Association of a Common Polymorphism in the Factor XIII Gene With Venous Thrombosis

By Andrew J. Catto, Hans P. Kohler, Julie Coore, Michael W. Mansfield, Max H. Stickland, and Peter J. Grant

We have shown an association between a common mutation in the factor XIII a-subunit gene, coding for an amino acid change, 3 amino acids from the thrombin activation site (factor XIII Val34Leu) that may protect against myocardial infarction and predisposes to intracranial hemorrhage. To investigate the possible role of factor XIII Val34Leu in the pathogenesis of venous thromboembolism (VTE) and potential interactions with factor V Leiden (FV:Q506R) and prothrombin G → A 20210, we studied 221 patients with a history of VTE and 254 healthy controls. Patients with VTE showed an increased frequency of the FXIII Val/Val genotype (63% v 49%) and a lower frequency of the Val/Leu genotype (31% v 42%) than controls (P = .007). FV:Q506 heterozygotes were more frequent in VTE patients (11%) than controls (5%; P = .04). The prothrombin G → A 20210 mutation was present in only 3 patients and no controls (P = .10). In a logistic regression model for a history of VTE, the odds ratio (95% confidence interval) for FXIII Val/Leu or Leu/Leu genotype was 0.63 (0.38 to 0.82) and for possession of FV:Q506 2.40 (1.17 to 4.90). There was no evidence for an interaction between factor XIII Val34Leu genotype and FV:Q506R, prothrombin G → A 20210, sex, or age. It is concluded that possession of the Leu allele at factor XIII Val34Leu is protective against deep venous thrombosis.

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GENETIC FACTORS may be implicated in the pathogenesis of at least 30% of cases of venous thrombosis. The most common inherited form of venous thrombosis is the result of resistance to activated protein C (APC). In the majority of cases, the molecular basis for APC resistance is a single point mutation arising in the factor V gene (FV:Q506R, Factor V Leiden).

We have recently reported that a common point mutation (G→T) in exon 2 of the factor XIII a-subunit gene is protective against myocardial infarction and predisposes to intracranial hemorrhage. This polymorphism codes for a valine (Val) to leucine (Leu) amino acid change 3 amino acids from the factor XIII thrombin activation site, and our results imply that this may interfere with fibrin cross-linking.

Fibrin formation is important in the development of venous thromboembolism (VTE), indicating that possession of the factor XIII 34Leu allele might interfere with this process. The aim of this study was to investigate an association of factor XIII Val34Leu with VTE and to evaluate possible interactions between FV:Q506R and prothrombin G → A 20210.

MATERIALS AND METHODS

Study Population

Patients. Two hundred twenty-six consecutive patients with a clinical diagnosis of VTE, attending the Leeds General Infirmary anticoagulation clinic between January 1, 1997 and October 31, 1997, which serves a population of approximately half a million, were recruited by one of the authors (A.J.C.). Patients with first ever as well as recurrent VTE were recruited. Five patients were excluded from the study (2 were not White North European, for 2 there was inadequate clinical information, and for 1 there was an insufficient sample for analysis), leaving a VTE study population of 221.

Controls. Two hundred fifty-four White North European patients were drawn from the same geographical area as the patients and identified at random from age-stratified registers of general medical practitioners serving the Leeds Family Health Authority. These community control patients were invited to participate by postal questionnaire, with a response rate of 71%. There were no demographic differences between those patients who participated and those who did not. At the time of recruitment, control patients were free from a personal history and family history of VTE.

The study was approved by the United Leeds Teaching Hospitals Trust Research Ethics Committee.

Clinical Details

In the patient group, 122 (55%) patients had sustained a deep venous thrombosis (DVT), confirmed on Doppler ultrasound venogram examination of the calf, popliteal, femoral, and iliac veins. Ninety-nine patients (45%) had a clinical diagnosis of pulmonary embolism (PE), of which 82 had no evidence of DVT and 17 had evidence of both DVT and PE (the latter were classified as cases of PE for the purpose of the analysis). Confirmation of the clinical diagnosis of PE was based on the presence of either two or more areas of ventilation-perfusion mismatch on a TechniGammagard lung scan or the presence of thrombus in the pulmonary vasculature confirmed by contrast-enhanced high-resolution spiral computed tomography (CT). CT examination was reserved for those patients with an intermediate probability of PE on lung scanning (n = 15).

DNA procedures. Genomic DNA was extracted from peripheral leukocytes in citrated blood using the Nucleon kit (Nucleon Biosciences, Lanarkshire, UK). Using a polymerase chain reaction (PCR)-single-stranded confirmation polymorphism (SSCP) method the FXIII Val34Leu polymorphism was reported as Val/Val (G/G), Val/Leu (G/T), or Leu/Leu (T/T). A PCR method1 and Mnl I digest were used to type the FV:Q506R mutation. The prothrombin G → A 20210 mutation was characterized by a mutagenic primer sequence and HindIII digest.4 Heterozygous prothrombin mutants were confirmed by direct sequencing. Each genotype was analyzed without knowledge of case or control status.

Statistical methods. Values for age were not normally distributed and are presented as median (range) and compared between groups using the Mann-Whitney test. The frequencies of dichotomous variables (sex, history of cancer, FV:Q506R, and prothrombin genotypes) were compared between patients and controls using Fisher’s exact test. Frequencies of Factor XIII genotypes were compared using the
chi-squared test. Logistic regression analysis was used to identify factors significantly and independently associated with a history of VTE.

RESULTS

As shown in Table 1, the 221 patients of VTE had a median age 2 years older than the 254 control patients, although there was a similar distribution of sexes in each group. Of the 221 patients, in 122 there was evidence of DVT only, whereas in the remaining 99 there was evidence of PE with or without clinically detectable DVT. A history of malignancy was more frequent in patients of VTE than in control patients.

The distribution of genotypes at the Factor XIII Val34Leu polymorphism was different between patients and controls with fewer Val/Leu and Leu/Leu patients and more Val/Val patients among patients. At the factor V polymorphism there were more carriers of the FV:Q506 mutation in patients than in controls. The prothrombin G → A 20210 mutation was infrequent, being found in 3 patients and no controls. This difference was not significant.

In a logistic regression model factor XIII Val34Leu genotype, FV:Q506, age, and a history of malignancy were independently related to VTE (Table 2). The assumption that the more frequent Val allele in relation to VTE gave the best fitting model. When Factor XIII Val34Leu genotype was assumed to have a codominant influence of risk of VTE, the odds ratio (95% confidence interval) for possession of each additional Leu allele was 0.63 (0.47 to 0.87; P = .004). When factor XIII genotype was entered with the Val allele being dominant, there was no significant relationship to history of VTE. Sex showed no independent association with VTE. In view of the low frequency of the prothrombin gene mutation, prothrombin was no independent association with VTE. In view of the low frequency of the prothrombin gene mutation, prothrombin was no independent association with VTE.

The associations with genotype were reexamined in the patients divided into those with DVT only and cases of PE. Both Factor XIII Val34Leu (P = .025) and FV:Q506 (P = .026) were related to DVT, but there was no significant association with PE (Table 3).

DISCUSSION

This study shows an inverse association between possession of the Leu allele at factor XIII Val34Leu and the occurrence of DVT. These results are consistent with the hypothesis that the previously described protective effect of factor XIII 34Leu against myocardial infarction2 extends to venous thrombosis. However, the findings should be interpreted in the context of a case-control study design, and our findings require confirmation in a prospective study.

The formation of cross-linked fibrin from fibrin monomer is pivotal in the development of a stable thrombus. This process requires the action of thrombin on fibrinogen to produce soluble fibrin and the activation of Factor XIII, which cross-links fibrin, rendering it more resistant to fibrinolysis. Until recently, there has been no knowledge regarding the role of factor XIII in vascular disease. We have shown a lower prevalence of carriage of the Leu allele at factor XIII Val34Leu in patients with myocardial infarction than without (32% v 50%). The odds ratio (OR) was 0.67 for a history of myocardial infarction in those carrying the Leu allele, which was similar to the OR of 0.56 in patients with VTE in the current study.

Further evidence for a biological effect of this polymorphism is derived from patients with primary intracerebral hemorrhage3 in whom we showed an excess of the factor XIII Leu allele.

Table 2. Logistic Regression Model for History of Venous Thromboembolism

<table>
<thead>
<tr>
<th>Factor XIII Val/Leu or Leu/Leu genotype</th>
<th>Odds Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.56 (0.38-0.82)</td>
<td>.003</td>
</tr>
<tr>
<td>Factor V:Q506 mutation carrier</td>
<td>2.40 (1.17-4.90)</td>
<td>.017</td>
</tr>
<tr>
<td>Age (for 10-yr increase)</td>
<td>1.17 (1.04-1.32)</td>
<td>.009</td>
</tr>
<tr>
<td>History of malignancy</td>
<td>3.29 (1.47-7.35)</td>
<td>.004</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.09 (0.74-1.60)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are shown for odds ratio with 95% confidence interval in brackets.

Abbreviation: NS, not significant.

The associations with genotype were reexamined in the patients divided into those with DVT only and cases of PE. Both Factor XIII Val34Leu (P = .025) and FV:Q506 (P = .026) were related to DVT, but there was no significant association with PE (Table 3).

Table 3. Genotype Frequencies at the Factor V:Q506 and Factor XIII Val34Leu Polymorphisms in Subjects With DVT and Pulmonary Embolism

<table>
<thead>
<tr>
<th>Factor</th>
<th>DVT Only</th>
<th>Pulmonary Embolism</th>
<th>Control Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>122</td>
<td>99</td>
<td>254</td>
</tr>
<tr>
<td>Factor V genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>104</td>
<td>87</td>
<td>237</td>
</tr>
<tr>
<td>FV:Q506</td>
<td>16</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Factor XIII Val34Leu genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val (G/G)</td>
<td>76</td>
<td>61</td>
<td>123</td>
</tr>
<tr>
<td>Val/Leu (G/T)</td>
<td>36</td>
<td>32</td>
<td>107</td>
</tr>
<tr>
<td>Leu/Leu (T/T)</td>
<td>7</td>
<td>5</td>
<td>22</td>
</tr>
</tbody>
</table>

DVT versus controls significant at P < .026 at both factor V and factor XIII polymorphisms. Other comparisons not significant.
These findings support the hypothesis that factor XIII Val34Leu is involved in the production of weaker fibrin structures, which might thereby protect against clot formation and predispose to hemorrhage.

Although we confirmed the expected association of FV:Q506 with DVT, there was no interaction between FV:Q506 and factor XIII Val34Leu, although this might be accounted for by the smaller numbers bearing the FV:Q506 mutation and requires further investigation.

There was no significant association between factor XIII Val34Leu or FV:Q506 and risk of PE, which is consistent with a previous study of APC resistance and PE. However, this may simply reflect the reduced power to detect differences when data are subdivided, particularly as there was no evidence of heterogeneity in factor XIII or factor V genotype frequencies between patients of DVT and PE, respectively. If confirmed, a difference in the relationship of factor XIII Val34Leu genotype with DVT and PE would raise the possibility that differences in fibrin cross-linking may affect the clinical presentation in terms of embolization.

Although this study does not provide direct evidence for functional effects of factor XIII Val34Leu, preliminary experiments from our Unit have shown that using a pentylamine incorporation assay (based on the method of Song6), factor XIII 34Leu shows an increased rate of activation by thrombin. Indeed, the rate of activation increases with increasing numbers of Leu alleles, and plasma from patients with the Leu/Leu genotype shows enhanced activation and degradation by α-thrombin compared with plasma from Val/Val patients, suggesting an alteration in FXIII function (unpublished observation, April 1998).

We have previously shown that in the small number of patients both carrying the factor XIII Leu allele and with acute myocardial infarction, PAI-1 levels were higher,2 suggesting that impairment of fibrinolysis negates the protective effect of factor XIII 34Leu. These findings indicate that the influence of factor XIII Val34Leu on myocardial infarction depends on complex interactions with other hemostatic factors. Although we have not shown this to be the case for factor V Leiden and prothrombin G → A 20210 in the present study, this is an area worthy of further investigation in relation to venous thrombosis.

In conclusion, these data suggest that possession of the Leu (T) allele at the factor XIII Val34Leu polymorphism is protective against VTE and provides further evidence for a role for factor XIII in thrombotic vascular disorders.

ACKNOWLEDGMENT

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REFERENCES

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