

Population differences in the frequency of the factor V Leiden variant among people with clinically symptomatic protein C deficiency

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Abstract

The factor V Leiden variant, responsible for the phenomenon of activated protein C resistance, was found to be less frequent among British (0.06) and Swedish/Danish (0.15) protein C deficiency patients than previously reported in a Dutch study (0.19). In the Swedish population, a significantly increased frequency of the factor V Leiden allele was apparent in protein C deficiency patients as compared to healthy controls. However, this was not found in the British population. Coinheritance of the factor V Leiden variant is therefore unlikely to be the sole determinant of whether a person with protein C deficiency will come to clinical attention. Nevertheless, when patient data were analysed by type of protein C deficiency, it was noted that the frequency of the factor V Leiden variant was 2.8-fold higher in type II patients compared to type I patients. A possible explanation of this disparity is discussed.

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A high prevalence of the newly discovered factor V (FV) Leiden variant (Arg506→Gln),¹ responsible for the phenomenon of activated protein C (APC) resistance,²⁻⁶ has been recently reported in subjects with clinically symptomatic protein C deficiency.⁷ Coinheritance of this frequent variant with a protein C gene lesion could therefore explain the difference in thrombotic risk noted between families with clinically symptomatic (overt) protein C deficiency and those with the much more common clinically asymptomatic (covert) deficiency state.^{8,9} In order to examine this postulate, we have determined the prevalence of the FV Leiden variant among unrelated protein C deficient patients of different ethnic/

geographical origin, all of whom shared a personal history of venous thrombosis.

Materials and methods

PROTEIN C DEFICIENCY PATIENTS

Blood samples from 120 unrelated patients with (1) a personal history of venous thrombosis, (2) a reduced level of protein C antigen or activity, and (3) a family history of protein C deficiency or venous thrombosis or both, were collected for analysis. The majority of these patients came from either Great Britain (white origin, n=47) or Scandinavia (Swedish/Danish, n=34) while the remainder was of variable geographical ethnic origin, including whites of other nationalities, AfroCaribbeans, and Asians. A sample of 173 healthy people (108 German, 65 British; all white) was selected as controls.

SCREENING FOR THE FACTOR V LEIDEN LESION

A 222 bp fragment of the factor V gene containing exon 10 (the location of the CGA→CAA transition responsible for the Arg506→Gln substitution in FV Leiden) was PCR amplified from DNA derived from the 120 protein C deficient patients and 173 controls as described.¹ The amplified fragments were slot blotted onto duplicate Genescreen filters which were then hybridised to end labelled sequence specific oligonucleotide probes to discriminate between the wild type allele and the FV Leiden variant.¹ Filters were subsequently washed at the discriminant temperature (58°C) for 20 minutes in 3 mol/l tetramethylammonium chloride before autoradiography.¹⁰

Results and discussion

FV LEIDEN ALLELE FREQUENCY IN PATIENTS AND CONTROLS

In our sample (table 1), the frequency of the FV Leiden variant was found to be significantly lower among British patients (0.064) than noted previously in a Dutch study⁷ (0.190, n=48). Comparison by means of a small sample Fisher permutation test assigned an error probability of p=0.009 to this finding. Among Swedish/Danish protein C deficient patients, however, the frequency of FV Leiden (0.147) was intermediate and failed to differ significantly from both the British and Dutch data, most probably because of the comparatively small sample size. In the control group, by contrast, the prevalence of FV Leiden

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Table 1 Frequency of FV Leiden variant in subjects with inherited protein C deficiency and controls by geographical origin

Group	No	FV Leiden heterozygotes	FV Leiden homozygotes	FV Leiden allele frequency
<i>Controls</i>				
British	65	4	0	0.031
German	108	7	0	0.032
<i>Patients</i>				
British	47	4	1 (4.2)	0.064
Swedish/Danish	34	6	2	0.147
Others	39	0	0	0.000

Figure in brackets denotes the relative risk of protein C deficiency conferred by homozygosity for the FV Leiden variant. Others: patients of diverse ethnic origin other than British or Swedish/Danish.

Table 2 Frequency of FV Leiden variant by protein C deficiency type

Protein C deficiency type	No	FV Leiden heterozygotes	FV Leiden homozygotes	FV Leiden allele frequency
I	80	5	2	0.056
II	13	2	1	0.154
Unknown	27	3	0	0.056

Type I: plasma activity and antigen values concomitantly reduced. Type II: greater reduction in activity than in antigen reflecting the synthesis of a dysfunctional protein.

variant (0.031) was similar to that noted in the normal Dutch (0.023),⁷ German (0.032, table 1), and Swedish populations (0.035).⁴

The extent of association between the FV Leiden allele and protein C deficiency thus appears contentious. On one hand, the frequency of the variant in British protein C deficient patients is only slightly higher than in controls from the same population (Fisher's $p=0.20$) and the relative risk¹¹ for protein C deficiency conferred by heterozygous or homozygous carriership of the FV Leiden allele is 1.8. In Swedish protein C deficient patients, however, the frequency of the FV Leiden allele is significantly higher than noted in healthy controls from the same population⁴ ($p=0.0001$, by reference to the underlying Bernoulli distribution), and a relative risk of 4.1, conferred by heterozygous or homozygous carriership of the FV Leiden allele, results when a Hardy-Weinberg equilibrium frequency of healthy non-carriers is assumed.

In summary, we conclude that the prevalence of the FV Leiden variant among patients with symptomatic protein C deficiency may not be consistently as high as previously suggested⁷ and that geographical differences in the pattern of coinheritance of the two traits may exist. Nevertheless, the higher relative risk for protein C deficiency conferred by homozygosity for the FV Leiden variant (table 1, British patients), although subject to considerable sampling variance, suggests that protein C deficient subjects who are homozygous for the FV Leiden variant are more likely to come to clinical attention than those who are heterozygous.

Two points are, however, worthy of further mention. Firstly, the relative importance of specific protein C defects could not be properly assessed without the comprehensive characterisation of all the mutations segregating in the 120 families. This rather laborious programme is under way but still incomplete. This notwithstanding, there is currently no evidence for protein C gene lesions differing in their likelihood of coming to clinical attention.¹²⁻¹⁴ Moreover, a recent study has revealed that founder effects are of relatively minor importance for the geographical distribution of known protein C gene lesions in European populations.¹⁴ On the whole, therefore, geographical differences in the protein C mutational spectrum are unlikely to represent a major contributory factor to the observed population differences in the frequency of the factor V Leiden variant.

ANALYSIS BY TYPE OF PROTEIN C DEFICIENCY

When the total patient data, including those of diverse ethnic/geographical origin ("Others" in

table 1), were analysed by type of protein C deficiency, it was noted that the frequency of the FV Leiden variant was 2.8-fold higher in type II patients than in type I patients (table 2). Although the significance of this observation was borderline (Fisher's $p=0.089$), and thus awaits confirmation by further studies, we offer the following possible explanation.

Together with protein S and factor V, APC forms a 1:1:1 stoichiometric complex on the phospholipid surface that is capable of inactivating factor VIIIa.¹⁵ The FV Leiden defect is likely to reduce the level of this inactivating complex. This is because thrombin generation is increased in APC resistant plasma owing to the excess of factor Va.¹⁵ Factor V activated by thrombin is, however, inefficient as a cofactor in the inactivation of factor VIIIa.¹⁵ Although the reduced synthesis of protein C, characteristic of type I protein C deficiency state, would presumably reduce the level of factor VIIIa inactivating complex still further, a normal level of factor V is still available to wild type protein C molecules for complex formation. By contrast, a dysfunctional (type II) protein C molecule might still be able to interact with protein S and factor V to generate a non-functional complex. Once formed, this inactive complex could sequester factor V and protein S thereby reducing the amount of factor V accessible to wild type protein C (it might also compete with its functional counterpart for access to factor VIIIa). This reduction might explain why carriership of the FV Leiden variant increases the likelihood of clinical detection more dramatically for type II protein C deficiency than for type I deficiency patients.

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