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The protective effect of the factor XIII Val34Leu mutation on the risk of deep venous thrombosis is dependent on the fibrinogen level

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Blood coagulation factor XIII (FXIII) cross-links fibrin on activation (i.e. cleavage) by thrombin to stabilize a fibrin clot. The common Val34Leu mutation in the FXIII A subunit is located only three amino acids from the thrombin cleavage site, and could thus potentially alter the clot-stabilizing propensity of FXIII. Previously, it has been shown that thrombin activates FXIII 34Leu more rapidly and at lower concentration than 34Val [1,2]. Early activation of FXIII leads to early cross-linking of fibrin, which seems to inhibit lateral aggregation of the fibrin fibres, and renders the fibrin clot less porous and made up of thinner fibers than a clot cross-linked by 34Val. Plasma clots consisting of thin fibrin fibers are relatively resistant to plasma degradation [3]; however, studies which determined the risk of venous thrombosis of carriers relative to non-carriers reported either a protective effect or no effect of the FXIII Val34Leu mutation on the risk of venous disease [4–8]. In the LETS study, carriers of the 34Leu allele showed higher FXIII activity, but no higher FXIII subunit levels. At most a weak protective effect for venous thrombosis was found for the 34Leu allele (OR 0.9; 95% CI 0.7–1.1), which was completely restricted to men, with a higher protective effect in homozygotes (OR 0.7; 95% CI 0.4–1.3) [9].

High fibrinogen levels lead, like the factor 34Leu variant, to a less porous and therefore less permeable fibrin clot with thin fibres, which is associated with an increased risk of myocardial infarction and premature coronary artery disease [10,11]. These effects seem contradictory, however, as Lim *et al.* [12] found that 34Leu homozygous patients form fibrin clots with lower permeability than 34Val homozygotes at low fibrinogen concentrations, but that the permeability was similar for both genotypes at intermediate fibrinogen levels and even higher for the 34Leu homozygotes at high fibrinogen levels. This would predict a protective effect of 34Leu at high fibrinogen levels. Therefore, we decided to determine the influence of fibrinogen levels on the effect of the FXIII Val34Leu mutation on venous thrombotic risk.

This study was performed using plasma samples from a large population-based case–control study on risk factors for venous thrombosis, the Leiden Thrombophilia Study (LETS) [13]. In this study, 474 consecutive patients with an objectively diagnosed first episode of deep venous thrombosis and 474 age- and

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Table 1 Risk of venous thrombosis associated with the FXIII 34Leu mutation according to fibrinogen levels

Fibrinogen levels	FXIII	OR* overall	OR men	OR women
Normal (< = 4.11)	Val34Leu	1.0 (0.7–1.3)	0.9 (0.6–1.4)	1.0 (0.7–1.5)
	Leu34Leu	0.6 (0.3–1.2)	0.4 (0.1–1.4)	0.8 (0.3–1.9)
	Val34Leu/ Leu34Leu	0.9 (0.7–1.2)	0.8 (0.5–1.3)	1.0 (0.7–1.4)
High (> 4.11)	Val34Leu	0.4 (0.2–0.9)	0.2 (0.1–0.7)	0.7 (0.3–1.9)
	Leu34Leu	0.7 (0.2–3.1)	0.2 (0.0–2.1)	1.8 (0.2–18.4)
	Val34Leu/ Leu34Leu	0.5 (0.2–1.0)	0.2 (0.1–0.7)	0.8 (0.3–2.1)

*Risks are calculated with individuals carrying Val34Val as reference group.

sex-matched friends or partners of these patients were included. For this analysis, we included all patients and controls tested for the FXIII Val34Leu variant (genotype information was missing for three patients). FXIII Val34Leu genotyping was performed using an allele-specific polymerase chain reaction as described previously [9,14]. Fibrinogen levels were measured with the Clauss method using Dade® thrombin (Baxter, Miami, FL, USA). We calculated odds ratios (ORs) and 95% confidence intervals (CI) for venous thrombosis associated with the 34Leu variant for those with high and normal levels of fibrinogen (using a 90th percentile cut-off point).

The mean age at the time of the blood draw was 45 years in the patients and the controls (range patients 15–68, range controls 15–71). Of the patients, 202 (43%) were men compared with 202 controls (43%). The allele frequency of the Val34Leu mutation was 0.20 in the 471 patients (165 carriers; 20 were homozygous) and 0.24 in the 474 controls (201 carriers; 27 homozygotes). The mean fibrinogen levels were 3.4 g L^{-1} (range 1.5–8.0) for cases and 3.3 g L^{-1} (range 1.7–6.3) for controls.

Table 1 shows the odds ratios for venous thrombosis for the Val34Leu variant stratified by normal (below the 90th percentile) or high (above the 90th percentile) levels of fibrinogen. The 34Leu variant does not confer a protective effect in heterozygote carriers of the 34Leu variant with normal fibrinogen levels, either overall or in men or women separately. In homozygote 34Leu carriers with normal fibrinogen levels we did find a protective effect, especially in men. A protective effect of 34Leu was present for individuals with high fibrinogen levels, again particularly for men (Table 1).

Earlier we found in the LETS that the risk of venous thrombosis associated with high levels of fibrinogen was increased mainly in individuals older than 45 years [15]. Stratification by age in the current analysis (below and above 45 years) did not change the ORs at normal fibrinogen levels in carriers, heterozygotes and homozygotes for the 34Leu variant (OR was 0.9 for those below and above the age of 45 years). However, at high fibrinogen levels the OR in carriers was 0.8 (95% CI 0.2–3.0) below 45 years old and 0.4 (95% CI 0.2–1.0) above 45 years old.

Our findings show clinical evidence for the observations of Lim *et al.* [12] suggesting that the protective effect of the 34Leu variant at high fibrinogen levels may be accounted for by the formation of more permeable fibrin clots at high fibrinogen concentrations than at low fibrinogen concentrations.

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