

Procoagulant mutations and venous thrombosis in Behçet's disease

SIR, Behçet's disease (BD) is a vasculitis of unknown aetiology characterized by involvement of both arteries and veins and vessels of all sizes [1]. Thrombosis of superficial and deep veins is more frequent than arterial aneurysms and thrombotic occlusions. Deep vein thrombosis of the lower extremities is seen in about one-fifth of Turkish patients with BD [2]. Thrombotic occlusions of inferior and superior venae cavae, hepatic veins and cerebral sinuses can also be observed, albeit less frequently.

We previously reported that coagulation factor V gene G1691A mutation (factor V Leiden), the most common inherited coagulation defect associated with venous thrombosis, could be detected in 37.5% of BD patients with a history of deep vein thrombosis in a case-control study [3]. The association of factor V Leiden with venous thrombosis in BD was later confirmed in different ethnic groups [4, 5].

Herein, we present our results of screening the same study group for the presence of a new mutation identified in the 3'-untranslated region of the prothrombin gene (20210 G→A). This mutation is associated with elevated plasma prothrombin levels and a 3-fold increased risk of venous thrombosis [6], and it has been reported as the second most common inherited hypercoagulable state [7]. The prevalence of heterozygous 20210A carriers varied from 1 to 5% in healthy Caucasians and was found to be 2.2–2.7% in healthy Turkish controls [7–9].

The study group consisted of 64 patients, 32 with a history of deep vein thrombosis (T+) of the lower extremities, and 32 age- and sex-matched patients without any thrombotic events (T-) during a minimum of 8 yr disease duration. All patients fulfilled three or more International Study Group (ISG) criteria for BD [2]. In the T+ group, three patients also had inferior vena cava, two had superior vena cava and three had sagittal sinus thromboses. Arterial involvement in pulmonary, brachial, iliac and femoral arteries was present in four patients. Clinical details of the patients were given previously [3].

The G20210A mutation in the 3' untranslated region of the prothrombin gene was detected by allele-specific polymerase chain reaction (PCR) as described previously [10]. Briefly, it was demonstrated by using a

forward consensus primer and two reverse primers, one of which is specific for the wild type and the other for the mutated alleles. The 340 bp PCR products were visualized on ethidium bromide-stained agarose gels. A second amplification was performed to confirm the presence of the mutation.

We compared the frequencies of the prothrombin and/or factor V gene mutations in the T+ and T- groups by χ^2 test, and calculated the odds ratio (OR) for estimation of thrombosis risk in the presence of procoagulant mutations.

Heterozygous prothrombin gene G20210A mutation was found in 10 (nine male, one female) of the T+ (31.3%) and in one (male) of the T- group (3.1%) ($P = 0.003$, OR = 14.1, 95% CI 1.7–118.2; Table 1). One of these 10 patients had sagittal sinus thrombosis, one had sagittal sinus + inferior vena cava and one superior vena cava thrombosis in addition to thrombosis of the lower extremities. The prothrombin gene mutation was found in two male patients, one with femoral + popliteal artery thrombosis and the other with brachial artery aneurysm + thrombosis.

Four patients (three male, one female) in the T+ group were carrying both factor V Leiden and prothrombin gene G20210A mutations, and one of them was the patient with femoral + popliteal artery thrombosis.

A total of 18 patients (56.3%) with BD and deep vein thrombosis were carrying factor V Leiden and/or the prothrombin gene G20210A mutation compared with only four of the BD patients without any thrombotic event ($P = 0.0002$, OR = 9, 95% CI 2.6–31.7).

The detection of the two most frequent hereditary prothrombotic mutations known in more than half of BD patients with deep vein thrombosis supports the important role of procoagulant mutations in the development of venous thrombosis in BD. We previously suggested that the tendency to thrombosis in BD could be explained by endothelial activation induced by immune-mediated vasculitis. Endothelial changes may trigger venous (and arterial) thrombosis in patients carrying factor V Leiden, the prothrombin G20210A mutation, or other yet unidentified mutations of the coagulation system, if the inflammatory response is strong enough. Cerebral vein and arterial thrombosis has been reported in individuals heterozygous for the prothrombin gene mutation [11, 12]. We identified two patients with sagittal sinus thrombosis, one of whom

TABLE 1. Prevalence of the factor V Leiden and prothrombin gene G20210A mutations in Behçet's disease patients with (T+) and without (T-) deep vein thrombosis

	n	Sex (M/F)	Factor V Leiden (%)		Prothrombin gene mutation (%)	
			G,G	G,A*	G,G	G,A†
T +	32	26/6	20 (62.5)	12 (37.5) ^a	22 (68.7)	10 (31.3) ^c
T -	32	26/6	29 (90.6)	3 (9.4) ^b	31 (96.9)	1 (3.1) ^d

*G,A, heterozygous for the factor V gene G1691A mutation (factor V Leiden); †G,A, heterozygous for the prothrombin gene G20210A mutation; G,G, no mutation.

a vs b, $P = 0.008$; c vs d, $P = 0.003$.

also had inferior vena cava thrombosis, and two males with arterial thrombosis carrying the prothrombin gene mutation in this series of patients with BD. Further studies are needed to show any predilection for the site of thrombosis in patients carrying a particular procoagulant mutation or a combination of mutations in larger series of patients.

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1. Lie JT. Vascular involvement in Behçet's disease: Arterial and venous and vessels of all sizes. *J Rheumatol* 1992;19:341–3.
2. International Study Group for Behçet's Disease. Evaluation of diagnostic ('Classification') criteria in Behçet's disease—towards internationally agreed criteria. *Br J Rheumatol* 1992;31:299–308.
3. Gül A, Özbek U, Öztürk C, Inanç M, Koniçe M, Özçelik T. Coagulation factor V gene mutation increases the risk of venous thrombosis in Behçet's disease. *Br J Rheumatol* 1996;35:1178–80.
4. Mammo L, Al-Dalaan A, Bahabri SS, Saour JN. Association of factor V Leiden with Behçet's disease. *J Rheumatol* 1997;24:2196–8.
5. Verity DH, Vaughan RW, Madanat W *et al.* Factor V Leiden mutation is associated with ocular involvement in Behçet disease. *Am J Ophthalmol* 1999;128:352–6.
6. Poort RS, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in 3'-untranslated region of prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996;88:3698–703.
7. Bertina RM. The prothrombin 20210 G to A variation and thrombosis. *Curr Opin Hematol* 1998;5:339–42.
8. Akar N, Misirlioglu M, Akar E, Avcu F, Yalcin A, Söztöz A. Prothrombin gene 20210 G-A mutation in the Turkish population. *Am J Hematol* 1998;58:249.
9. Gürgey A, Hicsönmez G, Parlak H, Balta G, Celiker A. Prothrombin gene 20210 G-A mutation in Turkish patients with thrombosis. *Am J Hematol* 1998;59:179–80.
10. Hézar N, Cornillet-Lefebvre P, Gillot L, Potron G, Nguyen P. Multiplex ASA PCR for a simultaneous determination of factor V Leiden gene, G→A 20210 prothrombin gene and C→T 677 MTHFR gene mutations. *Thromb Haemostasis* 1998;79:1054–5.
11. Arruda VR, Annichino-Bizzacchi JM, Gonçalves MS, Costa FF. Prevalence of the prothrombin gene variant (nt20210A) in venous thrombosis and arterial disease. *Thromb Haemostasis* 1997;78:1430–3.
12. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Mannucci PM. High risk of cerebral vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. *N Engl J Med* 1998;338:1793–7.