<th>No</th>
<th>Gender (F/M)</th>
<th>Age (Year)</th>
<th>Location</th>
<th>Mutation Site and Type</th>
<th>Polymorphism</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>40</td>
<td>Exon2</td>
<td>A6578T (Het/Mis)</td>
<td>No</td>
<td>Thr185Ser</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Promoter</td>
<td>C5156T (Het)</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>32</td>
<td>Promoter</td>
<td>C5156T (Het)</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>28</td>
<td>Promoter</td>
<td>C4867T (Het)</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exon2</td>
<td>AGA12702-12704del or 12705-12707del (Del)</td>
<td>No</td>
<td>Arg192del or Arg193del</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exon9</td>
<td>A15240G (Het/Mis)</td>
<td>No</td>
<td>His370Arg</td>
</tr>
</tbody>
</table>

Remarks: AA for amino acid change. Gender M for male and F for female. Mis for missense mutation, Del for deletion mutation, Het for heterozygous mutation.

...decreasing protein C activity. In case No. 2, mutation A5045T was localized on promoter region, though this region has no function of encoding amino acids. It was considered that the decrease in protein C activity was caused by decreasing protein S activity by APC-dependent pathways. In case No. 3, there was a deletion mutation on exon7, having a significant influence on encoding connection and activation peptides. Mutation G15240A on exon9 affected the two activation triangle regions in the protein structure. Previous studies have claimed that polymorphism sites could also decrease protein C activity to a certain extent [31]. In this study, mutation C4867T and C5156T on promoter region were polymorphism sites of protein C gene. They may result in decreasing protein activity.

In view of a higher prevalence of this disease in Chinese populations than in Caucasian populations, establishing a library of gene mutation spots of Chinese populations guiding of early intervention and future gene-targeting therapy is urgent. Individuals with a history of thrombosis could benefit from advanced thrombophilia testing and should be level-headed when making decisions using medicines influencing the anticoagulant process. The achievements of current domestic research include unearthing of the following mutations on PROC, homozygous mutation g. 1628T>G [32], c. 565C>T [33], c. 508G>T [33], c. 1157T>A [33], and c. 1218G>A [34]. Unfortunately, PROS mutation research among Chinese populations is extremely rare, with only a few studies having been published [25, 35]. The current study confirmed the mutation site C86066T, which had been reported in a Hong Kong study, previously. Other mutations sites, C68430T and G82512C on PROS, were first discovered worldwide. This investigation enriched the mutation spectrum of hereditary protein S deficiencies among Chinese populations. A notable limitation of the study is that researchers need to transflect vectors containing such mutations into cells, further revealing how gene mutations alter protein secretion and characteristics.

There were several limitations to the present study. First, the sample size was too small and hot mutations identified were not universally applicable. Due to foundation effects, mutations discovered in this region may have originated from the same ancestor, which cannot represent mutations in another region. Second, with respect to relatives subjective wills, family pedigree mutation information was not accessible. Third, this study merely detected mutations on protein C and protein S genes. Further research concerning mutations of antithrombin genes should be conducted.

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Disclosure of conflict of interest

None.

Abbreviations

PC, protein C; PS, protein S; PROC, protein C gene; PROS, protein S gene; PCR, polymerase chain reaction; APC, activated Protein C; VTE,
ravenous thrombus embolism; CTA, Computed Tomography Angiography; DIC, disseminated intravascular coagulation.

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Gene diagnosis of seven patients with hereditary protein S deficiencies


