FACTOR V AND ANTITHROMBIN GENE MUTATIONS IN PATIENTS WITH IDIOPATHIC CENTRAL RETINAL VEIN OCCLUSION

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SUMMARY

A number of anticoagulants are found in plasma, helping to maintain the balance between thrombosis and haemorrhage. Two of the most important are antithrombin and protein C, which inactivates factors V and VIII. Deficiencies are well-recognised predisposing factors for systemic thrombosis. To establish whether the factor V or Cambridge II antithrombin mutations were present with an increased frequency in patients with idiopathic central retinal vein occlusion (CRVO) we screened 50 such patients. DNA was isolated and the regions of the gene encoding for factor V and antithrombin were amplified by means of the polymerase chain reaction. Following digestion with restriction enzymes the products were electrophoresed in agarose gels. We identified a single patient with the factor V mutation and none with the antithrombin mutation. These findings suggest that resistance to activated protein C and antithrombin mutations does not play a major role in CRVO.

Central retinal vein occlusion (CRVO) is a common cause of acute visual loss. However, despite extensive research much of the pathophysiology remains unclear. The actual mechanism may be related to either structural problems at the lamina cribrosa,[1,2] haemostatic factors[3,4] or a combination of these.

Many systemic conditions have been associated with CRVO:[5] arteriosclerosis and cardiovascular disease,[6] arterial hypertension,[7,8] diabetes,[9] smoking and hyperlipidaemia.[8] The most important ocular condition is open angle glaucoma.[10,11] Despite excluding these causes there are still many patients without an obvious predisposition. It has been suggested that nocturnal hypotension may have a major role in this condition.[12]

The role of haemostatic factors in venous occlusion has not been fully clarified although our understanding of systemic venous thrombosis is improving. How this relates to CRVO is unclear. Blood has to remain in a fluid state yet, if a leak develops, it has to be arrested by coagulation, but only at that specific site. To maintain this homeostasis a complex series of reactions characterised by molecular and cellular amplification takes place. The role of the thrombomodulin/protein C anticoagulant pathway in this balance has now been defined (Fig. 1).[13] After its activation on the surface of endothelial cells, protein C inhibits coagulation by selectively degrading activated factors V and VIII. It has recently been described[14] that a mutation in factor V (Arg506Gln) can prevent its inactivation by activated protein C leaving its pro-coagulant activity unaffected.[14] The importance of this mutation has been established by it being found in up to 7% of the normal Swedish population,[15] up to 19% of a consecutive series of patients with venous thrombosis[14] and in 40% of patients referred to a centre for coagulation disorders.[16]

Antithrombin is a serine protease inhibitor produced by the liver and which binds irreversibly to thrombin. *In vitro* the activity of antithrombin is increased at least 1000-fold by binding to heparin. Congenital antithrombin deficiency (autosomal dominant) has a prevalence in young patients with venous thrombosis of up to 5%[17] and antithrombin deficiency has been reported in several patients with CRVO.[18,19] The antithrombin Cambridge II mutation (Ala384 Ser) has been described with a high frequency in the West of Scotland, and although the majority of individuals were asymptomatic blood donors, in two patients there was a history of CRVO. No formal search for the mutation has yet been undertaken in patients with CRVO.

To establish whether the factor V or the Cambridge II antithrombin gene mutation are present with an increased frequency in patients with central

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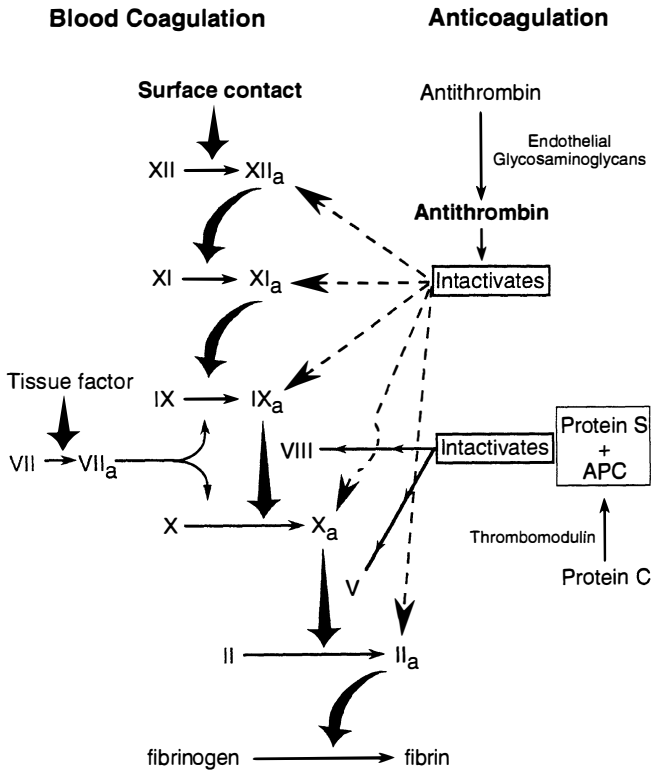

Fig. 1. *The blood coagulation and anticoagulation pathways.*

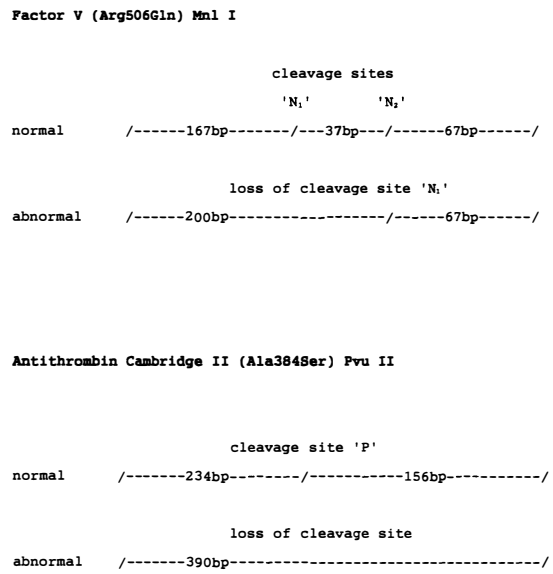

Fig. 2. *Loss of restriction enzyme cleavage sites as a result of mutation in factor V and antithrombin.*

retinal vein thrombosis, we screened 50 idiopathic cases (which allowed us to look at these factors in relative isolation) compared with a control group.

METHODS

Ethics approval was obtained for the study. Patients with a CRVO (ischaemic or non-ischaemic) were selected from the clinic database record at Addenbrooke's Hospital. Case notes were reviewed to identify idiopathic cases. Those patients with a CRVO without an association or abnormality on blood tests were recalled to provide 20 ml of citrated blood after an explanation of the research basis for the test.

DNA was isolated from the leucocytes of each of the patients. The factor V gene mutation is associated with the loss of an *Mnl*I restriction site and the antithrombin Cambridge II mutation with the loss of a *Pvu*II restriction site (Fig. 2). Following amplification and digestion, the resulting products were electrophoresed on a 1% agarose gel, stained with ethidium bromide and viewed under ultraviolet light.

RESULTS

One hundred and thirty-six patients with a CRVO were identified. Review of the notes revealed: 9 patients with diabetes, 21 patients with arterial hypertension (blood pressure greater than 160/95 mmHg), 2 patients with an abnormal full blood count, 3 patients with abnormal erythrocyte sedimentation rate (greater than half the age), 3 patients with glaucoma and 12 patients who had since died (9%). Eighty-eight (65%) patients with no systemic or ocular associations remained as suitable to be included in the study.

Fifty (50/88) of these patients (57%) re-attended for blood samples to be taken and were included in the analysis. There were 22 men of average age 68.4 years (range 58–83 years) and 28 women of average age 70.5 years (range 62–84 years).

We identified a single patient with the factor V Leiden mutation but no cases of the Cambridge II mutation. The one patient with the factor V mutation developed a severely ischaemic fundus that despite panretinal photocoagulation developed proliferative retinopathy with glaucoma.

DISCUSSION

Central retinal vein thrombosis remains a serious problem for the patient and ophthalmologist. Many risk factors have been identified and our study confirms the importance of hypertension. Recently, a major advance has been made in our understanding of the risk factors for systemic thrombosis.[14–16] Dahlback *et al.*[20] postulated that a defect in the protein C pathway interferes with the action of activated protein C (APC). Subsequent work has identified this resistance to APC as a single amino acid substitution within the factor V protein.[15]

The mutation associated with the factor V abnormality is at its site for cleavage by APC. This would normally lead to its inactivation (Fig. 1). These mutated factor V molecules therefore have normal pro-coagulant activity but are resistant to inactiva-

tion, leading to a thrombotic tendency. The mutation is inherited as an autosomal dominant trait.[15]

There are reports of protein C deficiency in relation to retinal vascular events. A 50-year-old man with bilateral retinal vein occlusions[21] was reported to be protein C deficient and Chung *et al.*[22] reported a statistically significant decrease in protein C in six patients with branch retinal vein occlusions. We can find no reference to APC resistance in retinal vascular occlusions. The antithrombin Cambridge II mutation has also been described as being associated with coagulation problems (D. Perry, unpublished data).

The early clotting-based assay for APC resistance was fraught with problems and a simpler approach to studying the defect is to analyse the DNA directly for the mutation using the polymerase chain reaction (PCR) and restriction enzymes with known cleavage sites. Both the antithrombin Cambridge II and factor V gene mutations are point mutations which as well as disrupting the function of the gene product also result in the loss of a restriction enzyme cutting site. This enables the mutant genes to be identified in a simplified fashion by amplifying the relevant segment of the gene using the PCR and then cutting the product with the appropriate restriction enzyme. Patients with the mutant form of the gene lack a cutting site and thus generate fewer fragments (Fig. 2).

This study indicates that neither the antithrombin Cambridge II mutation nor the factor V Leiden mutation has a major role in the pathogenesis of central retinal vein thrombosis. It remains that CRVO are probably multifactorial in origin and rarely does any single factor on its own cause an occlusion. Factor V and antithrombin III seem to be only part of this multifactorial origin when studied in isolation and they may play a more substantial role when there are other predisposing factors. Despite criticisms such as insufficient numbers or too selective a group of patients we suggest there is no requirement for these expensive investigations in this group of patients without other evidence of thrombosis or a family history of thrombosis, and that screening should be selective. These further studies are needed and we are currently undertaking them.

Key words: Antithrombin III, CRVO, Factor V.

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