Dear Sirs,

Deep venous thrombosis (DVT) usually starts in the calf veins, from where it may extend to the proximal veins, and subsequently embolise to the pulmonary arteries (1).

Factor V Leiden (FVL) and the prothrombin G20210A gene (PTM) mutations are the two most prevalent hereditary thrombophilic abnormalities (2). Previous studies have shown that both mutations are associated with an increased risk of venous thromboembolic events (VTE) (3, 4). Recently, a meta-analysis suggested that patients with FVL are significantly more likely to present with DVT than with PE, while the prevalence of PTM was similar in these two groups (5). It was hypothesised that FVL carriers have more frequently a distal location of DVT with a lower risk of embolisation than non-carriers (Leiden paradox) (6). Although subsequent studies produced conflicting results (7, 8), the possibility that the presence of FVL also influences the location of DVT, in particular predisposing to either proximal or distal locations, remains open. We therefore performed a systematic review and a meta-analysis of the literature to address this knowledge gap. Furthermore, we also included studies evaluating the prevalence of PTM in patients with different DVT location to assess if differences in DVT location also existed for carriers of this mutation.

Protocol detailing the specific objectives, criteria for study selection, approach to assess study quality, outcomes, and statistical methods was described in the Suppl. Material (available online at www.thrombosis-online.com).

Briefly, Medline and Embase databases were searched up to June 2012. Research was supplemented by manually reviewing abstract books from the Congress of the ISTH (2003–2012) and the reference lists of all retrieved articles. Only studies with objectively diagnosed DVT providing separate data for patients with distal DVT and proximal DVT and assessing FVL and PTM in an objectively and commonly accepted manner were included. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with a random-effects model. Statistical heterogeneity was evaluated through the use of I² statistics. The presence of publication bias was explored by using funnel plots of effect size against standard error.

The results of study identification and selection are described in the Suppl. Material (available online at www.thrombosis-online.com). At the end of this process, 12 studies for a total of 5,733 patients were included in our systematic review (7–18).

All included studies provided data on the prevalence of FVL mutation in patients with proximal and distal DVT; eight studies (9–11, 13–16, 18) provided data on the prevalence of PTM mutation in these two groups. Baseline characteristics of included studies are given in Table 1.

Quality assessment items are summarised in the Suppl. Material (available online at www.thrombosis-online.com). Eight of the 12 studies received at least seven stars according to Newcastle-Ottawa Scale and were considered of high quality (7, 8, 10–13, 15, 18).

FVL mutation was present in 877 of the 4,318 (20.3%) patients with proximal DVT and in 218 of the 1,415 (15.4%) patients with isolated distal DVT. Thus, the presence of FVL mutation was found significantly more often in patients presenting with proximal DVT than in patients presenting with distal DVT (OR 1.33 95% CI 1.07, 1.65; p = 0.01), with a low heterogeneity among the studies (I² = 16%) (Fig. 1A). Funnel plot appeared symmetric suggesting the absence of publication bias (see Suppl. Figure 1, available online at www.thrombosis-online.com). Considering only high quality studies, we obtained a pooled OR of 1.41 (95% CI 1.08, 1.84; p = 0.01).

PTM was present in 234 of the 2,158 (10.8%) patients with proximal DVT and in 46 of the 883 (5.2%) patients with distal DVT. In these studies, the prevalence of PTM was marginally significantly higher in patients presenting with proximal DVT than in patients presenting with distal DVT (OR 1.43, 95% CI 1.00, 2.04; p = 0.05) (Fig. 1B). There was no heterogeneity among the studies (I² = 0%). Funnel plot appeared symmetric suggesting the absence of publication bias (see Suppl. Figure 1, available online at www.thrombosis-online.com). Similar results were obtained when low-quality studies were excluded from the analysis (OR 1.49, 95% CI 0.99, 2.23; p = 0.05).

In this large meta-analysis we aimed to evaluate if the presence of FVL was associated with a different thrombus location in patients with DVT. The results of our study indicate that FVL mutation is significantly more common in patients with proximal DVT than in patients with distal DVT, and the strength of our observation is further increased by the results of the sensitivity analysis including high-quality studies only. In our study, also G20210A PTM was
<table>
<thead>
<tr>
<th>Study, year (ref.)</th>
<th>Design of the study</th>
<th>Thrombophilic abnormalities</th>
<th>Patients description</th>
<th>Exclusion criteria</th>
<th>Total number</th>
<th>Mean age, years (SD),</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsov, 2006 (7)</td>
<td>Case-control</td>
<td>FVL</td>
<td>Hospitalised patients with VTE</td>
<td>NR</td>
<td>145</td>
<td>FVL+ 33.7 (11.5) FVL- 41.1 (15.7)</td>
</tr>
<tr>
<td>Caprini, 2005 (9)</td>
<td>Case-control</td>
<td>FVL, PTM</td>
<td>Patients evaluated for SVT, DVT, venous disease, personal or family history of DVT who required elective surgery and needed prophylaxis</td>
<td>NR</td>
<td>44</td>
<td>NR</td>
</tr>
<tr>
<td>Gadelha, 2010 (10)</td>
<td>Case-control</td>
<td>FVL, PTM</td>
<td>Consecutive patients with first symptomatic VTE</td>
<td>Partecipation in a therapeutic clinical trial with blind mediation</td>
<td>1541</td>
<td>NR</td>
</tr>
<tr>
<td>Gonzales-Porras, 2006 (11)</td>
<td>Case-control</td>
<td>FVL, PTM</td>
<td>Consecutive patients with first episode of DVT of the limbs</td>
<td>Cancer, abnormalities in the coagulation or fibrinolytic system, APL syndrome, patients with extended anticoagulation therapy</td>
<td>181</td>
<td>FVL+/PTM+: 45 (22–69) ** FVL+/PTM-: 45 (13–85) ** FVL-/PTM+: 38 (14–69) ** FVL-/PTM-: 49 (3–84) **</td>
</tr>
<tr>
<td>Huisman, 2008 (12)</td>
<td>Case-control</td>
<td>FVL</td>
<td>Consecutive patients with DVT</td>
<td>NR</td>
<td>481</td>
<td>63 (29–83)*</td>
</tr>
<tr>
<td>Kovac, 2010 (13)</td>
<td>Case-control</td>
<td>FVL, PTM</td>
<td>Patients with symptomatic VTE referred to thrombosis center for thrombofilia screening</td>
<td>Patients with no medical records, or with other thrombophilic abnormalities (AT, PC, PS deficiency, antiphospholipid antibodies or combined abnormalities), or with diabetes or with cancer</td>
<td>192</td>
<td>NR</td>
</tr>
<tr>
<td>Kupeli, 2011 (14)</td>
<td>Case-control</td>
<td>FVL, PTM</td>
<td>Consecutive patients with VTE</td>
<td>NR</td>
<td>29</td>
<td>NR</td>
</tr>
<tr>
<td>Leroyer, 1997 (8)</td>
<td>Case-control</td>
<td>FVL</td>
<td>Consecutive patients with DVT on admission</td>
<td>Patients who developed DVT during the course of hospitalisation</td>
<td>165</td>
<td>FVL+ 51.2 (20.2) FVL- 59.9 (18.9)</td>
</tr>
<tr>
<td>Martinelli, 2007 (15)</td>
<td>Case-control</td>
<td>FVL, PTM</td>
<td>Consecutive patients with a first episode of symptomatic VTE referred to thrombosis center for thrombofilia screening</td>
<td>Patients with other thrombophilic abnormalities (Homozgyous FVL or PTM, AT, PC, PS, antiphospholipid antibodies or combined abnormalities)</td>
<td>330</td>
<td>NR</td>
</tr>
<tr>
<td>Schulman, 2007 (16)</td>
<td>Case-control</td>
<td>FVL, PTM</td>
<td>Patients with a first episode of VTE (population from the DURAC1 trial)</td>
<td>Patients with deficiency of AT, PC, PS</td>
<td>425</td>
<td>NR</td>
</tr>
<tr>
<td>Van Stralen, 2008 (17)</td>
<td>Case-control</td>
<td>FVL</td>
<td>Consecutive patients with a first VTE</td>
<td>Age &lt; 18, end-stage diseases, inability to fill in the questionnaire, VTE non objectively documented, refusal of informed consent</td>
<td>1888</td>
<td>Isolated DVT: 48.2 DVT+PE: 50.3</td>
</tr>
<tr>
<td>Wåhlander, 2002 (18)</td>
<td>Case-control</td>
<td>FVL, PTM</td>
<td>Patients underwent total hip or knee replacement</td>
<td>History of clinical DVT, PE or intra-cranial bleeding, impaired liver and kidney function, cancer, ischaemic stroke during previous year</td>
<td>312</td>
<td>NR</td>
</tr>
</tbody>
</table>

* Mean and range; ** Median and range; APL, antiphospholipid; AT, antithrombin; DVT, deep venous thrombosis; FVL, factor V Leiden mutation; NR, not reported; PC, protein C; PE, pulmonary embolism; PS, protein S; PTM, prothrombin G20210A mutation; SD, standard deviation; VTE, venous thromboembolic events.
more commonly detected in patients with proximal DVT, although this association was of marginal statistical significance.

Results of previous studies seem to suggest a different presentation of VTE in patients with and without this thrombophilic abnormality, partially challenging the traditional concept of DVT and PE as a single disease entity (5-19). As a possible explanation of these results, it has been suggested that carriers of this polymorphism develop a thrombus which is more stable and more adherent to the vessel wall and, thus, less likely to embolise (20). This hypothesis is supported by the knowledge that activated protein C (PC) has profibrinolytic effects, but activated PC-resistance due to FVL mutation produces an increased thrombin formation, which leads to an increased rate of activation of thrombin activatable fibrinolysis inhibitor and to a down regulation of fibrinolysis (21, 22). According to the results of our meta-analysis, FVL presence seems to be associated with a higher risk of involvement of the proximal veins in patients presenting with DVT. In patients with FVL mutation, hyperfibrinolysis and the increased thrombin formation may contribute to a more pronounced local extension of the thrombus. In patients with G20210A PTM, the higher risk of involvement of the proximal veins may be due to the higher levels of circulating prothrombin in these patients (23). Furthermore, G20210A PTM seems to inhibit plasma fibrinolysis through a TAFI-mediated mechanism (24). Thus, the opportunity of a general screening for these abnormalities in patients with a distal DVT appeared questionable. However, these hypotheses should be confirmed in appropriately planned studies.

Our meta-analysis has some potential limitations. First, studies included in our meta-analysis have different inclusion and exclusion criteria: some of the studies a priori excluded from the analysis patients with other thrombophilic abnormalities and it was not possible to obtain separate data of patients with provoked and unprovoked DVT. Furthermore, the prevalence of concomitant risk factors for VTE may be different in patients presenting with distal or proximal DVT in some of the studies included in our systematic review. Thus, to combine results across studies may be inappropriate. However, the heterogeneity

Figure 1: Forrest Plots evaluating the association different VTE location and the prevalence of FVL mutation (A) and G20210A mutation of the prothrombin (B).
among the studies, calculated using the I² statistic, was low. Second, in a minority of the studies included in our meta-analysis the research of FVL and G20210A PTM mutation was not performed in all the patients, and a selection bias could not be definitively excluded. Thus, caution is necessary in the interpretation of the results. Last, we failed to identify any unpublished study. Because it is recognised that publication bias can affect the results of meta-analyses, we attempted to assess this potential bias using funnel plots, which appeared symmetric, suggesting the absence of publication bias.

In conclusion, the results of this large systematic review and meta-analysis of the literature suggest that patients with FVL and G2010A PTM are significantly more likely to present with proximal than distal DVT. Future research might focus on the possible mechanisms underlying these findings.

Conflicts of interest
None declared.

References