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Deep-vein thrombosis is not associated with the P/S186 polymorphism of histidine-rich glycoprotein

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Summary *Background*: In several studies, higher plasma levels of histidine-rich glycoprotein (HRG) have been observed in patients with venous thrombosis than in healthy subjects. Apart from environmental factors, such as the use of oral contraceptives, the plasma HRG levels are mainly determined genetically. The most important genetic determinant is P/S186 polymorphisms in exon 5 of the HRG gene which is associated with 40% higher HRG levels. In this study we investigated the relationship between the HRG P/S 186 polymorphism and venous thrombosis.

Methods and Results: DNA was available from 466 patients and 471 controls of the Leiden Thrombophilia Study (LETS), a population-based case-control study on venous thrombosis. Both in patients and controls, the genotype distribution of the P/S186 polymorphism was not different from that predicted by the Hardy–Weinberg equilibrium. No association between the genotypes of the P/S186 polymorphism and deep-vein thrombosis was found (PS 186 genotype: OR: 0.97 (CI:0.24,1.70); SS 186 genotype: OR: 1.12 (CI:0.21,2.04), PP 186 is the reference category).

Conclusion: The results of this study suggest that the HRG P/S 186 polymorphism is not associated with first venous thrombotic events.

INTRODUCTION

Histidine-rich glycoprotein (HRG) is a single-chain non-enzymatic plasma glycoprotein that is synthesized by the parenchymal cells of the liver.[1,2] HRG may be active in many physiological processes and it is considered to be able to act as a modulator of coagulation and fibrinolysis through binding with heparin and plasminogen (reviewed in Koide).[3] Whether HRG really contributes to the *in vivo* fibrinolysis is still not clear,[4,5] but in several cross-sectional studies elevated plasma concentrations of HRG were associated with venous thrombosis.[6–10]

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HRG concentrations are influenced by genetic factors,[11,12] and by environmental factors such as oestrogens,[13,14] pregnancy[15,16] and age.[7] Hennis et al.[17] identified a common molecular variant of HRG, which is caused by a single base pair substitution in exon 5 of the HRG gene, resulting in a substitution of proline 186 by serine.[18] This substitution is associated with an increase in the molecular weight, most likely by glycosylation of serine 186 (S 186) (unpublished results). The S186 allele was found to be associated with ±40% higher levels of HRG in plasma when measured with the immunodiffusion technique.[19]

Since the S/P 186 polymorphism accounts for 84% of the total genetic influence of the HRG locus on the plasma HRG level[18] we postulated that the S/P 186 polymorphism may be used to evaluate the relationship between plasma levels of HRG and thrombosis. In this study we investigated the association between the S 186 allele and the occurrence of a first thrombotic event.

MATERIALS AND METHODS

Subjects

The Leiden Thrombophilia Study (LETS) is a population-based case-control study on venous thrombosis. LETS was set up with the aim of providing a direct comparison between unselected patients with venous thrombosis and an appropriate population-based venous-thrombosis-free control group. The selection procedures for patients and control subjects have previously been described in detail.[20] Briefly, consecutive patients, less than 70 years, who were referred for anticoagulant treatment after a first, objectively confirmed, episode of deep-vein thrombosis occurring between January 1988 and January 1993, were selected from the files of the anticoagulation clinics in Leiden, Amsterdam and Rotterdam. Each thrombosis patient was asked to find their own healthy control subject according to the following criteria: same sex, about the same age (plus/minus 5 years), no biological relative and no history of venous thromboembolism, no use of coumarins over the previous 3 months, not known to have a malignant disorder and an inhabitant of the same geographical area. Partners of patients were also invited to serve as control subjects for other patients who were unable to find a control subject. For the present study DNA was available from 466 patients and 471 controls.

DNA procedures

Enzymatic amplification of genomic DNA was performed by polymerase chain reaction (PCR) in a final volume of 25 µl containing 100 ng genomic DNA, 20 mmol/l Tris-HCl pH 9.4, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 50 µmol dNTPs, 50 ng of each primer and 0.1 unit Super Taq polymerase (HT Biotechnology Ltd., UK) according to the manufacturer's instructions. The PCR reactions were performed in a Hybaid Omnigene thermal cycler (Hybaid Teddington, UK). The nucleotide sequences of the PCR primers were 5'-CTGTTCTTGAAACTATTTGATCC-3' and 5'-TGACTCTAGTCAACGATCAC-3' (Pharmacia Biotech, the Netherlands) The PCR reaction started with 4 min at 95°C and proceeded for 30 cycles, each with a denaturation step of 1 min at 95°C, annealing for 1 min at 55°C and extension for 1 min at 72°C. The PCR product was digested overnight with 1 2 units of *BamI* (New England Biolabs, MA, USA) at 37°C. After digestion, the PCR product was separated by electrophoresis using 4% agarose gels in 0.5 × TBE buffer (0.045 mol/l Tris, 0 044 mol/l boric acid and 1 mmol/l EDTA) containing ethidium bromide, and visualized under uv light. *BamI* digestion yielded one band of 156 bp in the absence of the restriction site (common allele, P 186) and two bands of 84 bp and 72 bp in the presence of the restriction site (rare allele, S 186)

Statistical analysis

A χ^2 test was used to compare the observed numbers of each genotype with those expected for a population in Hardy–Weinberg equilibrium Logistic regression was used to evaluate the risk for venous thrombosis in those carrying the S 186 allele

RESULTS

The general characteristics of the total population and separately for each genotype are shown in Table 1 There was no difference in the frequency of the S 186-allele in patients {0.34 (95% CI 0 31,0 37)} and in controls {0 35 (95% CI: 0.32,0 38)}, and also the genotype distribution was similar. The genotype distributions were in Hardy–Weinberg equilibrium both for patients and controls. In both the patients and the control group the mean age and percentage of females was similar in each genotype group.

No association between the P/S 186 polymorphism and venous thrombosis was observed The odds ratios were 0.97 (95% CI: 0 24,1.70) for the PS Genotype and 1 12 (95% CI: 0.21,2.04) for the SS genotype when the PP genotype was taken as a reference

Table 1 General characteristics of patients and controls, for the total group and according to genotype

		Genotype		
	Total group	**PP186**	**PS186**	**SS186**
Patients				
number of subjects	466	197	219	50
age (y) {mean(SD)}	45.1 (13.7)	45.6 (14.2)	44 9 (13 3)	43 5 (13 8)
females (%)	56.9	56.9	56 6	58 0
Controls				
number of subjects	471	199	215	57
age (y) {mean(SD)}	44.7 (13.5)	45.1 (13.3)	44.6 (13.7)	43.3 (13.9)
females (%)	57.3	62.8	54.9	47 4

DISCUSSION

In the present study no association has been observed between the HRG P/S 186 polymorphism and venous thrombosis

The allele frequencies of the polymorphism in the controls were similar to those in healthy volunteers reported in a previous study[17] The genotype distribution for patients and controls was similar which suggests that patients and controls originated from the same source population This supports our finding of no increased risk of venous thrombosis in those carrying the S allele

Twin studies have shown that 70% of the variance in plasma HRG levels, as measured by radial immunodiffusion, can be explained by genetic factors, mainly (84%) by the P/S 186 polymorphism The rest is explained by other, as yet unknown, genetic factors[18] Recently, it was reported that the radial immunodiffusion assay for plasma HRG, which is based on a polyclonal antibody against HRG, shows a different specificity towards the two variants of the P/S 186 polymorphism It has been found that higher HRG levels were recorded for the molecular form containing the glycosylated S 186 than that containing the non-glycosylated P186[19] In subjects with the SS 186 genotype the HRG levels, as measured using immunodiffusion, appear to be 40% higher than the levels in subjects with the PP 186 genotype, while heterozygotes have levels that are intermediate

This high contribution of the P/S186 polymorphism to plasma HRG levels, as measured with immunodiffusion, combined with our observation that there is no relationship between the S 186 allele and the incidence of venous thrombosis, may suggest that there is also no association between plasma concentrations of HRG and venous thrombosis This conclusion would be in line with results from some previous studies,[5,21] however, in other studies a positive relationship between HRG levels and thrombosis was reported[6-10] This suggests that other factors which influence plasma HRG levels may play a role in the association of HRG and thrombosis as observed in previous studies[6-10] In conclusion, the results presented here indicate that the HRG P/S186 polymorphism is not related to venous thrombosis

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