State-of-the-Art Article

Venous thromboembolic risk and protein S deficiency: ethnic difference and remaining issues

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Abstract  Protein S deficiency is an autosomal dominant disorder that results from mutations in the protein S gene (PROS1). Inherited deficiency of protein S constitutes a risk factor for venous thromboembolism. Protein S functions as a nonenzymatic cofactor for activated protein C in the proteolytic degradation of coagulation factors V a and VIIIa. The frequency of protein S deficiency seems to differ between populations. More than 200 rare mutations in PROS1 have been identified in patients with protein S deficiency. Among the prevalent mutations within PROS1, the S460P substitution (known as Heerlen polymorphism) detected in Caucasians and the K196E substitution (known as protein S Tokushima) found in Japanese have been intensively studied for their structures and potential functions in the disorder of protein S deficiency. Until now, causative mutations in PROS1 have been found in only approximately 50% of cases with protein S deficiency. Co-segregation analysis of microsatellite haplotypes with protein S deficiency in families with protein S deficiency suggests that the causative defects in the PROS1 mutation-negative patients are located in or close to the PROS1 gene. Large PROS1 gene deletions have been identified in 3 out of 9 PROS1 mutation-negative Swedish VTE families with protein S deficiency and 1 out of 6 PROS1 mutation-negative Japanese patients with protein S deficiency. Intensive sequencing of the entire PROS1 gene, including introns, may be needed to identify the cryptic mutations in those patients, and these efforts might uncover the pathogenesis of protein S deficiency. (J Geriatr Cardiol 2009; 6:11-19)

Key words  protein S deficiency; PROS1; thrombophilia; mutation; genetic defects

Introduction

Thrombin generation is critical to hemostasis and thrombosis; it is therefore tightly and synergistically regulated by two independent anticoagulant systems. One system is known as the protein C anticoagulant pathway, and the other is the heparan sulfate-dependent protease inhibitor system. In the protein C anticoagulant system, activation of both factor X and prothrombin is down-regulated by activated protein C (APC) through proteolytic inactivation of activated factors V (FVa) and VIII (FVIIIa). Protein S is a vitamin K-dependent plasma protein that suppresses blood coagulation by serving as a cofactor for APC and promoting the factor Xa inhibition by tissue factor pathway inhibitor.

Genetic or acquired deficiency of protein S is one of the major risk factors for venous thromboembolism (VTE). Individuals with hereditary protein S deficiency have a predisposition to recurrent VTE. The activity assay for plasma protein S in the general population and patients with VTE showed that the prevalence of protein S deficiency is much higher in Japanese than in Caucasians, although we must take into consideration the different assay methods and different criteria for the deficiency. In addition, the K196E mutation in the protein S molecule with decreased activity shows a relatively high prevalence in the Japanese population.

Screening for mutations in individuals with protein S deficiency has been performed in a number of studies, and more than 200 rare mutations have been reported thus far. Although many laboratories have intensively analyzed the PROS1 gene to identify the causative mutation for protein S deficiency, mutations were only found in approximately 50% of the patients and the rest remained to be determined. Most of the genetic defects identified thus far are missense, nonsense, and frameshift mutations, and these are scattered throughout the coding region of the gene. Several large gene deletions have also been described.

In this review, characteristics of protein S, thrombotic diseases caused by protein S deficiency, and the genomic changes underlying protein S deficiency are focused on. We have adopted the recommendations of the Human Genome Organization and the Nomenclature Working Group throughout this article, in which A of the initiation codon ATG is denoted as nucleotide +1 and the first translated Met is amino acid +1.

Physiological roles of protein S in anticoagulant pathway

Genomic structure of human PROS1 gene

The human PROS1 gene is located near the centromere...
on chromosome 3q11.2. It spans approximately 101 kb of genomic DNA and comprises 15 exons and 14 introns. The human genome contains two protein S genes on chromosome 3: the active PROS1 gene and a closely related pseudo gene (PROSP). PROSP is located at 3pl1-1 and shows 96.5% homology to exons 2 to 15 of the PROS1 gene. PROSP does not encode protein S.

Characteristics of protein S

Protein S is a 69-kD vitamin K-dependent glycoprotein that in its N-terminal domain has 11 γ-carboxyglutamic acids, abbreviated as Gla. Mature protein S has a discrete domain structure: a Gla domain (residues 42-87), a region sensitive to cleavage by thrombin (residues 88-283), four domains homologous to epidermal growth factor (EGF-like domains, residues 76-242), and a region homologous to sex hormone binding globulin (residues 284-676). Protein S is mainly produced in the liver and is also synthesized in endothelial cells. Leydig cells of the testis, megakaryocytes, osteoblasts, and the central nervous system.16-18

Physiological role of protein S

The blood coagulation cascade is regulated by two independent pathways, the protein C anticoagulant pathway and the heparan sulfate-dependent protease inhibitor system (Fig. 1).11,12 Protein C is proteolytically activated by thrombin bound to thrombomodulin on the endothelial cell surface, and the resulting APC proteolytically degrades procoagulant FVIIa and FVIIIa in the presence of protein S, thereby suppressing the coagulation. Thus, both protein C and protein S are anticoagulant proteins (Fig. 1).19,20 In addition to the APC-dependent anticoagulant activity, protein S has been recently reported to inhibit the tissue factor pathway by stimulating factor Xa inhibition by tissue factor pathway inhibitor.21 Protein S may also be involved in the regulation of cell growth and in the prevention of inflammation.22,23

Protein S exists in two forms in plasma: the free form and the bound form with C4b binding protein (C4BP) (Fig. 2). The free form has anticoagulant activity and the C4BP bound form does not. Quite recently, a kinetic study has revealed that the protein S-C4BP complex expresses low but significant APC-catalyzed proteolysis and to some extent stimulates APC-catalyzed proteolysis of FVIIa.23 Approximately 40% of plasma protein S circulates as the free form, while the remaining 60% is the bound form. Human C4BP exists in several forms in plasma. C4BP contains either 6 or 7 α-chains and either one or no β-chain held together by disulfide bonds.24 The β-chain contains the protein S binding site. Thus, protein S binds to β-chain containing C4BP, known as C4BPβ+, and does not bind to C4BP with no β-chain. Protein S and C4BPβ+ form a 1:1 stoichiometric complex. C4BP is an acute-phase protein. The concentrations of C4BP dramatically increase up to 400% during inflammatory disorders. This rise is largely due to an increase in the C4BPα form, and the concentrations of the protein S-C4BPβ+ complex are altered mildly.25

Classification of protein S deficiency

Protein S deficiency is classified into three types. Type I is characterized by low levels of both total and free protein S (quantitative deficiency).26 Type II entails reduced activity of protein S but normal levels of free and total protein S

![Blood coagulation and regulation](image)

An extrinsic coagulation pathway is initiated by the binding of activated factor VII (VIIa) with cell-surface procoagulant protein, tissue factor (TF). The coagulation reaction is amplified on the negative phospholipid (PL) surface, such as with activated platelets in the presence of cofactor proteins. In this amplification reaction, activated factors VIII (VIIIa) and V (Va) facilitate the reaction, leading to the massive production of multi-functional protease, thrombin. Activated protein C (APC), generated by the proteolytic activation of protein C (PC) by thrombin-thrombomodulin complex, proteolytically cleaves VIIIa and Va and regulates their activities with the help of protein S (PS). Thus, protein C and protein S serve as anticoagulant proteins. Protease inhibitors, tissue factor pathway inhibitor (TFPI) and antithrombin (AT), can bind to the heparan sulfate glycosaminoglycan on the endothelium and regulate the coagulation reaction. Protein S inhibits TF activity by promoting the inhibition of Xa by TFPI.
(qualitative deficiency). Type III deficiency is characterized by low levels of free protein S and normal levels of total protein S. Type I and type III deficiencies have been shown to coexist in many families indicating that these two types are phenotypic variants of the same genetic disease.

Diagnosis of protein S deficiency is complicated by the inter- and intra-individual variation in plasma protein S levels and by the overlap in levels between unaffected individuals and those having heterozygous deficiencies. To obtain the actual protein S activity in the plasma samples, the temperature of the assay is very important. Activity assay at the room temperature gave consistent protein S activity, but increased temperatures such as 37°C gave a false high activity. Several factors, including age, sex, hormonal state, pregnancy, liver disease, and inflammation, can influence plasma protein S levels. In particular, the protein S activity of pregnant women is consistently low, which hampers the definite identification of protein S deficiency in pregnant women.

Protein S deficiency and thrombotic diseases

With venous thrombosis diseases

Inherited protein S deficiency is an autosomal dominant disorder. Many studies have indicated that hereditary protein S deficiency is a confirmed risk factor of VTE, as described below. Individuals with hereditary protein S deficiency have a predisposition to recurrent VTE. Protein S deficiency was found in approximately 5% of VTE patients and 1-2% of consecutive patients with the first episode of VTE. A population-based, patient-control study showed that protein C and antithrombin deficiency are clearly associated with an increase in thrombosis risk, but that total protein S or free protein S levels are not associated with VTE risk. In a case-control study, individuals with low free protein S levels had a relative risk of 2.4 for VTE. This paradox suggests that the screening methods for determining protein S deficiency do not effectively identify most patients.

Thrombosis risk for individuals with hereditary protein S deficiency (risk ratio, 8.5) was significantly higher than that for normal individuals and was much higher than that for individuals with factor V Leiden mutation. A retrospective study in a single extended family consisting of 122 members, including 44 mutation carriers of the PROS1 G336V mutation, has been reported. The G336V mutation in this family was a strong independent risk factor for VTE with a hazard ratio of 11.5. A study using a cohort of patients from a single center where the diagnosis was confirmed at the genetic level showed that, in relatives, a low free protein S level is the most reliable predictor of a PROS1 gene defect. First-degree relatives with a PROS1 gene defect were found to have a 5-fold higher risk of thrombosis than those with a normal PROS1 gene and no other recognized thrombophilic defect.

A large multicenter prospective follow-up study showed that relatives with hereditary protein S deficiency had experienced a VTE with an incidence of 7.1 per 1,000 person years and that the risk of VTE associated with hereditary protein S deficiency was 26.1. The highest incidence per 1,000 person years was found in relatives with combined defects, and the lowest incidence was found in those with the FV Leiden mutation, indicating considerable differences in the lifetime risk of VTE observed among individuals with different thrombophilic defects.

With arterial thrombosis diseases

The relationship between protein S deficiency and arterial thrombosis is less obvious. A few patients with arterial occlusions showed protein S deficiency. Therefore, hereditary protein S deficiency may be a risk factor for the development of arterial thrombosis, especially cerebrovascular occlusion. More extensive studies are needed to
demonstrate whether or not protein S deficiency is a risk factor for the development of arterial thrombosis.

**Frequency of hereditary protein S deficiency in different ethnicities**

The frequency of protein S deficiency seems to be quite different between Caucasians and Japanese. Heterozygous protein S deficiency is present in approximately 2-5% in Caucasian thrombosis patients,\(^9\)_14 whereas the prevalence in the general Caucasian population has been estimated to be between 0.16% and 0.21%.\(^5\)_44 Protein S deficiency in Japanese both in the general population and in patients with VTE is much higher. The prevalence of protein S deficiency, defined as low protein S activity, in the Japanese general population has been estimated to be 1.12% in men and 1.60% in women.\(^5\) The reasons for the frequency difference of protein S deficiency between Caucasians and Japanese were not clear. The use of different assay methods and a different cut-off value for protein S deficiency may explain the differences. There are no available data regarding the frequency of protein S deficiency in other east-Asian countries; it is therefore not clear whether the difference is intrinsic to the relation between Japanese and Caucasian populations.

**Genetic changes underlying protein S deficiency**

The large size of the gene and the presence of a pseudogene (PROSP) complicate the genetic analysis of the PROS1 gene. Nevertheless, screening for PROS1 gene mutations has been performed in a number of studies. To date, more than 200 rare mutations in PROS1 have been identified as causative PROS1 gene defects.\(^4\) Most of the genetic defects are missense, nonsense, or frameshift mutations scattered throughout the coding region of the gene.

Two missense mutations, the S460P mutation (known as Heerlen polymorphism) found in Caucasians and the K196E mutation (known as protein S Tokushima) found in Japanese, have been intensively studied.

**Protein S S460P polymorphism (Heerlen polymorphism)**

A S460P missense mutation known as the Heerlen polymorphism is present in the sex hormone-binding globulin domain of protein S molecule. The protein S mutant molecule carrying Pro at position 460 is characterized by a lower molecular weight than normal protein S molecule due to the loss of the N-glycosylation consensus sequence at N458. Many studies have indicated that the Heerlen polymorphism is not a risk factor for thrombosis.\(^15\)_47

**Protein S K196E as a risk factor for VTE in Japanese**

Two independent case-control studies carried out in Japan have indicated that PROS1 K196E is a genetic risk factor for VTE.\(^12\)_48 We have summarized the characteristics of PROS1 K196E in Table 1. One of the studies was large scale, including 161 VTE patients and 3,655 population-based controls.\(^12\) In this study, five functional genetic variants, including PROS1 K196E, plasminogen A610T, ADAMTS13 P475S, factor XII -4C>T, and plasminogen activator inhibitor-1 4G/5G, were evaluated by comparing their frequencies among VTE patients and controls. It was found that only PROS1 K196E was a genetic risk factor for VTE, with an odds ratio of 5.58.\(^12\) Another study reached the same conclusion, showing PROS1 K196E to be a risk factor for VTE with an odds ratio of 3.74.\(^48\)

The protein S K196E mutation is a missense mutation causing Lys196 to be replaced by Glu, formerly known as protein S Tokushima, and referred to as the K155E mutation by the nomenclature system of mature protein numbering. It is present in the second EGF-like domain of the protein S molecule. This mutation was originally identified independently in two different Japanese patients with VTE.\(^10\)_11 The allele frequency is approximately 0.9% in the Japanese population, which means that 1 out of 55 Japanese carries the mutation as a heterozygote.\(^10\)_12\(^48\) Thus, a substantial number of Japanese carry the mutant E allele for protein S and are at risk of developing VTE. The protein S K196E mutation has thus far been identified only in Japanese, but may be present in eastern Asian populations as well. A genetic study of this mutation should be carried out in other eastern Asian populations.

**Functional analysis of the protein S K196E mutant**

In vitro studies using the recombinant proteins have shown that mutant protein S with Glu196 had impaired APC cofactor function.\(^49\)_50 Plasma protein S activities in carriers of the K196E mutation show reduced activity, but antigen levels are within normal limits.\(^10\)_51\(^52\) In the Japanese general population, 34 out of 1,862 individuals were heterozygous for the K196E mutation. They showed a 16% mean reduction in plasma protein S activity, indicating that it is not as dramatic as conventional protein S deficiency.\(^52\) Actually, the measurable protein S activities of the carriers were not always reduced but were intensively overlapped with those of normal individuals. This result indicated that protein S activity itself is not a proper tool for detecting this type of protein S deficiency. Therefore, genetic analysis or other direct discrimination methods must be used for identification of protein S K196E carriers.\(^13\) Homozygote carriers with this mutation have not been identified in the general population thus far, but they have in the VTE group.

**Failure of detection of causative mutations in protein S-deficient families**

It is widely recognized that causative mutations in PROS1 in patients with protein S deficiency are only found in approximately 50% of cases. The proportion of cases where no mutations are detected varies widely between studies.\(^35\) When all cases are pooled together, PROS1 gene
mutations have not been detected in 47% of protein S-deficient families. The reason for the missing identification of causative mutations in patients with well-characterized protein S deficiency is currently unclear.

This situation might implicate the causative genetic defects in other loci or other genes involved in protein S deficiency. However, cosegregation between certain microsatellite haplotypes and protein S deficiency has been observed in families without any causative mutations in the PROS1 gene, indicating that the causative genetic defects for protein S deficiency are located in or close to the PROS1 gene.33

Another explanation for the absence of identified mutations might be mutations in the promoter region or in introns. Transcription of protein S mRNA was directed at multiple start sites, and the PROS1 promoter was shown to contain a forkhead box A2-binding site and an Sp1-binding site. The PROS1 promoter also has liver-specific cis-acting DNA elements for regulation of transcription. Binding sites for various transcription factors within the first 400-bp proximal to the PROS1 translational start codon were further identified, among which only Sp1 and Sp3 play a crucial role in basal expression of the PROS1 gene. A mutation in the PROS1 promoter region responsible for protein S deficiency has been reported in a patient. The C-to-T mutation was within the core binding site of Sp1 and disrupted the Sp1 binding to the promoter, thereby causing reduced PROS1 expression.

Other possible causes of protein S deficiency are the existence of large deletions, duplications, or inversions covering the whole or part of the PROS1 gene, which are difficult to identify by sequencing. They may explain the genetic abnormalities in patients with protein S deficiency.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Description</th>
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<tbody>
<tr>
<td>Genetic mutation</td>
<td>586A&gt;G in cDNA</td>
</tr>
<tr>
<td>Amino acid change</td>
<td>Lys-to-Glu (K196E) mutation in the second EGF-like domain</td>
</tr>
<tr>
<td></td>
<td>(Lys155Glu using the nomenclature system of mature protein numbering)</td>
</tr>
<tr>
<td>Allele frequency</td>
<td>0.009 in 3,651 Japanese 17</td>
</tr>
<tr>
<td></td>
<td>0.008 in 183 Japanese 10</td>
</tr>
<tr>
<td>Odds ratio (95% confidence interval) to VTE</td>
<td>5.58 (3.11-10.01) 12</td>
</tr>
<tr>
<td></td>
<td>3.74 (1.06-13.2) 48</td>
</tr>
<tr>
<td>Plasma protein S activity</td>
<td>71.9%±17.6% (mean±SD) in 34 heterozygotes</td>
</tr>
<tr>
<td></td>
<td>(16% lower than the mean of normal individuals)</td>
</tr>
<tr>
<td>Detection methods</td>
<td>Method 1: Restriction fragment length polymorphism method using mutagenic primer. Amplify the target 434-bp region by PCR using primer 5'-CAATTTTAGATTCATGACATGAGA and mutagenic primer 5'-CCATCTGGCTCTTTACCTTACATCTGACT. The PCR fragments are digested with Hinf I restriction enzyme. The E-allele gives rise to the 404-bp and 30-bp fragments. Method 2: TaqMan genotype discrimination method. PCR primers, 5'-ACCACTGTTCTGTAATATGTTT, 5'-TGTGTTTTAATTCTACCATCCTGCT. Probes, 5'-VIC-CAATGAGAAAGATTTGAAAG-MGB (mutant E-allele)/ 5'-FAM-CAATGAGAAAGATTTGAAAG-MGB (wild-type allele).52</td>
</tr>
<tr>
<td>Functional study using recombinant protein</td>
<td>Molecular weight: same with the wild-type</td>
</tr>
<tr>
<td>S E 196-mutant</td>
<td>Activated protein C does not bind to the mutant.49</td>
</tr>
</tbody>
</table>

Large deletions of PROS1 in mutation-negative patients with protein S deficiency

Hereditary thrombophilia associated with protein S deficiency might result from deletion of the PROS1 gene. By now, six large gene deletions have been described in families with protein S deficiency (Table 2).

Using Southern blotting, a patient with protein S deficiency was found to have a deletion of the middle portion of the PROS1 coding sequence. Subsequently, the precise location of the deletion within introns 7 and 11 was identified. This deletion resulted in removal of approximately 14 kb of sequence. The data for this family show for the first time
the association between protein S deficiency and the gene deletion in PROS1.

A 5.3-kb deletion spanning over 90% of intron 12, all of exon 13, and approximately a quarter of intron 13, was detected in two families with protein S deficiency. This deletion would generate a stop codon at position 463.

In 2005, a highly important study was published regarding the frequency of a large deletion of PROS1 in mutation-negative Swedish patient families with protein S deficiency. Prior to this study, the genetic mutations in PROS1 were first investigated by a PCR-based method in 17 families with protein S deficiency and identified 7 causative mutations in 9 families. The causative mutations in the remaining 8 families were not identified. There was then an attempt to identify the large PROS1 gene deletions in these 8 patients using a dense set of SNP and microsatellite markers in segregation analysis. Finally, gene deletions were identified in 3 out of 8 investigated families. Three deletions encompassed at least 35 kb including exons 5-15, 437 kb including exons 1-11, and 449 kb including the whole PROS1 gene, respectively (Table 2). The last two large deletions included 3 genes, ARL2L1(ARL13B), DHFRL1, and NSUN3, but these deletions were not regarded as the cause of the protein S deficiency. Thus, an important finding of this study is that the frequency of PROS1 gene deletion in PROS1 mutation-negative Swedish VTE patients was 38% (3 out of 9 families). This result translates to an overall frequency of large deletions in patients with protein S deficiency of 18% (3 out of 17 families).

We investigated whether large deletions may exist in PROS1 in Japanese VTE patients. A multiplex ligation-dependent probe amplification (MLPA) analysis was employed to identify the deletion in PROS1 in 163 Japanese patients with VTE. A large gene deletion was identified in only 1 patient who showed 16% protein S activity and did not carry causative point mutations in PROS1 by conventional DNA sequencing (Table 2). The deletion was validated by the quantitative PCR method. The deletion spanned at least the whole PROS1 gene (107 kb) and at most from the centromere located downstream of PROS1, to before the D3S3619 marker, the first heterozygous marker in the upstream region of PROS1.

As for the frequency of the PROS1 gene deletion, we unfortunately did not measure the protein S activity in all 163 patients with VTE, but measured the activities of protein S and protein C in only 34 patients. Among them, we found 11 patients with protein S deficiency with low protein S activity of less than 50% and with protein C activity of more than 90%. Among these patients, five carried missense or splice site mutations. Among the remaining 6 patients, we found 1 with the PROS1 gene deletion. Therefore, the frequency of the PROS1 gene deletion in PROS1 mutation-negative Japanese VTE patients was estimated to be 17% (1 out of 6 patients). This result translates to an overall frequency of large deletions in patients with protein S deficiency of 9% (1 out of 11 families). Both studies suggested that large deletions are likely to be found in protein S-deficient families where conventional PCR-based mutation screening has failed. Thus, screening for gene deletions in PROS1 might be warranted in PROS1 causative point mutation-negative VTE patients with protein S deficiency.

### Table 2 Large gene deletions in PROS1 found in patients with protein S deficiency

<table>
<thead>
<tr>
<th>Length of deletion</th>
<th>Position of deletion</th>
<th>Analytical methods</th>
<th>Number of PROS1-deficiency families</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3 kb</td>
<td>intron 12 to intron 13</td>
<td>Southern blotting</td>
<td>two</td>
<td>60</td>
</tr>
<tr>
<td>14 kb</td>
<td>intron 7 to intron 11</td>
<td>Southern blotting, long PCR</td>
<td>one</td>
<td>59</td>
</tr>
<tr>
<td>35 kb</td>
<td>exon 5 to exon 15</td>
<td>SNP and microsatellite markers</td>
<td>one</td>
<td>61</td>
</tr>
<tr>
<td>437 kb</td>
<td>exon 1 to exon 11</td>
<td>segregation analysis</td>
<td>one</td>
<td></td>
</tr>
<tr>
<td>449 kb</td>
<td>exon 1 to exon 15</td>
<td>MLPA, Q-PCR, microsatellite analysis, SNPs linkage</td>
<td>one</td>
<td>63</td>
</tr>
<tr>
<td>107-729 kb</td>
<td>at least from the promoter to exon 15, at most from centromere to before D3S3619'</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*First heterozygous marker in the upstream of PROS1
MLPA: multiplex ligation-dependent probe amplification, Q-PCR: quantitative PCR

**Perspective on the detection of genomic alterations of PROS1 in protein S deficiency**

With a few exceptions, gene dosage measurements are not routine in most mutation scanning studies; alterations of gene dosage on the kb scale are therefore almost certainly under-estimated. As mentioned above, causative mutations in PROS1 could not be identified in approximately half of the cases with protein S deficiency by conventional sequencing of the protein coding region using the PCR-based methods, suggesting the possible existence of undetected genomic aberrations, including duplications, deletions, or inversions, that can involve gain or loss of genetic material of PROS1 in protein S deficiency. In addition, cryptic mutations occurred in introns may generate a new splice acceptor site that disturbs the correct mRNA transcript. In this case, intensive sequencing of the entire
PROS1 gene, including introns, may be needed to identify the cryptic mutations in those patients, and these efforts might uncover the pathogenesis of protein S deficiency. A wide range of traditional methods, including Southern blotting, long PCR, real-time quantitative PCR, microsatellite and SNP linkage analyses, and MLPA, are useful for gene-dosage measurement, but none offers an overwhelming advantage. An array-based comparative genomic hybridization method containing densely selected large insert DNA fragments distributed roughly every 1 Mb throughout the human genome was developed to detect large-scale copy-number variations that involve gains or losses of several to hundreds of kb of genomic DNA. This method has the potential to perform whole genome scans for altered gene dosage. A molecular copy-number counting method could precisely delineate regions with known or cryptic genomic alterations. Haplotype-fusion PCR followed by bead-based single-molecule haplotyping on repeat-specific markers method could be used for genotyping inversion events with breakpoints embedded within long (>100 kb) inverted repeats. Recently, the high-density array chip technology is available for genome-wide linkage analysis, association, and copy number studies. Those high-density arrays contain more than 1.8 million markers for SNPs and copy number variations. Even though those genome-wide approaches have high throughput, their copy number variation algorithms are indirect because they depend on signal-intensity differences to predict regions of variation. In addition, they cannot detect inversions. Quite recently, intermediate scale structural variations were reported in eight individuals. The methodology utilized in this study appears promising. These types of new methodologies will lead to a more accurate understanding of the extent of both pathogenic and nonpathogenic gene-dosage changes in PROS1 underlying protein S deficiency.

Acknowledgements

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