Factor V Leiden (G1691A) and Prothrombin Gene G20210A Mutations as Potential Risk Factors for Venous Thromboembolism after Total Hip or Total Knee Replacement Surgery

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Keywords

- Factor V Leiden mutation
- prothrombin gene mutation
- pulmonary embolism
- deep vein thrombosis
- anticoagulation

Summary

Patients (n = 1600) from 12 European countries, scheduled for elective orthopaedic hip or knee surgery, were screened for Factor V Leiden and prothrombin gene G20210A mutations, found in 5.5% and 2.9% of the populations, respectively. All patients underwent prophylactic treatment with one of four doses of melagatran or ximelagatran or dalteparin, starting pre-operatively. Bilateral ascending venography was performed on study day 8-11. The patients were subsequently treated according to local routines and followed for 4-6 weeks post-operatively. The composite endpoint of screened deep vein thrombosis (DVT) and symptomatic pulmonary embolism (PE) during prophylaxis did not differ significantly between patients with or without these mutations. Symptomatic venous thromboembolism (VTE) during prophylaxis and follow-up (1.9%) was significantly over-represented among patients with the prothrombin gene G20210A mutation (p = 0.0002). A tendency towards increased risk of VTE was found with the Factor V Leiden mutation (p = 0.09). PE were few, but significantly over-represented in both the Factor V Leiden and prothrombin gene G20210A mutated patients (p = 0.03 and p = 0.05, respectively). However, since 90% of the patients with these genetic risk factors will not suffer a VTE event, a general pre-operative genotyping is, in our opinion, of questionable value.

Introduction

Following total hip replacement (THR) or total knee replacement (TKR) surgery the incidence of venographically verified deep vein thrombosis (DVT) is within the range of 50-60% if no prophylactic anticoagulation is given (1, 2) and post-operative mortality due to PE could be as high as 1.65% without prophylactic treatment (3). This rate can be decreased by the administration of subcutaneous (s.c.) heparin or low-molecular-weight heparin (LMWH) (2-4). A decrease in the incidence of DVT, comparable to standard treatment, has been observed with recombinant hirudin and the novel, oral direct thrombin inhibitor ximelagatran (5-7).

The Factor V Leiden mutation (G1691A, Arg→Gln) is the most common genetic risk factor for venous thromboembolism (VTE) among Caucasians, with a prevalence of between 2 and 15% in the general population (8). This amino acid substitution slows down the inactivation of Factor Va by activated protein C (APC), thus resulting in impaired control of the coagulation cascade. This is known as APC resistance (APCR) (8-12). Over 80% of the APCR phenotype can be explained by the Factor V Leiden mutation (9, 12).

Recent studies have shown that a point mutation in the prothrombin gene, G20210A, is also associated with a high risk of VTE (13, 14). The overall prevalence of this mutation in Caucasians is ~2%, with higher frequencies being found in southern Europe compared with northern Europe (15). The mechanism of action of this mutation is not understood fully, although it is associated with a slight elevation in plasma prothrombin levels (13).

Recent studies have suggested that patients with APCR/Factor V Leiden mutation are more likely to suffer from VTE following THR or TKR surgery compared with patients without APCR (16, 17). However, other studies have failed to confirm this association (18, 19). It is still unknown whether the prothrombin gene G20210A mutation increases the risk for VTE after this type of surgery. To date only one small study has addressed this question (20). The objective of the present study was, therefore, to measure the prevalence of Factor V Leiden and prothrombin gene G20210A mutations in a European population scheduled for THR or TKR surgery and to investigate whether carriers of these mutations are more likely to suffer from post-operative VTE. These two mutations were selected since they are reasonably common, and screening for these mutations could be warranted.

Methods

Patients

The study population was obtained from a prospective, controlled, randomised, double-blind, dose-ranging study for patients scheduled for elective THR or TKR surgery, conducted at 59 centres in 13 European countries (6). A total of 1876 patients underwent THR and TKR, and blood samples for the mutation analyses were obtained from 1600 patients. One country did not participate in the genotype screening.
Patients (male and female) were between 18 and 85 years and weighed between 46 and 110 kg. Patients were excluded if they had a history of clinical DVT, pulmonary embolism (PE) or intra-cranial bleeding. Patients were also excluded if they had suffered from ischaemic stroke during the last year, or had impaired liver and kidney function or ongoing malignancy.

Ethical approval for the protocol was obtained from the local Ethics Committees in the participating countries, and the study was performed in accordance with the Declaration of Helsinki. All patients gave written informed consent.

Study Design

Patients were randomised to receive either dalteparin 5000 IU s.c. once daily or one of four s.c. doses of melagatran (1.0, 1.5, 2.25 and 3.0 mg b.i.d.) followed by oral ximelagatran (8, 12, 18 and 24 mg b.i.d.). Prophylaxis with dalteparin was initiated the night before surgery, and with melagatran immediately prior to surgery. Prophylaxis was maintained until bilateral venography was performed on day 8-11. Patients were followed for 4-6 weeks post-operatively. During the follow-up period they were treated according to local clinical practice.

Thromboembolic Evaluations

DVT was confirmed by mandatory bilateral ascending venography on day 8-11, as described by Kälebo et al. (21). Venograms were centrally assessed by two independent experts who determined the examinations as DVT, no DVT or non-evaluable. Thrombi were classified into proximal and distal (below the popliteal trifurcation). If a patient experienced more than one event, only the most severe was included.

Clinical symptoms of PE were verified by pulmonary perfusion and ventilation scintigraphy, using the Biello criteria for high probability of PE (22), pulmonary angiography or spiral computed tomography (CT). PE was defined as such in patients with symptoms of PE where the objective examinations could not exclude PE. Symptomatic DVT during the follow-up period was confirmed by either compression sonography or venography. The final decision of an accurate diagnosis of PE or DVT during follow-up was taken by an adjudication committee of independent experts, blinded to study treatment.

Factor V Leiden and Prothrombin Gene G20210A Mutation

DNA was extracted from whole blood and the genotypes for Factor V Leiden (G1691A), and prothrombin gene G20210A mutations were determined as described previously (10, 12, 13).

Statistics

Statistical analyses were performed on the intent-to-treat (ITT) population. All randomised patients who underwent THR or TKR are included in all analyses unless otherwise indicated. Descriptions of patient characteristics are given in numbers and percentage of patients, or as mean ± standard deviation (SD). The hypothesis of equal proportions of thromboembolic events among patients with either of the two mutations under investigation versus those without these mutations was tested using the Fisher’s exact test, adopting a two-sided 5% significance level. A logistic regression model was used to estimate the odds ratios, with 95% confidence intervals, for VTE frequencies among patients with, relative to those without, either the Factor V Leiden or the prothrombin gene G20210A mutations.

Results

Characteristics of the Study Population

TKR was performed in 35% (n = 567) and THR in 65% (n = 1033) of the patients. Sixty percent (n = 952) of the patients were female, and the mean (± SD) age was 66 ± 9 years. Patient demographic characteristics (age, gender, weight, height, type of surgery or renal function) were similar regardless of the presence of gene mutations. Nearly all patients were Caucasian (1597/1600).

Presence of Factor V Leiden and Prothrombin Gene G20210A Mutation

Of the 1600 patients (Table 1), either of the mutations were found in 133 patients (8.3%). The Factor V Leiden mutation was found in 88 patients (5.5%) and the prothrombin gene G20210A mutation was found in 46 patients (2.9%). One patient had a homozygous Factor V Leiden mutation, and one had a homozygous prothrombin gene G20210A mutation. One patient was heterozygous for both mutations. There was wide variation in the prevalence of these mutations between different countries (Table 1). The highest prevalence for the mutations was observed in Austria, with 10.4% for Factor V Leiden and 6.1% for the prothrombin gene G20210A mutation. The lowest prevalence was found in Finland, with 0.9% for Factor V Leiden and no patients with the prothrombin gene G20210A mutation.

Table 1 The frequencies of the Factor V Leiden and prothrombin gene G20210A mutations in the countries involved in the study

<table>
<thead>
<tr>
<th>Country</th>
<th>Any mutation</th>
<th>Factor V Leiden mutation</th>
<th>Prothrombin gene G20210A mutation</th>
<th>Total number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>19 (16.5%)</td>
<td>12 (10.4%)</td>
<td>7 (6.1%)</td>
<td>115</td>
</tr>
<tr>
<td>Belgium</td>
<td>4 (7.1%)</td>
<td>3 (5.4%)</td>
<td>1 (1.8%)</td>
<td>56</td>
</tr>
<tr>
<td>Denmark</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>England</td>
<td>5 (10.6%)</td>
<td>3 (6.4%)</td>
<td>2 (4.3%)</td>
<td>47</td>
</tr>
<tr>
<td>Finland</td>
<td>1 (0.9%)</td>
<td>1 (0.9%)</td>
<td>0</td>
<td>111</td>
</tr>
<tr>
<td>France</td>
<td>3 (3.6%)</td>
<td>1 (1.2%)</td>
<td>2 (2.4%)</td>
<td>83</td>
</tr>
<tr>
<td>Germany</td>
<td>38 (9.0%)†</td>
<td>22 (5.2%)</td>
<td>17 (4.0%)</td>
<td>421</td>
</tr>
<tr>
<td>Hungary</td>
<td>14 (9.2%)</td>
<td>9 (5.9%)</td>
<td>5 (3.3%)</td>
<td>152</td>
</tr>
<tr>
<td>Poland</td>
<td>6 (4.7%)</td>
<td>3 (2.4%)</td>
<td>3 (2.4%)†</td>
<td>127</td>
</tr>
<tr>
<td>Spain</td>
<td>1 (4.8%)</td>
<td>1 (4.8%)</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Sweden</td>
<td>40 (9.4%)†††</td>
<td>31 (7.3%)†††</td>
<td>9 (2.1%)</td>
<td>427</td>
</tr>
<tr>
<td>Switzerland</td>
<td>1 (10.0%)</td>
<td>1 (10.0%)</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>133 (8.3%)</td>
<td>88 (5.5%)</td>
<td>46 (2.9%)</td>
<td>1600</td>
</tr>
</tbody>
</table>

†One patient was heterozygous for Factor V Leiden and prothrombin gene G20210A mutations
††One patient was homozygous for prothrombin gene G20210A mutation
†††One patient was homozygous for Factor V Leiden mutation
The two mutations were equally distributed between the dalteparin (27/327, 8%) and the melagatran and ximelagatran groups [from low to high dose: 29/314 (9%), 26/319 (8%), 28/317 (9%), 23/323 (7%)] as well as between the patients with (83/952, 9%) and without (50/648, 8%) prolonged prophylaxis/treatment. The frequencies of the Factor V Leiden and prothrombin gene G20210A mutations were equally distributed between the treatment regimens, with 19 and 9 patients, respectively, in the dalteparin group (22% and 20%) and 69 and 37 patients, respectively, in the melagatran and ximelagatran groups (78% and 80%), which is in accordance with the randomisation ratio used in the study (1:4).

VTE during Obligatory Anticoagulation Prophylaxis – Relationship to Gene Mutations

Of the 1600 patients evaluable for gene mutations, 1251 had evaluable venograms, and four patients without evaluable venograms had PE. Thus, in total, 1255 patients were evaluable for VTE. Of these patients, 104/1255 (8.3%) had one or both of the mutations, 70 were heterozygous and one homozygous for the Factor V Leiden mutation (5.7%), and 33 were heterozygous and one homozygous for the prothrombin gene G20210A mutation (2.7%). One patient was heterozygous for both mutations.

There was no significant difference in the incidence of total VTE rate (dominated by asymptomatic venographic DVT) between patients with (31/104, 29.8%; Table 2) and without (292/1151, 25.4%) either of the gene mutations during prophylaxis (odds ratio 1.25, 95% CI 0.84–1.94, p = 0.35; Table 3). VTE occurred in 32.4% (23/71) of patients with, and 25.3% (300/1184) without the Factor V Leiden mutation (odds ratio 1.41, 95% CI 0.84–2.36, p = 0.21). The single patient with a homozygous Factor V Leiden mutation did not have a VTE. In total, 26.5% (9/34) of patients with, and 25.7% (314/1221) without the prothrombin gene G20210A mutation had VTE (odds ratio 1.04, 95% CI 0.48–2.25, p = 1.0). Table 3. The prothrombin gene G20210A mutation was shown to be a risk factor for symptomatic VTE during the follow-up period, when compared with patients without this mutation (odds ratio 10.48, 95% CI 3.31–33.17, p = 0.0014), but the Factor V Leiden mutation did not reach statistical significance (odds ratio 2.17, 95% CI 0.49–9.61, p = 0.26).

During follow-up, 952 of the 1600 patients (59.5%) received prophylactic anticoagulation (ranging from 3 days to 5 weeks) according to local clinical practice, usually with a LMWH. The patients who received treatment during this period are also included in these figures. In the group without anticoagulation (648/1600), VTE was observed in 7/598 (1.2%) of patients without the mutations and 4/50 (8.0%) of patients with the mutations. In the group with prolonged prophylaxis/treatment (952/1600) there were 5/869 (0.6%) VTE in patients without the mutations and 2/83 (2.4%) in patients with the mutations. However, even excluding the patients with prolonged prophylaxis/treatment from the statistical analyses, the prothrombin gene G20210A mutation was still a significant risk factor for VTE during follow-up, while the presence of Factor V Leiden mutation did not reach statistical significance (p = 0.001 and p = 0.47, respectively).

Symptomatic VTE during Prophylaxis and Follow-up – Relationship to Gene Mutations

During the combined study treatment and follow-up period, there were 32 symptomatic VTEs (19 DVT and 13 PE). One patient had a symptomatic VTE both during treatment and at follow-up. Thus, in total there were 31 (1.9%) patients with VTE. Among these 10 patients having any of the mutations, six carried the prothrombin gene G20210A mutation, and four carried the Factor V Leiden gene mutation. Carrying either of these mutations represented a significant risk factor for a post-operative symptomatic VTE (odds ratio 5.60, 95% CI 2.58–12.15, p = 0.0001), as seen in Table 3. The prothrombin gene G20210A mutation was a statistically significant risk factor for post-operative symptomatic VTE (odds ratio 9.17, 95% CI 3.57–23.60, p = 0.0002). The Factor V Leiden mutation showed a trend towards increased risk of post-operative symptomatic VTE (odds ratio 2.62, 95% CI 0.89–7.66, p = 0.09).

An analysis of symptomatic PE showed that carrying either of the two mutations was a significant risk factor (odds ratio 7.12, 95% CI 2.30–22.09, p = 0.003). This was significant also for the prothrombin gene G20210A mutation (odds ratio 6.38, 95% CI 1.37–29.63, p = 0.05) and the Factor V Leiden mutation (odds ratio 5.30, 95% CI 1.43–19.62, p = 0.03).

### Table 2. Objectively verified DVT, using mandatory bilateral venography (study day 8-11), and objectively verified symptomatic PE. Almost all DVTs were asymptomatic. In total, 1255 patients were evaluable for VTE

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Any mutation n = 104</th>
<th>Factor V Leiden mutation n = 71</th>
<th>Prothrombin gene G20210A mutation n = 34</th>
<th>No mutation n = 1151</th>
<th>All patients n = 1255</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal DVT</td>
<td>20.2% (23)</td>
<td>23.9% (17)</td>
<td>14.7% (5)††</td>
<td>19.9% (229)</td>
<td>19.9% (250)</td>
</tr>
<tr>
<td>Proximal DVT</td>
<td>5.8% (6)</td>
<td>5.6% (4)</td>
<td>5.9% (2)</td>
<td>4.9% (56)</td>
<td>4.9% (62)</td>
</tr>
<tr>
<td>PE</td>
<td>3.8% (4)</td>
<td>2.8% (2)</td>
<td>5.9% (2)</td>
<td>0.6% (7)</td>
<td>0.9% (11)</td>
</tr>
<tr>
<td>Total VTE</td>
<td>29.8% (31)</td>
<td>32.4% (23)</td>
<td>26.5% (9)</td>
<td>25.4% (292)</td>
<td>25.7% (323)</td>
</tr>
</tbody>
</table>

† One patient was heterozygous for Factor V Leiden and prothrombin gene G20210A mutations
†† One patient was homozygous for prothrombin gene G20210A mutation

**Symptomatic VTE during Follow-up Period – Relationship to Gene Mutations**

During the follow-up period after the cessation of study drug treatment, 18 of 1600 patients (1.1%) had VTE (16 DVT and 2 PE). Six (33%) of these patients – five with DVT and one with PE – had either the prothrombin gene G20210A (n = 4) or Factor V Leiden (n = 2) mutations. When combining both mutations, a significantly increased risk for VTE was seen (odds ratio 5.73, 95% CI 2.11–15.52, p = 0.002;
VTE in Relation to Drug Treatments

The clinical outcome of this study has been reported previously (6) and was not influenced significantly by the presence or absence of the two gene mutations. Among the 31 patients with symptomatic events, 8 patients were randomised to dalteparin (26%) and 23 (74%) to melagatran and ximelagatran, which is in accordance with the randomisation ratio of dalteparin:melagatran and ximelagatran used in the study (1:4).

Mortality

Four of the 1600 patients died post-operatively. One patient randomised to the dalteparin group died during the treatment period due to pneumonia. This patient was heterozygous for Factor V Leiden mutation. Three patients, randomised to the melagatran and ximelagatran groups, died during follow-up due to PE, myocarditis and heart failure. None of these patients carried either of the gene mutations.

Discussion

Of the 1600 patients investigated in this study, the prevalence of Factor V Leiden and prothrombin gene G20210A mutations was 5.5% and 2.9%, respectively. These values are largely consistent with previous studies (8, 15), although slightly lower than expected in Sweden regarding the Factor V Leiden mutation (23). There was a large variation in the prevalence of these mutations between different countries. This difference between countries probably reflects a true difference between geographical regions but could also be a chance finding, since some of the countries had only a limited number of patients enrolled in the study.

The Factor V Leiden and prothrombin gene G20210A mutations (alone or taken together) were not found to be risk factors for VTE at the end of the obligatory anticoagulation period, until study day 8-11. The endpoint at this time is dominated by the results from the mandatory bilateral venography (asymptomatic DVT). However, for symptomatic VTE, both mutations taken together and the prothrombin gene G20210A mutation alone were shown to be risk factors during the follow-up period and/or the total observation period. A tendency towards a higher risk for symptomatic VTE among the patients carrying the Factor V Leiden mutation was seen, although this did not reach statistical significance. Both mutations taken together and alone were risk factors for PE.

The literature is somewhat contradictory on this issue. Ryan et al. (18), in a meta-analysis of five studies involving 825 patients, could not find a statistically significant increase in the risk for the Factor V Leiden mutation in joint replacement surgery. Likewise, Philipp et al. (19) and Campos et al. (20) also reported no increased risk for the Factor V Leiden mutation. Conversely, Svensson et al. (16) and Lindahl et al. (17) both found a statistically significant risk for APCR or the Factor V Leiden mutation for post-operative VTE following major orthopaedic surgery. For the prothrombin gene G20210A mutation we are only aware of one study in orthopaedic surgery (20), and this study did not show an increased risk for post-operative VTE.

A number of factors could have contributed to the different outcome with regard to the prothrombin gene G20210A and Factor V Leiden mutations. The sample size in our study was much larger than the sample size of the previous studies (16-20). Another factor that might play an important role is the fact that 59% of the patients in our study received some form of prolonged anticoagulation after the mandatory study treatment. Early treatment will reduce the number of later symptomatic VTEs, although most of the asymptomatic VTEs will not progress to become symptomatic (1, 2, 17, 24). The clinical significance of asymptomatic DVT has been questioned, since the majority of these events are located in the distal calf veins. It has been shown that approximately 20-30% of these thrombi may propagate into the proximal veins and thereby constitute an increased risk for PE (25). Additio-
nally, isolated calf vein thrombi may embolise to the lungs (26). How-
ever, there are major differences between asymptomatic DVT found at venographic screening and symptomatic VTE. Of the previous studies with the mutations in ortho-
paedic surgery, Ryan et al. and Philipp et al. (18, 19) used early veno-
graphic screening as an endpoint with similar results for the Factor V Leiden mutation as were observed in our study. Svensson et al. (16) al-
so used venographic screening, but it was performed 3 weeks post-
operatively. Lindahl et al. and Campos et al. (17, 20) used symptomatic VTE as an endpoint.

The effect of prolonged anticoagulation on the results from the present study is difficult to estimate in terms of odds ratios since there
were only two symptomatic VTEs in the group of patients with muta-
tions who were given prolonged anticoagulation. However, the relative frequency of symptomatic VTE indicates that prolonged prophylaxis/treatment might have reduced the risk increase associated with the mutations. In patients having any of the mutations, the VTE rate was 4/50 (8.0%) without prolonged anticoagulation compared with 2/83 (2.4%) with prolonged anticoagulation. A possible explanation is that only patients with multiple risk factors will develop VTE during anti-
coagulation, whereas without anticoagulation, the prothrombin gene G20210A and Factor V Leiden mutation may increase the risk enough for the development of a VTE. Given the fact that so many patients received prolonged prophylaxis, the result from our study is probably an under-estimation of the risk connected with these mutations.

In the previous studies, Ryan et al. and Philipp et al. (18, 19) only in-
vestigated the venographic endpoints, and no follow-up result is stated.
Although Campos et al. (20) investigated clinical endpoints both with regard to Factor V Leiden and the prothrombin gene G20210A muta-
tion, the results are not conclusive since the patient population was very small (123 patients). Svensson et al. (16) found that the increased risk of developing VTE with the Factor V Leiden mutation was reduced markedly by prolongation of the anticoagulation treatment and sugges-
ted a prolonged period of prophylactic treatment for these patients. In
the Lindahl study (17) no prolonged anticoagulation was given.

It could be argued that the patients in our cohort were not evaluated for other risk factors. Furthermore, our study excluded patients with a previous thromboembolism and ongoing malignancy. However, with a study population with an average age of approximately 60 years, it is unlikely that these patients would suffer from a severe hereditary risk factor such as antithrombin, Protein C or S deficiency, as they are rare in an unselected population and frequently result in thromboembolic complications at young age (27-29). Prospective studies, beyond the scope of this investigation, are required, however, to elucidate fully the complex issue of the risks/benefits of pre-operative screening for all sub-groups of patients undergoing orthopaedic replacement surgery.

Although we have shown that patients with the Factor V Leiden or the prothrombin gene G20210A mutations have an elevated risk for symptomatic VTE post-operatively, approximately 90% of these patients will not have such an event. It is, therefore, our opinion that general pre-operative screening for such genotypes is of questionable value.

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Received September 10, 2001 Accepted after revision January 8, 2002