Thrombophilic risk factors and homocysteine levels in Behçet’s disease in eastern Spain and their association with thrombotic events

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Summary

Behçet’s disease (BD) is a chronic inflammatory disorder in which thrombosis occurs in about 30% of patients. The pro-thrombotic mechanisms are unknown. Thrombophilic defects and hyperhomocysteinaemia may be involved in the pathogenesis of thrombotic events, although results have been controversial. Moreover, no information is available on this issue for eastern Spain. We studied the prevalence of inherited and acquired thrombophilic risk factors in 79 patients with BD (43 men, 36 women) who had (n = 23) or did not have (n = 56) thrombosis, and in 84 healthy control subjects (42 men, 42 women). Risk factors examined were antithrombin, protein C, and protein S levels, factor V Leiden, the prothrombin G20210A mutation, the methylenetetrahydrofolate reductase C677T polymorphism, and acquired thrombophilic risk factors, including anticardiolipin antibodies, lupus anticoagulant, and serum homocysteine levels.

There were no differences between patients and controls in any of the parameters studied. When we studied BD patients with and without thrombotic events, the only thrombophilic defect that differed was the prothrombin G20210A mutation: Three out of 23 patients with thrombosis were carriers, compared with none of 56 patients without thrombosis (p = 0.022). Two of the three carriers developed catastrophic or recurrent thrombotic episodes; one was a homozygous carrier of the G20210A prothrombin mutation and the other was doubly heterozygous for the G20210A prothrombin mutation and factor V Leiden. A meta-analysis demonstrated an association of factor V Leiden and prothrombin mutation with thrombosis in BD. When studies from Turkey were excluded from the meta-analysis, only the prothrombin G20210A mutation was associated with thrombosis.

Keywords

Behçet’s disease, hyperhomocysteinaemia, thrombophilic risk factors, thrombosis, meta-analysis

Vasculitis underlies the thrombotic tendency in BD, but it is unclear why some patients present with thrombosis and others do not. Impaired coagulation (4–9), defective fibrinolysis and endothelial injury or dysfunction (10), as well as rheological changes (11) have all been proposed as contributors.

Regarding hypercoagulability, the role played by deficiencies in natural anticoagulants such as antithrombin (AT), protein C, and protein S, as well as anticardiolipin antibodies (ACAs)
and lupus anticoagulant (LA), have been suggested to contribute to thrombosis in BD, although results have been conflicting (12, 13) and little information is available about this issue in Spain (14). Data are also conflicting regarding the role played by the most prevalent thrombophilic defects in the development of thrombotic events, such as factor V (FV) Leiden and the prothrombin G20210A (PTG20210A) mutation. Some authors have found that FV Leiden increases thrombotic risk in patients with BD (9, 15–17), as does the PTG20210A mutation (16), whereas other authors found no association between thrombotic events and FV Leiden (3, 4, 14, 18–21) or the PTG20210A mutation (3, 4, 9, 14, 18–20).

The possible association between homozygosity for the C677T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene and thrombotic events has been scarcely investigated in BD (3, 14, 18, 22). Moreover, whether hyperhomocysteinaemia in BD is largely due to the presence of this mutation (23), with the aforementioned thrombotic manifestations, is debatable (3, 8, 22, 24–27). To our knowledge, there have been only four studies of patients with BD that examined whether the C677T polymorphism of the MTHFR gene is a thrombotic risk factor (3, 14, 22, 18). However, only two of these (3, 22) simultaneously determined plasma homocysteine levels. They found no association between these factors, possibly because the elevation of plasma homocysteine levels is also caused by nutritional deficiencies in folate, vitamin B_{12}, or vitamin B_6, which are involved in homocystein metabolism (28).

The aim of this study was i) to determine the prevalence of inherited thrombophilic risk factors, including AT, protein C, protein S, FV Leiden, the PTG20210A mutation, and the homozygous MTHFR 677TT mutation, and acquired risk factors such as ACAs, LA, and serum homocysteine in patients with BD and in a control group, ii) to evaluate the possible association of these thrombophilic defects and serum homocysteine levels with thrombotic events in patients with BD, and iii) to conduct a meta-analysis of studies on the FV Leiden, the PTG20210A mutation and the homozygous 677TT mutation and venous thrombosis in BD.

**Methods**

**Patients and controls**

Records of patients with BD recruited from La Fe, General, and Peset University Hospitals in Valencia between 1990 and 2005 were reviewed. From the initial cohort of 115 patients, 12 patients had died, 10 did not meet the inclusion criteria, and 14 were lost to follow-up. Therefore, the patient group comprised 79 patients with BD (43 men and 36 women, aged 45 ± 12 years). All patients fulfilled three or more of the International Study Group criteria for the diagnosis of BD (29). Patients were interviewed, and a detailed history was taken. The mean duration of the disease was 9.4 ± 6.3 years (range 1–24 years). Age, sex, body mass index (BMI), disease duration, symptoms, drugs, and past thrombotic events were recorded. Based on their histories and medical records, patients were categorised into those with a history of venous or arterial thrombosis and those with no such history. No subjects were taking drugs that could interfere with the metabolism of vitamin B_{12}, or folic acid, or vitamin supplements, when sampled. Sixteen patients were taking corticosteroids, six immunosuppressives, 14 corticosteroids and immunosuppressives, 12 colchicine, and 30 patients were taking no drugs. None of the patients had malignancies, or renal or hepatic dysfunction.

Of these 79 patients, 22 had suffered deep vein thrombosis and/or phlebitis, and two had also suffered angina pectoris and ischaemic stroke. One patient had suffered an ischaemic stroke but no thrombosis of the venous system. All thrombotic events had been assessed clinically and were confirmed using objective methods (Doppler ultrasonography, venography, computed tomography, or magnetic resonance imaging). Sampling took place at least six months after any thrombotic event to avoid the reactant phase. The mean time elapsed since the thrombotic events was 6.4 ± 4.1 years (range 1–11 years). Three patients were taking oral anticoagulants for recurrent deep vein thrombosis. They had been previously referred to our unit for a thrombophilia work-up. Therefore, only biochemical tests were performed in these patients. When sampled, the patients were not in an active phase of the disease, or displayed only minimum activity (mild aphthosis and/or arthralgia).

The control group comprised 84 healthy subjects (42 men and 42 women, aged 43 ± 11 years) from the staff of La Fe University Hospital or outpatients from the Dermatology Clinic at La Fe University Hospital with minimal dermatological problems, such as seborrhoeic keratosis, warts, or pityriasis versicolor. As we knew in advance sex and age of BD patients, consecutive control individuals from the above mentioned sources were recruited to match with BD patients. In these subjects, the absence of previous thrombotic events was confirmed with Frezza’s (30) validated questionnaire.

Controls and patients were from the same geographical area (eastern Spain), were all Caucasians, and sampling and analytical tests were performed at the same time. Informed consent was obtained from all the participants, and the Ethics Committee of our hospital approved the study. Given the influence of cardiovascular risk factors on serum homocysteine levels, these factors were recorded for both groups. Subjects were considered to have a cardiovascular risk factor if they were obese (BMI > 30 kg/m²), were smokers (> 1 cigarette/day), or if they had hypertension (diastolic blood pressure > 90 mmHg), hyperlipidaemia (total cholesterol > 220 mg/dl or triglycerides > 175 mg/dl), fasting glucose concentration > 126 mg/dl, or in receipt of pharmacological treatment for hypertension, hyperlipidaemia, or diabetes.

**Blood collection**

Blood was collected from the antecubital vein with minimum stasis between 08:00 and 10:00 after 12 h fasting, into vacuum tubes with 0.129 trisodium citrate as anticoagulant. Samples were centrifuged at 1500 x g for 15 min to obtain platelet-poor plasma, which was stored in aliquots at −70°C until tested. Dry tubes were used to collect samples for biochemical analysis, and K3EDTA tubes were used for samples for DNA studies.

**Laboratory methods**

AT antigen was measured by radial immunodiffusion using a monospecific antiserum to human AT (Behringwerke AG, Marburg, Germany). Anti-Xa activity was measured in the presence
of heparin using the Comatric AntiThrombin Kit (Chromogenix AB, Mölndal, Sweden). Protein C activity was determined with Comatec PC (Chromogenix AB). A one-step enzyme immunoassay (Diagnostica Stago, Asnières, France) was used to measure total protein S and free protein S. The IL Test™ (Instrumentation Laboratory, Milan, Italy) was used to assess functional protein S. The assay for LA was performed according to the criteria proposed by the Subcommittee for Lupus Anticoagulants of the International Society on Thrombosis and Haemostasis (ISTH, 1995). The ACA titre and IgG and IgM isotypes were analysed using enzyme-linked immunosorbent assays (ELISAs; Cheshire Diagnostic Ltd, Chester, UK). Those subjects with ACA titres above 3SD from the upper normal limit were considered positive (IgG≥25GPL or IgM≥20MPL).

DNA was extracted from whole blood samples using the Genomic Purification System (Promega, Madison, WI, USA) following the manufacturer’s protocol. The FV Leiden mutation was detected using polymerase chain reaction (PCR) and restriction analysis of a fragment of FV Leiden DNA (31). The PTG20210A gene variant was detected as reported (32). Based upon the method described by Skibola, et al. (33), a 198-bp genomic DNA fragment of exon 4 of the MTHFR gene was amplified by PCR and genotyped using Hind III restriction enzyme digestion.

Serum homocysteine levels were determined by fluorosence polarization immunoassay (FPIA), and folic acid and vitamin B₁₂ levels by chemiluminescence (Abbott Laboratories, USA). Total cholesterol, triglycerides and glucose were determined by enzymatic techniques and creatinine by a colorimetric technique in a DAX 72 autoanalyser (Bayer Diagnostics, Tarrytown, NY, USA).

**Statistical analysis**

All continuous variables were evaluated for normality of distribution. Glucose and homocysteine distributions were markedly skewed and these data were logarithmically transformed before statistical analysis. Student’s t tests for independent groups were used to compare differences in age, BMI, glucose, lipids, and homocysteine concentrations between patients and controls and between BD patients with and without thrombosis. Two standard deviations (SD) above the mean serum homocysteine concentration in the healthy control group (15 µM) was taken as the cut-off value used to classify subjects as having hyperhomocystinaemia. The Fisher’s exact test was used to evaluate differences in the percentage of carriers with inherited (AT, protein C, protein S, FV Leiden, PTG20210A mutation, and homozygous MTHFR C677T polymorphism) or acquired (ACAs, LA, serum homocysteine >15 µM) thrombophilic risk factors between patients and controls or between BD patients with and without thrombosis. A chi²-test was conducted to evaluate differences in percentage in the other dichotomic variables. Multivariate stepwise logistic regression was used to estimate the odds ratio for the occurrence of thrombosis associated with potential risk factors in subjects with BD. The data are expressed as means ± one SD. A bilateral P value of less than 0.05 was considered statistically significant. All analyses were performed with the Statistical Package for Social Sciences (SPSS, version 10) for Windows.

**Data sources and statistics for meta-analysis**

In addition to our original work, we have carried out a meta-analysis (34) with the inclusion of forest plots for the most prevalent risk factors (FV Leiden, PTG20210A mutation, and the MTHFR C677T polymorphism). Eligible studies for the meta-analyses were identified by searching the electronic literature (Medline and PubMed) for reports analysing the association between these factors and BD published between 1990 and October 2005. We extracted individual risk estimates and standard errors from each study, and then we combined these estimates using a random effects model. For the pooled OR we used the DerSimonian and Laird’s random effect model (34). Study results, their relative size, precision, pattern of effects and degree of heterogeneity, were explored visually using forest plots, in which the confidence interval for each study is represented by a horizontal line and the point estimate (OR) is represented by a square. The size of the square corresponds to the relative size of the study in the meta-analysis. The confidence interval for totals is represented by a diamond shape (StatsDirect, version 2.2.0, Cambridge, UK). A statistical test of heterogeneity was also calculated, estimating a Q statistic, which follows a Chi²-distribution with degrees of freedom of n-1, n being the number of studies included in the corresponding analysis. A two-tailed P value < 0.05 for this statistic parameter indicates the presence of heterogeneity, which somewhat compromises the validity of the pooled estimates. Then we analysed two sub-groups to prevent heterogeneity.

**Results**

Table 1 shows the general characteristics of the study participants. No differences in age, gender, or BMI were observed between patients and controls. The only biochemical parameter significantly higher in patients than in controls was serum triglyceride level (P = 0.003). No differences in glucose, total cholesterol, serum homocysteine, folic acid, or vitamin B₁₂ were observed. In controls, men had higher homocysteine levels than women: 11.32 ± 2.39 vs. 8.98 ± 2.79, respectively (P < 0.01). There was no difference by age (P = 0.933; data not shown).

The prevalence of hyperhomocystinaemia did not differ between patients and controls (P = 0.302).

No subjects showed a deficiency in AT, protein C, or protein S. Only one patient presented with LA. There were no differences between patients and controls in the prevalence of FV Leiden, the PTG20210A mutation, or homozygous carriers of the MTHFR C677T polymorphism (Table 1).

Patients had a higher percentage of cardiovascular risk factors such as hyperlipidaemia (P = 0.003), hypertension (P = 0.047) and diabetes (P = 0.015) than controls, whereas no differences were observed in the percentage of smokers (P = 0.828) or obesity (P = 0.156).

We also analysed the differences in the above factors in patients with BD with and without thrombosis. Table 2 shows the results of these comparisons. Among BD patients, no differences in age, or BMI were observed between those with and without thrombosis, but the risk of thrombosis tended to be higher in men than in women. Similarly, the biochemical parameters analysed did not differ between these groups. In both groups, men had
higher homocysteine levels than women (13.41 ± 7.62 vs. 9.30 ± 2.68, respectively; P = 0.003), but there were no differences by age (P = 0.182; data not shown). No difference in hyperhomocysteinemia was observed between BD patients with and without thrombosis (P = 1.000).

No differences in AT, protein C, protein S, LA, or ACAs were observed between patients with and without thrombosis, nor in the prevalence of FV Leiden (Table 2). Three of the 23 BD patients with thrombosis were carriers of the PTG20210A mutation, whereas none of the 56 patients without thrombosis were carriers. In one of the carriers, the mutation was homozygous and in the other it was associated with FV Leiden (double heterozygosity). There was no difference in the prevalence of the homozygous MTHFR C677T polymorphism in patients with and without thrombosis.

There was no association between the homozygous MTHFR C677T polymorphism and hyperhomocysteinemia in either the controls or patients. Of the 14 control subjects with the MTHFR 677TT genotype, only two had hyperhomocysteinemia (P = 0.213); and of the 11 BD patients carrying the MTHFR 677TT genotype, only two had hyperhomocysteinemia (P = 0.608).

When BD patients with and without thrombosis were compared, no differences were observed in the percentage of hyperlipidaemia (P = 0.538), hypertension (P = 0.350), obesity (P = 0.719), or diabetes (P = 0.426). The percentage of smokers was higher in BD patients without thrombosis (P = 0.016).

The Pearson bivariate test showed a positive correlation between homocysteine and both total cholesterol (r = 0.196) and triglycerides (r = 0.250) (P < 0.05), and with creatinine (r = 0.424; P < 0.01), and a negative correlation between homocysteine and folic acid (r = −0.377) and vitamin B12 (r = −0.281) (P < 0.01).

As several studies have already addressed the issue of the association between thrombophilic risk factors and thrombosis in BD, we performed a meta-analysis as indicated in Methods, to assess the influence of FV Leiden, PTG20210A mutation and MTHFR 677TT mutation on thrombotic risk in BD patients. We found statistically significant heterogeneity between studies from Turkey, as compared with other studies, when we estimated the risk associated with FV Leiden (P < 0.05 for Q statistics). Thus, we analysed two sub-groups (Turkish origin and non-Turkish). Although no statistically significant heterogeneity (P > 0.05) was found for PTG20210A and MTHFR 677TT variations, we also present the forest plot stratified for the Turkish origin to compare results. Figure 1 shows the results obtained. Overall, in the pooled analysis, FV Leiden was associated with a 230% (OR 2.3; 95% CI 1.5–3.5) higher risk of thrombosis in BD patients. However, no homogeneity was observed between studies. Thus, in Turkish studies, FV Leiden was significantly associated with a 320% (OR 3.2; 95% CI 1.8–5.8) increased thrombotic risk, whereas in studies carried out elsewhere the mutation did not influence the risk (OR 1.2; 95% CI 0.6–2.3) (Fig. 1A). With respect to the PTG20210A mutation, it was almost significantly associated with a higher thrombotic risk in Turkish studies (OR 2.5; 95% CI 1.0–6.5) whereas it significantly increased the risk of thrombosis in studies performed elsewhere (OR 2.8; 95% CI 1.1–7.2). As no statistically significant heterogeneity was found, in the pooled analysis, the PTG20210A mutation significantly

### Table 1: Age, gender, BMI, biochemical parameters, and thrombophilic defects in patients with BD and in the control group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BD patients (n = 79)</th>
<th>Control group (n = 84)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45 ± 12</td>
<td>43 ± 11</td>
<td>0.330</td>
<td>-</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>43/36</td>
<td>42/42</td>
<td>0.517</td>
<td>-</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 ± 4.4</td>
<td>25 ± 3.4</td>
<td>0.364</td>
<td>-</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>93 ± 18</td>
<td>98 ± 14</td>
<td>0.109</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>213 ± 38</td>
<td>209 ± 33</td>
<td>0.536</td>
<td>-</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>132 ± 67</td>
<td>102 ± 58</td>
<td>0.003</td>
<td>-</td>
</tr>
<tr>
<td>Homocysteine (µM)</td>
<td>11.5 ± 6.2</td>
<td>10.2 ± 2.4</td>
<td>0.112</td>
<td>-</td>
</tr>
<tr>
<td>Folic acid (µg/ml)</td>
<td>7.2 ± 3.6</td>
<td>6.9 ± 2.9</td>
<td>0.504</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B12 (µg/ml)</td>
<td>459 ± 159</td>
<td>458 ± 173</td>
<td>0.962</td>
<td>-</td>
</tr>
<tr>
<td>Creatinine (µg/dl)</td>
<td>0.96 ± 0.18</td>
<td>0.93 ± 0.15</td>
<td>0.307</td>
<td>-</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>132 ± 67</td>
<td>102 ± 58</td>
<td>0.003</td>
<td>-</td>
</tr>
<tr>
<td>Homocysteine &gt;15 µM (%)</td>
<td>8/77 (10%)</td>
<td>5/84 (6%)</td>
<td>0.302</td>
<td>-</td>
</tr>
</tbody>
</table>

*One patient was double heterozygous for FVL and PTG20210A and one patient was homozygous for FVL.

### Table 2: Age, gender, BMI, biochemical parameters, and thrombophilic defects in BD patients with and without thrombosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BD with thrombosis (n = 23)</th>
<th>BD without thrombosis (n = 56)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48 ± 13</td>
<td>44 ± 12</td>
<td>0.142</td>
<td>-</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>18/5</td>
<td>28/28</td>
<td>0.197</td>
<td>-</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 ± 4.2</td>
<td>26 ± 4.6</td>
<td>0.787</td>
<td>-</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>94 ± 16</td>
<td>96 ± 19</td>
<td>0.498</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>213 ± 49</td>
<td>241 ± 34</td>
<td>0.946</td>
<td>-</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>129 ± 61</td>
<td>134 ± 34</td>
<td>0.946</td>
<td>-</td>
</tr>
<tr>
<td>Homocysteine (µM)</td>
<td>12.7 ± 8.5</td>
<td>11.1 ± 5.0</td>
<td>0.302</td>
<td>-</td>
</tr>
<tr>
<td>Creatinine (µg/dl)</td>
<td>0.98 ± 0.19</td>
<td>0.96 ± 0.18</td>
<td>0.582</td>
<td>-</td>
</tr>
<tr>
<td>Folic acid (µg/ml)</td>
<td>7.8 ± 4.2</td>
<td>7.1 ± 3.3</td>
<td>0.470</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B12 (µg/ml)</td>
<td>488 ± 143</td>
<td>448 ± 167</td>
<td>0.351</td>
<td>-</td>
</tr>
<tr>
<td>Homocysteine &gt;15 µM (%)</td>
<td>2/23 (9%)</td>
<td>6/54 (11%)</td>
<td>1.000</td>
<td>-</td>
</tr>
<tr>
<td>AT, proteins C or S deficiency (%)</td>
<td>0/23 (0%)</td>
<td>0/56 (0%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*One patient was double heterozygous for FVL and PTG20210A and one patient was homozygous for FVL. The MTHFR C677T polymorphism was not determined in two patients.
increased the risk of thrombosis in BD patients (OR 2.7; 95% CI 1.4–5.2) (Fig. 1B). The presence of the MTHFR 677TT genotype was not associated with an increased risk of thrombosis in overall or in any of the subgroups studied (Fig. 1C).

Discussion

Like most earlier studies (12, 35, 36), we found normal values for protein C, protein S, and AT activity in patients with BD. Only a few cases of BD with venous thrombosis and protein C or protein S deficiency have been reported (6). None of these studies, including ours, could demonstrate an association between a deficiency in these physiological anticoagulant inhibitors and thrombotic events in patients with BD (12, 14, 35, 36). Therefore, congenital deficiencies in these thrombophilic factors seem to have a minor, if any, role in the pathobiology of thrombosis in BD patients.

Most (13, 35) but not all studies (37) have reported no correlation between ACAs and LA antibodies and thrombosis in BD patients. In the present study, the prevalence of ACAs and LA in BD patients did not differ from that in controls. Therefore, these acquired thrombophilic effects seem to play no role in the development of thrombotic events in BD.

Although not significant, we observed that men with BD tended to show a higher thrombotic risk than women. This is in agreement with previous reports (4, 18, 38).

One of the most controversial issues regarding thrombotic tendencies in BD is the role played by the most prevalent procoagulant mutations, such as FV Leiden, the PTG20210A mutation, and the homozygous MTHFR 677TT mutation. Previous reports of an association between FV Leiden and thrombotic events in BD have shown contradictory results, possibly related to the different populations examined, with studies favouring (7, 9, 15–17, 24, 39) or opposing (3, 4, 8, 14, 18–21, 35). These discrepancies may have arisen because patients included in some studies (9, 15–17, 24, 39) were of Turkish origin, where the prevalence of these mutations is high (4, 9, 15–17). Furthermore, in some studies that supported an association, the number of patients with thrombosis was small (7, 17, 24, 39). Some studies have determined activated protein C (APC) resistance, but not the genetic mutation, so some cases of APC resistance could be acquired (24, 39). In our study, the prevalence of FV Leiden in BD was not higher than that in controls, nor did it differ between patients with and without thrombosis, consistent with most research in our geographical area (14, 19, 20), as well as in Israel (3). The meta-analysis performed in the present study showed that the association between the presence of FV Leiden and thrombosis in BD may be attributed to the Turkish studies, since studies elsewhere did not show significant association.

The PTG20210A mutation has also been investigated in BD patients, with conflicting results regarding thrombotic events (3, 4, 9, 14, 16, 18, 20). In the present study, 3 (13%) of BD patients with thrombosis were carriers of the PTG210A mutation, whereas no carrier was found among the BD patients without thrombosis (OR 19.3, 0.9–390.1). The PTG20210A mutation is the most frequent genetic prothrombotic defect in Spain (40), with a prevalence of 4–5% in eastern Spain (41), which may partly explain this association. The meta-analysis showed an almost sig-

![Figure 1: Forest plot for meta-analysis of effect of factor V Leiden (A), prothrombin G20210A (B) and methylenetetrahydrofolate reductase 677TT (C) mutations on the thrombotic risk in patients with Behçet's disease.](https://www.thrombosis-online.com)
nificant association of this mutation with thrombotic risk in Turkish studies and a significant association in the pooled analysis.

In the present study, the prevalence of the homozygous MTHFR 677TT genotype was not significantly higher in BD patients (14%) than in controls (17%), or higher in BD patients with thrombosis (18%) than in those without thrombosis (13%). The prevalence in controls (17%) is consistent with the finding of a previous study in our geographical area (15.5%) (42). Only four other studies have determined this polymorphism in BD patients (3, 14, 18, 22), and they also found no association between this mutation and thrombotic events in BD patients. Overall, the meta-analysis showed that this polymorphism does not seem to increase the thrombotic risk in BD patients. However, whether hyperhomocysteinaemia is a prothrombotic factor in BD remains a highly controversial issue. Levels of homocysteine depend partly on the MTHFR 677TT genotype (23), and on the effects of environmental factors, primarily folic acid and vitamin B12 levels, deficits in which increase plasma homocysteine levels (43).

Like other authors (3, 26, 27), we found no difference in the levels of homocysteine in patients with and without thrombosis. However, most studies carried out in Turkey reported higher levels of homocysteine in patients with thrombosis than in those without (22, 24, 25), as Lee, et al. also reported in Koreans (8). Furthermore, a large proportion of the patients in these studies were in the active phase of the disease (22, 25, 27), which can also increase homocysteine levels (8). Interestingly, Lee, et al. (8) found higher homocysteine levels in BD patients with active disease than in those who had suffered thrombotic events. Our patients were not in the active phase of the disease or displayed minimum activity when sampled, and this may account in part for the discrepancies.

On the other hand, not all the studies that reported higher homocysteine levels in BD patients with thrombosis had made the appropriate adjustments for all those factors that can influence homocysteine levels, such as diabetes (44), hyperlipidaemia (45), tobacco (28), folic acid, and vitamin B12 (44). Given that the Mediterranean diet is rich in folic acid and vitamin B12, we found no differences in the levels of these nutrients in patients and controls or in BD patients with and without thrombosis. Another important factor is the time elapsed since the last thrombotic event, which varies within several studies and may also account for discrepancies (26). Lastly, in those studies in which an association was found between hyperhomocysteinaemia and thrombosis, the number of patients with thrombosis was too small to draw the conclusion that hyperhomocysteinaemia constitutes an independent thrombotic risk factor (8, 22, 24).

In conclusion, the results obtained in the present study, regarding thrombotic risk associated with the most frequent thrombophilic defects in BD, agree with those obtained in the meta-analysis when the Turkish studies were excluded, therefore establishing the PTG20210A mutation as a thrombotic risk factor for BD patients.

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