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BRIEF REPORT

COAGULATION FACTOR V GENE MUTATION INCREASES THE RISK OF VENOUS THROMBOSIS IN BEHÇET'S DISEASE

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SUMMARY

We investigated the prevalence of the coagulation factor V gene G1691A mutation in 64 patients with Behçet's disease (BD) and in 107 apparently healthy individuals. The mutation was present in the heterozygous state in 37.5% of the patients with a history of deep vein thrombosis (12/32) and in 9.4% of the patients without any thrombotic event (3/32). Eleven healthy individuals were also heterozygous for the mutation (10.3%). The prevalence of the mutation in BD patients with and without thrombosis was significantly different ($P = 0.0079$). We conclude that the factor V gene mutation may play a major role in the development of venous thrombosis in BD.

KEY WORDS: Behçet's disease, Factor V gene mutation, Activated protein C resistance, Deep vein thrombosis, Vasculitis.

BEHÇET'S disease (BD) is a multisystemic chronic inflammatory disorder of unknown aetiology, with vasculitis as the main pathological process. Although involvement of blood vessels of all types and sizes is a prominent feature of the disease, venous involvement is seen more frequently (20–40%) than arterial disease [1]. Superficial thrombophlebitis is the most frequent form of vascular disease, followed by deep vein thrombosis of especially the lower extremities, which can be seen in 19% of Turkish patients [1, 2]. Additionally, vascular disease is more frequent and severe in males [1].

Although a decrease in fibrinolysis was described, a specific molecular defect leading to thrombosis has not been identified in patients with BD [3]. Endothelial dysfunction resulting from immune-mediated vasculitis is suggested as the basis of thrombotic vascular complications [4].

Resistance to activated protein C (APC) has been described as the most common inherited coagulation defect associated with venous thrombosis [5]. The molecular basis of APC resistance has recently been shown to be a single point mutation (G1691A) in the coagulation factor V gene [6]. This molecular defect was found in ~20–40% of patients with idiopathic deep vein thrombosis [5, 6].

We investigated whether the presence of the factor V gene G1691A mutation was associated with the development of venous thrombosis in BD by this case–control study.

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PATIENTS AND METHODS

Patients and healthy individuals

A total of 64 patients, 32 with a history of deep vein thrombosis (T+), and 32 age- and sex-matched patients without any thrombotic events (T−), all fulfilling three or more International Study Group (ISG) criteria for BD, were included (Table I) [2]. All T+ patients had deep vein thrombosis of the lower extremities. In addition, 10 patients also had superficial thrombophlebitis, and three had inferior vena cava, two had superior vena cava and two had sagittal sinus thromboses. Arterial involvement with aneurysm formation, and thrombotic occlusion in pulmonary, brachial, iliac and femoral arteries, was present in four patients. Patients with no history of thrombosis during a disease duration of at least 5 yr were selected as the T− group since the vascular involvement appears to decrease after 5 yr from the onset of the disease [1]. Frequencies of oral and genital ulcers, eye disease, skin manifestations and a positive pathergy test were quite similar in both groups. Arthritis was more frequent in patients without thrombosis (24 patients in T− *vs* 11 patients in T+), whereas the number of patients with central nervous system disease was higher in the other group (five patients in T+ *vs* one patient in T−).

We also screened 107 apparently healthy unrelated subjects for estimation of the prevalence of the factor V gene mutation in the Turkish population.

DNA analysis

The presence of the G1691A mutation in exon 10 of the factor V gene was detected by polymerase chain reaction as described previously [7]. Briefly, it was demonstrated by digestion of amplified factor V DNA with *MnlI* and visualization of the cleavage products on ethidium bromide-stained agarose gels. A second

TABLE I
Prevalence of the factor V gene G1691A mutation in BD patients with (T+) and without (T−) thrombosis, and in an apparently healthy Turkish control group

	n	Sex (M/F)	Age (yr) mean ± S.D. (range)	Disease duration (yr) mean ± S.D. (range)	Genotype* G,A	Genotype* G,G	% G,A
Behçet's disease							
T+	32	26/6	36 ± 9 (20–53)	9 ± 4 (2–20)	12	20	37.5[a]
T−	32	26/6	40 ± 9 (22–63)	11 ± 6 (5–28)	3	29	9.4[b]
Healthy controls	107	57/50	28 ± 7 (16–48)	–	11	96	10.3

*Genotype: G,A = heterozygous for the G1691A mutation; G,G = no mutation. [a] versus [b], $P = 0.0079$.

amplification and digestion was performed to confirm the presence of the mutation.

Statistical analysis

We compared the percentages of the mutation in the T+ and T− groups by χ^2 test, and calculated the odds ratio for estimation of thrombosis risk in the presence of the factor V gene mutation. The effects of male dominance on the results were tested by logistic regression.

RESULTS

The factor V gene mutation was found in 12 (10 male, 2 female) of the T+ (37.5%) and in three (two male, one female) of the T− group (9.4%) (Table I). All of the patients who tested positive were heterozygous for the mutation (Fig. 1). In the T+ group, all patients with superior and inferior venae cavae, and sagittal sinus thromboses lacked the mutation, whereas two out of four patients with arterial and venous involvement (in iliac and femoral arteries) were found to be positive for the mutation.

All BD patients carrying the mutation had a history of oral and genital ulcerations, and skin manifestations, including the pathergy reaction. Among the T+ patients, two also had uveitis and four had recurrent attacks of oligoarthritis. Two of the patients in the T− group experienced one attack of acute oligoarthritis. Although all three T− patients with the mutation fulfilled the ISG criteria for BD, they remained asymptomatic for years, except for rarely recurring oral aphthous ulcerations, and had a very mild disease course.

The mutation frequency was significantly higher in BD patients with than without thrombosis ($P = 0.0079$). Heterozygosity for the factor V gene mutation was found to be associated with an almost 6-fold increase in the risk of venous thrombosis (odds ratio 5.8, 95% CI 1.4–23.2). Logistic regression analysis revealed no significant effect of male dominance on the results.

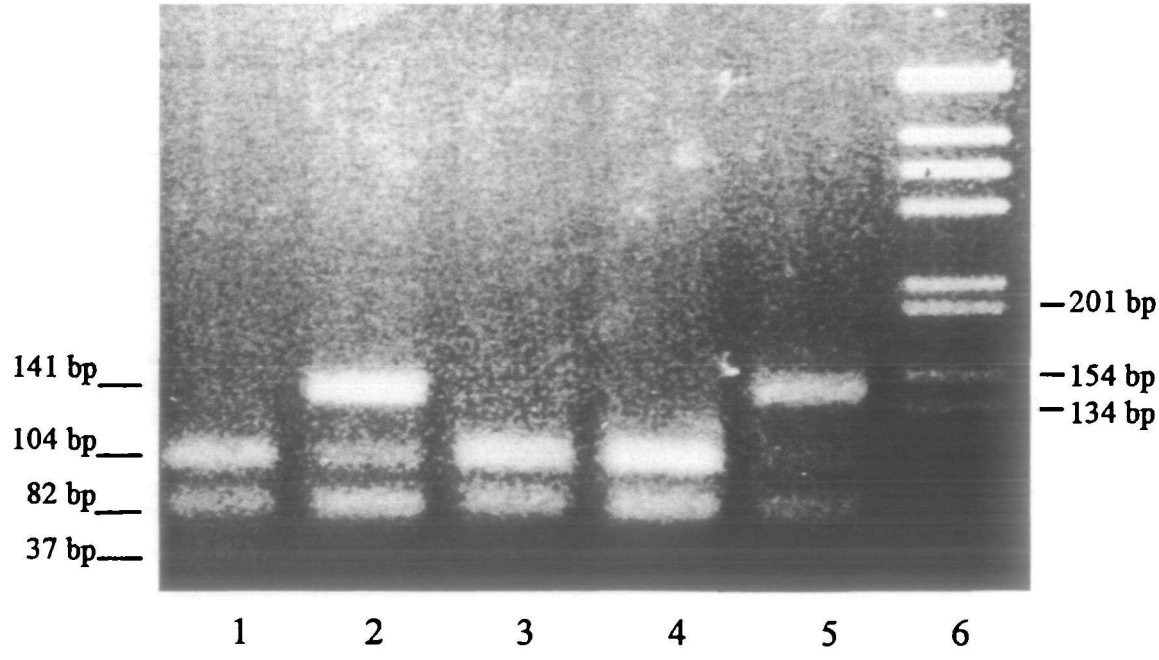

FIG. 1.—A 223 base pair (bp) fragment was amplified from genomic DNA and digested with *MnlI* as described previously [7]. The products were separated on a 2% agarose gel and stained with ethidium bromide. The fragments measuring 37, 82 and 104 bp indicate the 1691G allele, and those of 82 and 141 bp the 1691A allele. Lanes 1, 3 and 4 show BD patients without mutation; lanes 2 and 5, BD patients heterozygous for the mutation; and the last lane the 1 kb marker.

In the healthy controls, we identified 11 (six male, five female) individuals heterozygous for the mutation. This gives an allelic frequency of ~5.1% in the Turkish population. The healthy control group and BD patients without thrombosis had similar mutation frequencies.

DISCUSSION

In patients with an inherited predisposition to thrombosis, a secondary insult such as surgery or pregnancy is needed to trigger a thrombotic event. The episodic nature of thrombosis, even in patients with more than one prothrombotic mutation, indicates the involvement of secondary factors. Endothelial microenvironment changes in BD may act as a secondary thrombogenic stimulus which results in thrombosis in patients with the factor V gene mutation. However, similar to the idiopathic deep vein thrombosis, the factor V gene mutation can explain the pathogenesis of thrombosis in 37.5% of our T+ patients. Therefore, the involvement of other known or as yet unidentified procoagulant genetic defects might be expected in the remaining T+ patients. In fact, protein C and protein S deficiency have previously been documented in cases of BD with extensive venous thrombosis [8, 9].

Male dominance in the T+ patients is consistent with previous reports [1, 2]. Since male sex and younger age of onset are associated with more severe disease in BD [10, 11], a higher prevalence of venous thrombosis could be explained by the severity of inflammation that might affect the extent of the endothelial dysfunction and cause a stronger thrombogenic insult. The absence of thrombosis in three patients with the mutation in the T− group might be due to their very mild disease course.

No factor V gene mutation could be shown in 12 BD patients with venous thrombosis in a recently published letter. Although ethnic diversity might have contributed to this contradictory finding, the small number in the study group prevents any conclusions being reached [12].

CONCLUSION

In conclusion, molecular defects of the coagulation system may play a major role in the development of venous thrombosis in BD. The factor V gene mutation appears to account for almost 38% of these genetic defects and is associated with a 6-fold increase in the risk of venous thrombosis in BD. Demonstration of this mutation at the time of diagnosis can identify those patients with BD who have a high risk of deep vein thrombosis.

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REFERENCES

1. Koç Y, Güllü I, Akpek G *et al.* Vascular involvement in Behçet's disease. *J Rheumatol* 1992;**19**:402–10.
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. Hampton KK, Chamberlain MA, Menon DK *et al.* Coagulation and fibrinolytic activity in Behçet's disease. *Thromb Haemostasis* 1991;**66**:292–4.
4. Kansu E, Sivri B, Sahin G *et al.* Endothelial cell dysfunction in Behçet's disease. In: O'Duffy JD, Kökmen E, eds. *Behçet's disease—basic and clinical aspects*. New York: Marcel Dekker, 1991:523–30.
5. Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med* 1994;**330**:517–22.
6. Bertina RM, Koeleman BPC, Koster T *et al.* Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994;**369**:64–7.
7. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N Engl J Med* 1995;**332**:912–7.
8. Chafa O, Fischer AM, Marianne F *et al.* Behçet syndrome associated with protein S deficiency. *Thromb Haemostasis* 1992;**67**:1–3.
9. Shehto NM, Ghosh K, Abdul Kader B, al Assad HS. Extensive venous thrombosis in a case of BD associated with heterozygous protein C deficiency. *Thromb Haemostasis* 1992;**67**:283.
10. Yazici H, Yurdakul S, Tüzün Y *et al.* Influence of age of onset and patient's sex on the prevalence and severity of manifestations of Behçet's disease. *Ann Rheum Dis* 1984;**43**:783–9.
11. Dilsen N, Koniçe M, Aral O, Öcal L, Inanç M, Gül A. Risk factors for vital organ involvement in Behçet's disease. In: Wechsler B, Godeau P, eds. *Behçet's disease*. Amsterdam: Excerpta Medica, 1993:165–9.
12. Lesprit P, Wechsler B, Piette J-C *et al.* Activated protein C resistance caused by factor V Arg 506→Gln mutation has no role in thrombotic manifestations of BD. *Ann Rheum Dis* 1995;**54**:860.