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Clinical and genetic features of protein C deficiency in 23 unrelated Chinese patients☆,☆☆

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ABSTRACT

In this study, we investigated the clinical and genetic features of protein C deficiency in the Chinese population. A total of 23 symptomatic patients with protein C deficiency were identified by thrombophilic assays. Detailed clinical data about the patients with respect to their personal and family history of venous thromboembolism (VTE) were collected. Mutational analysis was then performed by direct sequencing of the protein C gene (*PROC*) in the patients and their family members. Of the 23 patients, 30.4% (7/23) had additional risk factors, 51.2% (12/23) suffered from recurrent thrombotic episodes, and 50.0% (6/12) of the patients with recurrent thrombosis had more than one heterozygous mutation in *PROC* itself or combined with protein S gene (*PROS*). The sex distribution of male:female was 19:4 in the 23 symptomatic patients and 10:2 in the 12 recurrent patients. Almost all patients (22/23) had lower extremity deep vein thrombosis (DVT) and one had pulmonary embolism (PE) only. A total of 15 different causative mutations were identified from the 23 subjects with 6 (40.0%) of the mutations being novel. Among the mutations identified, the Arg147Trp substitution was hotspot mutation in the Chinese population with a high frequency of 43.5%. Our finding suggests that complex genotypes of *PROC* or combined with protein S deficiency are primarily responsible for an increased risk of recurrent VTE. Our data further provides a framework for correlating the clinical pathogenesis of protein C deficiency to ethnic backgrounds in the Chinese population.

Introduction

Venous thromboembolism (VTE) is a common multifactorial disease resulting from the interaction of genetic and environmental risk factors. Genetic abnormalities of proteins involving the coagulation pathway that lead to hypercoagulability have been found in subjects suffering from thrombophilic disease. Factor V Leiden (FV Leiden) and prothrombin G20210A gene mutations are highly prevalent in the Caucasian population but are almost non-existent among the Asian population [1]. In contrast, a higher prevalence of protein S and protein C deficiency has been reported in the Asian population [2–5]. Two studies have reported that protein C and protein S deficiencies are the most important risk factors associated with thrombosis in Taiwanese and Japanese populations [3,4].

Protein C is a vitamin K-dependent serine protease zymogen synthesized in the liver. It is activated by thrombin in complex with thrombomodulin on the surface of endothelial cells. The activated protein C (APC), in complex with its cofactor protein S, inactivates procoagulant cofactors Va and VIIIa, thereby down-regulating thrombin generation in coagulation cascade.

Protein C deficiency has an autosomal dominant pattern of inheritance and is associated with an increased risk of VTE. Heterozygosity for the disease has been shown to increase the risk of venous thrombosis by 5- to 10-fold [6]. Homozygosity and compound heterozygosity of protein C are associated with neonatal purpura fulminans or severe thromboembolic complications after birth. In rare cases, thrombotic episodes develop in childhood or adulthood. The prevalence of protein C deficiency defined by plasma levels is between 0.2% and 0.5% of the healthy population [7,8]. Most of the affected individuals remain asymptomatic throughout their life and 2–5% present clinically symptomatic protein C deficiencies, suggesting that protein C deficiency alone is a relatively low risk factor for thrombosis [9,10]. In Caucasians,

Abbreviations: VTE, venous thromboembolism; DVT, deep venous thrombosis; PE, pulmonary embolism; PVT, portal venous thrombosis; MVT, mesenteric venous thrombosis; AVT, axillary vein thrombosis; PROC, protein C gene; PROS, protein S gene; PC:A, activity of protein C; PC:Ag, antigen of protein C.

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the presence of the two common mutations, factor V Leiden and prothrombin G20210A, has been associated with a more severe phenotype in some protein C deficient patients [11,12]. However, available data are scarce regarding which kinds of additional genetic and environmental risk factors contribute to the onset of first-time and recurrent thrombotic episodes in patients with protein C deficiency in the Asian population.

The human protein C gene (*PROC*) is localized in chromosome 2q13–q14 and comprises nine exons spanning more than 11 kb of genomic DNA. More than 300 mutations have been reported so far, and the spectrum of *PROC* mutations has been reported in several studies. Most reports are restricted to Western populations with only a few having been reported in the Asian population [11,13–15]. Results of these studies have indicated that the mutation profile of *PROC* is significantly influenced by ethnic background, thus, some mutations (Arg230Cys, Arg178Trp, Gln132X, Val297Met and Pro168Leu) are commonly found in the Caucasian population, while the others (Phe139Val, Arg169Trp, Val297Met, Met364Ile, and G8857del) are recurrent defects in the Japanese population. So far, few studies have been published on the spectrum of *PROC* defects and its thrombotic manifestation in patients with protein C deficiency in mainland China.

In this study, we addressed this question by analyzing the molecular defects of the *PROC* in the 23 symptomatic subjects. Our results demonstrate a correlation between the ethnic background and the occurrence of thrombotic episodes stemming from protein C deficiency in the Chinese population.

Materials and methods

Patients

This study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine. Beginning in January 2002–January 2012, 28 unrelated patients with first-time or recurrent VTE episodes were referred to our thrombosis center due to their predisposition to the risk factors for VTE and finally were found that they were with protein C deficiency. All the index subjects were interviewed with respect to their medical history. The diagnosis of VTE and the presence of acquired risk factors, including immobilization, fractures, pregnancy, puerperium, oral contraceptives, hormone replacement, surgery, and malignancy, at the time of all episodes of VTE were recorded. The VTE diagnosis was based only on the results of objective investigations employing the following approaches: compression or color Doppler ultrasonography was used to diagnose deep venous thrombosis (DVT) and abdominal venous thrombosis; a high-probability ventilation–perfusion scan, pulmonary angiography, or computed tomography (CT) was used to diagnose pulmonary embolism (PE); magnetic resonance venography (MRV) or CT was used to diagnose occlusion of cerebral or abdominal veins. A detailed family history was obtained from the family members with special emphasis on the occurrence of prior VTE events.

Hemostatic assays

Venous blood samples from the patients and their family members were collected in 0.109 mmol/L sodium citrate after informed consent was obtained. Tests for thrombophilia including antithrombin (AT), protein C, protein S, plasminogen (PLG), tissue plasminogen activator (t-PA), lupus anticoagulant (LA), anticardiolipin antibody (ACA), anti-β2 glycoprotein I (anti-β2GPI), fibrinogen (Fg), and total homocysteine (Hcy). The plasma levels of ACA and anti-β2GPI (Euroimmun, Lübeck, Germany), and t-PA (Instrumentation Laboratory, Milan, Italy) were detected using enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions. LA was detected using a diluted viper venom time (DVVT) assay (Instrumentation Laboratory). The Hcy levels were determined using the AxSYM homocysteine kit (Abbott, Lake County, IL, USA) based on a fluorescence polarization immunoassay (FPIA). The functional and antigenic fibrinogen levels were detected using the Clauss method on a Sysmex CA7000 analyzer (Sysmex Corporation, Tokyo, Japan) and immunoturbidimetric assay, respectively. The activities of protein C, AT, and PLG (PC:A, AT:A, and PLG:A) were analyzed using chromogenic substrate methods (Instrumentation Laboratory). The activity of free protein S (FPS:A) was assayed using a clotting method based on prothrombin time (Instrumentation Laboratory). Free protein S antigen (FPS:Ag) was measured with a polyclonal anti-human protein S antibody using the ZYMUTEST free protein S kit (Hyphen BioMed, Neuville-Sur-Oise, France). Protein C antigen (PC:Ag) was determined using a sandwich ELISA method with a rabbit anti-human protein C polyclonal antibody (Dako, Glostrup, Denmark) as a capture antibody and a horseradish peroxidase (HRP) conjugated antibody as a detection antibody. Protein C deficiency was diagnosed only in cases where the activity of protein C (PC:A) was lower than the normal level and both liver and renal functions were normal.

Genetic analysis of PROC

Genomic DNA was extracted from the peripheral blood leukocytes using the QIAamp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Mutations in the *PROC* and protein S gene (*PROS*) were identified as previously described [5]. The detected mutations were confirmed by reverse sequencing and were verified using a second amplicon. To rule out polymorphisms, the novel missense mutations were screened in 50 normal individuals. Only the corresponding sequence was amplified and sequenced in family members. The genetic alterations were reported according to both the standard international nomenclature guidelines recommended by the Human Genome Variation Society (HGVS; http://www.hgvs.org/mutnomen/recs.html), with nucleotide +1 as the A of the ATG translation initiation codon and Foster's numbering system. The genomic DNA (GenBank: NM_000312.2) and cDNA (GenBank: P04070) sequences of *PROC* were used as references.

Results

Association between the genetic variants of PROC and onset of thrombotic episodes

A total of 28 patients were diagnosed as having protein C deficiency, 2 patients had combined protein C and protein S deficiency, defined by the levels of PC:A and PS:A in their plasma. Genetic defects in the *PROC* were identified in 23 unrelated patients. No *PROC* mutation was identified in the other five patients, who were diagnosed with acquired protein C deficiency due to liver diseases. One heterozygous mutation in the *PROS* was found in one of the two patients with combined protein C and protein S deficiency, but no mutation was identified in the other patient with an FPS:A level of 18.0% and a FPS:Ag level of 23.7%, respectively. The subject's mother had a similarly low FPS level, suggesting that the protein S deficiency may be hereditary in the family. The characteristics of the genetic defects and the onset of thrombotic episodes in the 23 unrelated symptomatic patients with protein C deficiency are shown in Table 1.

Of the 23 symptomatic subjects, 30.4% (7/23) had additional risk factors. Of the seven patients, four presented the acquired risk factors at the time of onset of VTE: surgery in patient 7 and patient 15; puerperium in patient 13; and the thrombotic complication related to the use of a permanent filter and/or advanced age in patient 10. Three patients exhibited other genetic risk factors: an elevated homocyteine level in patient 21, protein C and protein S deficiency coexisted in patients 5 and 6. Results of all the other thrombophilic assays were normal in the 23 symptomatic patients (data not shown). Twelve patients (51.2%) suffered from recurrent thrombotic episodes, six of