Brief report

α-Fibrinogen Thr312Ala polymorphism and venous thromboembolism

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The α-fibrinogen Thr312Ala polymorphism, which occurs in a region involved in factor XIII (FXIII)-dependent cross-linking processes, is associated with poststroke mortality in subjects with atrial fibrillation, suggesting an influence either on intraatrial clot formation or embolization. We have determined the association of Thr312Ala with deep vein thrombosis (DVT) and pulmonary embolism (PE) and have assessed the interaction of Thr312Ala with the FXIII Val34Leu polymorphism in 122 patients with DVT, 99 patients with PE, and 254 healthy control subjects. The genotype distribution of patients with PE (TT = 49%, TA = 36%, AA = 15%), but not DVT (TT = 50%, TA = 42%, AA = 8%), differed significantly from healthy control subjects (TT = 60%, TA = 34%, AA = 6%, P = .02). A significant interaction of Thr312Ala and Val34Leu was also identified (P = .01), indicating an inverse association between Leu34 and Ala312. These results support the hypothesis that Thr312Ala alters FXIII-dependent cross-linking, making formed fibrin clot more susceptible to embolization. (Blood. 2000;96:1177-1179)

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Introduction

The hemostatic system maintains a delicate balance between coagulation/anticoagulation, platelet activation/inhibition, and fibrinolysis to maintain vascular patency. Abnormalities in hemostatic factors may either lead to bleeding or to excessive fibrin production and thrombosis. The FV Leiden and prothrombin G20210A polymorphisms have been related to venous thromboembolism (VTE), and we have shown that the Leu allele of the factor XIII (FXIII) Val34Leu polymorphism is inversely associated with risk of VTE and also of myocardial infarction.4

The α-fibrinogen Thr312Ala polymorphism occurs in a region important for FXIII-dependent cross-linking processes and may, therefore, influence clot structure or rigidity. We have found a significant association of Thr312Ala with poststroke mortality in subjects with atrial fibrillation, with increased mortality in subjects possessing Ala312 compared with those homozygous for Thr312.7 These results indicate that Thr312Ala may influence either intraatrial clot formation or embolization.

To further clarify the association of Thr312Ala with either thrombus formation or embolization, the aim of the present study was to determine the association of Thr312Ala with deep vein thrombosis (DVT) and pulmonary embolism (PE) and additionally to identify any significant interaction with FXIIIVal34Leu.

Study design

Subjects

In the patient group, 122 (55%) subjects had sustained a DVT, confirmed on doppler ultrasound venogram examination of calf, popliteal, femoral, and iliac veins. Ninety-nine (45%) subjects had a clinical diagnosis of PE, of which 17 had both DVT and PE (classified as PE for analysis); the remaining 82 subjects with PE had no clinical evidence of DVT. PE was confirmed by the presence of two or more areas of ventilation-perfusion mismatch on a Technicium-99 lung scan or of thrombus in the pulmonary vasculature, confirmed by contrast-enhanced high resolution spiral computed tomography (reserved for subjects with intermediate probability of PE on lung scanning [n = 15]).

Healthy control subjects (n = 254) were from the same geographical area as patients and were free of a personal and family history of VTE.3 The study was approved by the United Leeds Teaching Hospitals Trust Research Ethics Committee.

DNA and biochemical analyses

Thr312Ala and FXIII Val34Leu genotypes were determined as previously described.3,7 DNA from 3 patients and 4 control subjects did not amplify for Thr312Ala. FXIII activity was determined as previously described.9

Statistics

Genotype distributions were compared by χ2 test. Logistic regression models (backward stepwise selection) were used to identify factors significantly associated with VTE (results presented as odds ratios [OR] and 95% confidence interval [CI]). Interaction terms were created in these models to identify significant interactions of Thr312Ala with other risk factors. Statistical analyses were performed, using the SPSS statistics package V7.0 (SPSS Inc.).

Results and discussion

As previously reported,3 FV Leiden was significantly associated with VTE, and the FXIII Leu34 allele was inversely associated with VTE (data not shown). There was a significant difference in the Thr312Ala genotype distributions of patients (TT = 49%, TA = 39%, AA = 12%) and healthy control subjects (TT = 60%, TA = 34%, TT = 6%, P = .04). However, in a logistic regression
model including age, sex, malignancy, FV Leiden, FXIII Val34Leu, and Thr312Ala. Thr312Ala was not independently associated with VTE. ORs for factors independently associated with VTE were as follows: 1.17 (95% CI = 1.04-1.32) for a 10-year increase in age; 2.93 (95% CI = 1.31-6.59) for those with versus without malignancy; 2.32 (95% CI = 1.12-4.79) for carriers of FV Leiden versus wild type; 0.37 (95% CI = 0.23-0.61) for subjects possessing FXIII Leu34 versus those homozygous for Val34.

Subclassification of patients into those with DVT or PE revealed a significant difference in Thr312Ala genotype distributions of patients with PE versus healthy control subjects, but no difference of patients with DVT versus control subjects, or patients with DVT versus PE (see Table 1). In a logistic regression model comparing patients with PE to healthy control subjects, Thr312Ala was independently associated with PE (OR = 2.71 [95% CI = 1.23-6.01]) for subjects homozygous for Ala312 versus those homozygous for Thr312. ORs for other factors significantly associated with PE were as follows: 1.26 (95% CI = 1.08-1.49) for a 10-year increase in age; 0.58 (95% CI = 0.36-0.95) for subjects possessing FXIII Leu34 versus those homozygous for Val34. We previously reported no significant association of Val34Leu with PE in univariate analyses; however, in this logistic regression model in which the Val allele was considered to be recessive in relation to PE (as previously shown for VTE), Leu34 was inversely associated with PE.

FXIII-dependent cross-linking processes are essential for stabilizing fibrin and for forming a fibrin clot more resistant to fibrinolysis. Therefore, it is not unreasonable to expect that any factor interfering with these processes may adversely influence the mechanical integrity or plasmin susceptibility of a fibrin clot. Because Thr312Ala was related to PE but not to DVT (compared with control subjects) in this study, it is plausible that Thr312Ala increases the susceptibility of fibrin clot to embolize, although further work on fibrin structure is required to test such a hypothesis.

To investigate the possible role of Thr312Ala in FXIII-dependent cross-linking processes, we introduced the interaction term Thr312Ala*Val34Leu (plus each individual term) into the PE versus control subject logistic regression model. A significant interaction between Thr312Ala and FXIII Val34Leu was indicated by this model (P = .01). Other factors significantly associated with PE were age (OR = 1.28 [95% CI = 1.09-1.51]) and FXIII Val34Leu (OR for those possessing Leu34 versus those homozygous for Val34 = 0.33 [95% CI = 0.17-0.64]), but not cigarette smoking. To investigate this interaction, the association of Val34Leu with PE was determined separately in those possessing Thr312Ala versus those possessing Ala312. These analyses indicated that, in subjects homozygous for Thr312, possession of Leu34 is inversely associated with PE (χ², P = .002), whereas, in those possessing the Ala312 allele, the association between FXIII Leu34 and PE is lost (χ², P = .95), as shown in Figure 1. This finding supports the possibility that Thr312Ala influences FXIII-dependent processes and also points to a likely difference in the influence of each of these polymorphisms on the stabilization of a fibrin clot.

FXIII Val34Leu occurs close to the thrombin cleavage site of FXIII and may influence thrombin activation of FXIII. In 113 of the healthy control subjects, FXIII cross-linking activity was available and significantly associated with Val34Leu (Val/Val = 81.4% [77.1%-86.0%]; Val/Leu = 124.4% [113.6%-136.2%]; Leu/Leu = 175.7% [152.2%-203.0%], P < .0001), as we have previously demonstrated. This finding indicates that Val34Leu influences FXIII activity or activation rates, although it remains unclear at present how increased activity in those individuals possessing Leu34 is related to protection from thrombosis.

Thr312Ala occurs close to the α-fibrin/α-fibrin cross-linking site (Aa328) as well as to the α2-antiplasmin/α-fibrin cross-linking site (Aa303) and occurs within the region of α-fibrinogen (Aa242-424) that has been suggested to be involved in FXIII activation. Thus, Thr312Ala could potentially interfere with any one of these processes. We have previously shown no association of Thr312Ala with α2-antiplasmin incorporation. Similarly, we found no association of Thr312Ala with FXIII activity (TT = 105.9% [97.6%-114.8%]; TA = 105.0% [93.3%-120.8%]; AA = 92.2% [70.5%-120.7%], P = ns), even when patients were characterized by Val34Leu genotype (data not shown). Thus, it would appear that Thr312Ala is most likely to influence α-fibrin/α-fibrin cross-linking. Because α-fibrin cross-linking contributes to approximately 65% of overall clot stability, it would be expected that any factor specifically influencing this cross-linking would lead to a reduction in the overall clot strength and potentially an increased likelihood of embolization. Further in vitro studies are currently under way to determine the influence of Thr312Ala on α-fibrin/α-fibrin cross-linking.

This study does have some limitations. In subjects classified with DVT alone, it is possible that a small proportion of subjects (particularly those with more proximal venous thrombosis) would be likely to embolize asymptptomatically, and, therefore, the incidence of PE might be underrepresented in this group. In addition, because of the relatively small sample size, a larger study would be required to confirm these findings.

Table 1. Thr312Ala genotype distributions of patients with VTE classified as those with DVT or PE compared with healthy control subjects

<table>
<thead>
<tr>
<th>Thr312Ala genotype</th>
<th>DVT (n = 120)</th>
<th>PE (n = 98)</th>
<th>Control subjects (n = 250)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>60 (0.50)</td>
<td>48 (0.49)</td>
<td>149 (0.59)</td>
</tr>
<tr>
<td>TA</td>
<td>50 (0.42)</td>
<td>35 (0.36)</td>
<td>85 (0.34)</td>
</tr>
<tr>
<td>AA</td>
<td>10 (0.08)</td>
<td>15 (0.15)</td>
<td>16 (0.06)</td>
</tr>
</tbody>
</table>

VTE indicates venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism. χ² testing: VTE overall vs. healthy control subjects, P = .04. Pairwise comparisons: DVT versus controls, P = .2; PE versus controls, P = .02; DVT versus PE, P = .06.
References


