A Quantitative Trait Locus Influencing Free Plasma Protein S Levels on Human Chromosome 1q

Results From the Genetic Analysis of Idiopathic Thrombophilia (GAIT) Project

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Objective—Protein S (PS) is a component of the protein C anticoagulant system. PS deficiency is associated with myocardial infarction and venous thromboembolism, two highly prevalent causes of death in industrialized nations. As part of the Genetic Analysis of Idiopathic Thrombophilia (GAIT) project, we conducted a genome-wide linkage screen to localize genes influencing variation in free PS (fPS) plasma levels.

Methods and Results—fPS levels were measured in 397 individuals in 21 Spanish families. A total of 363 highly informative microsatellite markers were genotyped to provide a 10-cM genetic map, and variance component linkage methods were used. A region on chromosome 1q32, flanked by markers D1S425 and D1S213, showed strong evidence of linkage with fPS levels (LOD score, 4.07; nominal P = 7.5 × 10⁻⁵; genome-wide P = 0.0024). This region contains two positional candidate genes, the complement component 4-binding protein α and β chains, which encode the principal binding protein for PS. Suggestive evidence for linkage was also observed on chromosomes 11p and 19p.

Conclusions—These results represent one of the first genomic screens for quantitative variation in a component of the hemostatic pathway and provide strong evidence for a locus on chromosome 1q influencing fPS levels. (Arterioscler Thromb Vasc Biol. 2003;23:508-511.)

Key Words: protein S ▪ linkage ▪ quantitative trait locus
which was spontaneous. The probands’ thrombophilia was considered idiopathic because known biological causes of thrombosis had been excluded, including PS and PC deficiencies and activated PC resistance. Blood was obtained from fasting subjects and was collected in 1/10 volume of 0.129 mol/L sodium citrate. Thrombophilic participants were not taking anticoagulants at the time of sampling. Plasma samples were stored at −80°C before measurement of fPS levels (in duplicate), as previously described.9 Free protein S was assayed using ELISA methods (Stago). Normal ranges for PS (total, free, and functional) were obtained from the analysis of 100 healthy local blood donors (mean ± 2 SD) and were 73% to 124% for total PS, 72% to 149% for free PS, and 63% to 142% for functional PS. In young women (<46 years), the lower levels for total, free, and functional PS were 63%, 54%, and 62%, respectively. Potential probands were excluded on the basis of protein S deficiency when the plasma level of total, free, or functional PS was below the lower limit of the normal range in at least 2 different samples. All procedures were reviewed by the Institutional Review Board of the Hospital de la Santa Creu i Sant Pau (Barcelona). Adult subjects gave informed consent for themselves and for their minor children, when applicable.

Subjects were genotyped for an autosomal genome-wide scan with 363 highly informative DNA markers. DNA extraction was performed according to standard protocols.9 Microsatellites consisted primarily of the ABI-Prism genotyping set MD-10. Linkage mapping was undertaken with the PE LMS II fluorescent marker set with multiplex polymerase chain reaction as described; in a few instances, nearby Genethon markers were substituted for LMS II markers to improve robustness (http://www.cng.fr/). Polymerase chain reaction products were analyzed on PE 310, PE 377, and PE 3700 automated sequencers and genotyped using the PE Genotyper software. The average heterozygosity of the microsatellite markers was 0.79, and the average interval between markers was 9.5 cM.

Markers in or near several hemostasis-related candidate genes were used to augment this genome screen. The PS-Heerlen mutation was amplified as previously reported.10 Information on the genotyped marker in the factor V gene (a short tandem repeat in intron 11) can be found in the publicly accessible Genome Database (http://www.gdb.org).

The genotypic data were entered into a database and were analyzed for discrepancies (ie, violations of Mendelian inheritance) using the PEDSYS program INFER.11 Discrepancies were checked in the laboratory for mistyping, and markers for discrepant individuals were either corrected or excluded from the analysis. Allele frequencies were estimated from the GAIT sample using maximum likelihood techniques. Marker maps for multipoint analyses were obtained from ABI-Prism (http://www.appliedbiosystems.com/ molecularbiology/) and from the Marshfield Medical Research Organization (http://research.marshfieldclinic.org/genetics/).

Standard multipoint variance component linkage methods, as implemented in SOLAR,12 were used to assess linkage between autosomal markers and quantitative levels of plasma PS. Previous studies have suggested that such methods may be vulnerable to deviations from multivariate normality and particularly to high levels of kurtosis in the trait distribution.13 Levels of fPS in the GAIT sample exhibited a kurtosis of 0.05. Recent statistical genetic theory demonstrates that this level of kurtosis will not affect the distribution of logarithm of odds (LOD) scores and that the standard nominal probability values for LOD scores are appropriate for the fPS linkage screen.14

Because 12 of the families were ascertained through thrombophilic probands, all analyses included an ascertainment correction achieved by conditioning the likelihood of these pedigrees on the likelihoods of their respective probands.15 Genome-wide probability values were calculated using the method of Feingold et al.16 Sex and sex-specific age were used as covariates in all analyses, and their effects were estimated simultaneously with the genetic effects.

Results

In the GAIT sample, the mean age-corrected fPS plasma level in males was 109.4%, measured relative to a normal pooled sample. Females exhibited significantly lower plasma levels, averaging 20.2% less than males. Oral contraceptive use and cigarette smoking had no significant effects on fPS in this sample. The range of fPS values in the GAIT sample was 54% to 166%, with the lowest values occurring in young females. Using appropriate age- and sex-specific PS thresholds, no GAIT participants had PS deficiency. Levels of fPS increased significantly with age in both sexes but more so in females than males. Age and sex accounted for 22.5% of the variation in fPS levels.

A previous study suggested that a missense mutation in the PROS1 gene (PS-Heerlen) may account for a small amount of phenotypic variation in fPS levels.17 The PS-Heerlen mutation was present in 2 GAIT families, and the allele frequency was 0.007. The 7 heterozygous carriers of this mutation exhibited a slightly lower mean fPS level (93.3% versus 109.4%) than noncarriers. The PS-Heerlen mutation accounted for ≈1% of the phenotypic variation in fPS plasma levels in this sample. PS-Heerlen status was included as a covariate in the genome-wide linkage analyses.

The results of the genome scan for QTLs influencing fPS levels are shown in Figure 1. Three areas showed suggestive or significant evidence of linkage. A LOD of 1.69 (nominal P = 0.0026) was observed on chromosome 11p and a LOD of 1.68 (nominal P = 0.0027) on chromosome 19p. Such LODs would be expected to occur by chance approximately once every genome scan and must be considered suggestive. The highest LOD observed in the genome screen was 3.79 on chromosome 1q. Examining the LOD scores by family, both the randomly ascertained and the thrombophilic pedigrees are contributing to the chromosome 1 linkage signal, with 60% of the overall LOD coming from the former and 40% from the latter. When 2 microsatellite markers in or completely linked to hemostasis-related candidate genes on chromosome 1q (Factor V, FV, and the complement component 4-binding protein alpha chain, C4BPA) were added to the analyses, the LOD score increased to 4.07 (nominal P = 7.5 × 10⁻⁶; genome-wide P = 0.0024). The peak LOD occurred near C4BPA in the interval flanked by markers D1S425 and
Linkage analysis in some of these extreme families excluded thrombophilia, genetic abnormalities in cause profound PS deficiencies associated with familial. Although mutations have been identified in this gene that acting as a cofactor of activated PC in the inactivation of Protein S plays a central role in the PC anticoagulant system, on chromosome 3, were 1, indicating little support for a PROS1 QTL influencing normal variation in fPS levels.

Discussion

Protein S plays a central role in the PC anticoagulant system, acting as a cofactor of activated PC in the inactivation of activated Factor V (FVa) and VIII (FVIIa). The PS structural gene (PROS1) is located on chromosome 3. Although mutations have been identified in this gene that cause profound PS deficiencies associated with familial thrombophilia, genetic abnormalities in PROS1 can only be detected in 40% to 90% of families with PS deficiency. Linkage analysis in some of these extreme families excluded PROS1 from being responsible for the observed PS-deficient phenotype. This suggests that additional genes, other than PROS1, are also involved in this monogenic deficiency.

Frank deficiency of PS in plasma is rare and represents a known risk factor for inherited thrombophilia. Unfortunately, little is known about the relationship between normal quantitative variation in PS and risk of common idiopathic thrombosis, although it is likely that a continuous inverse functional relationship exists. Recently, evidence has been presented that normal fPS levels (ie, levels that are higher than those seen in clear PS deficiency) are inversely correlated with risk of myocardial infarction, but no such data are available for venous thrombosis.

The genetic components determining normal variation in PS levels are still largely unknown. Our results from the present study suggest that variation at the PROS1 structural locus is not a primary determinant of the observed quantitative variation. Instead, our genome scan suggests that the major QTL influencing variation in fPS levels is located at chromosome 1q32. Two major positional candidate genes (C4BPA and C4BPB), encoding the complement 4b-binding protein (C4BP), are located in this region, within 5 cM of the LOD score peak. Our linkage analyses included a short tandem repeat marker completely linked to C4BPA.

The C4BP genes are part of a larger gene cluster involved in the regulation of complement activation. The C4BP is composed of 7 identical α-chains (C4BPα+) and 1 β-chain (C4BPβ+) covalently linked by their C-terminal regions. In human plasma, PS forms a noncovalent 1:1 stoichiometric complex with C4BP via binding to the C4BP β-chain. In vivo, all C4BPβ+ isoform molecules circulate bound to PS. Only the molar excess of PS (≈40%) over C4BPβ+ circulates in a free form and is active as a cofactor for activated PC. The PS-C4BP complex may also be functional. For example, an activated PC-independent anticoagulant mechanism has been ascribed to the PS-C4BP complex based on its ability to inhibit both the prothrombinase complex and the factor X activating complex.

Recently, it has been shown that differential regulation of the C4BPA and C4BPB genes by acute phase cytokines leads to alterations in the synthesis of C4BP isoforms. The resultant variations in the concentration of C4BP may alter the equilibrium between bound and free PS. This could be interpreted as a mechanism to maintain steady levels of PS, preserving the normal function of the PC anticoagulant pathway.

Given the evidence for linkage of a locus influencing fPS levels to chromosome 1q32 and the known relationships between C4BP and PS, the C4BPA and C4BPB genes must be considered strong candidates for a major PS regulatory locus. Additionally, the associations of PS levels or deficiency with myocardial infarction and thrombosis imply that the C4BP genes may also be potentially important modulators of an individual’s susceptibility to disease.

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References

4. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation...
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