Factor XIII Val34Leu polymorphism, factor XIII antigen levels and activity and the risk of deep venous thrombosis

Rosendaal, F.R.

Citation

Rosendaal, F. R. (2002). Factor XIII Val34Leu polymorphism, factor XIII antigen levels and activity and the risk of deep venous thrombosis, 169-175. Retrieved from https://hdl.handle.net/1887/1601

Version: Not Applicable (or Unknown)

License:

Downloaded from: https://hdl.handle.net/1887/1601

Note: To cite this publication please use the final published version (if applicable).

British Journal of Haematology, 2002, 119, 169–175

Factor XIII Val34Leu polymorphism, factor XIII antigen levels and activity and the risk of deep venous thrombosis

ASTRID VAN HYLCKAMA VLIEG,[1,2] NANTARAT KOMANASIN,[3] ROBERT A. S. ARIËNS,[3] SWIBERTUS R. POORT,[2] PETER J. GRANT,[3] ROGIER M. BERTINA[2] AND FRITS R. ROSENDAAL[1,2] [1]*Department of Clinical Epidemiology and* [2]*Haemostasis and Thrombosis Research Centre, Leiden University Medical Centre, Leiden, the Netherlands, and* [3]*Academic Unit of Molecular Vascular Medicine, University of Leeds, Leeds, UK*

Received 6 February 2002; accepted for publication 1 May 2002

Summary. Varying results on the effect of factor XIII (FXIII) Val34Leu on venous thrombotic risk have been reported. The probability of a true association between this polymorphism and venous thrombotic risk would be enhanced by a laboratory phenotype associated with this polymorphism and with the thrombotic risk. The aim of this study was to assess the effect of FXIII Val34Leu, FXIII activity and subunit levels on venous thrombotic risk in a large case–control study, The Leiden Thrombophilia study (LETS). We found higher FXIII activity for 34Leu carriers (Leu/Leu: 158·0, Val/Val: 95·0). FXIII subunit levels were not associated with genotype. Higher FXIII activity was associated with a slightly decreased thrombotic risk [Odds ratio (OR): 0·8, 95% confidence intervals (CI): 0·5–1·3]. This effect was not present for elevated FXIII subunit levels. Higher FXIII activity was also associated with a higher dissociation index (percentage A_2B_2 complex dissociated after activation by thrombin for a fixed time interval). This index was higher for FXIII 34Leu carriers. The risk of deep venous thrombosis was slightly decreased for carriers of the 34Leu allele [OR: 0·9 (95%CI: 0·7–1·1)]. For homozygous 34Leu carriers the OR was 0·7 (95%CI: 0·4–1·3). This finding, suggesting a weak protective effect, was completely restricted to men. An overall estimate of thrombotic risk was calculated by using earlier reports on the risk of FXIII Val34Leu. The overall risk estimate for homozygous 34Leu carriers was 0·8 (95%CI: 0·6–1·0). In this study, a weak protective effect against venous thrombosis was found, of FXIII 34Leu as well as of increased FXIII activity.

Keywords: factor XIII activity, factor XIII subunit levels, Val34Leu polymorphism, venous thrombosis.

Earlier studies revealed varying results on the thrombotic effect of a common polymorphism (G → T) in exon 2 of the gene coding for the A subunit of factor XIII (FXIII). Some studies indicated that this polymorphism may be associated with protection against venous thrombosis (Catto *et al*, 1999; Franco *et al*, 1999; Alhenc-Gelas *et al*, 2000; Renner *et al*, 2000) while other studies reported no effect of this polymorphism on the risk of deep venous thrombosis (Balogh *et al*, 2000; Corral *et al*, 2000; Margaglione *et al*, 2000).

Several studies also suggested that the factor XIII 34Leu allele protects against myocardial infarction (Kohler *et al*, 1998a; Wartiovaara *et al*, 1999; Franco *et al*, 2000a) and ischaemic stroke (Elbaz *et al*, 2000), whereas it has been associated with an increased risk for haemorrhagic stroke (Catto *et al*, 1998).

Correspondence: F. R. Rosendaal, Department of Clinical Epidemiology, Leiden University Medical Centre, C9-P, PO Box 9600, 2300 RC Leiden, The Netherlands. E-mail: f.r.rosendaal@lumc.nl

The G to T polymorphism predicts the replacement of Valine-34 by Leucine in the activation peptide, only three amino acids from the thrombin cleavage site (Mikkola *et al*, 1994).

As described by Lane & Grant (2000), in order for a genetic polymorphism to have an effect on disease, it has to be mediated through a phenotype.

Factor XIII Val34Leu is one of several common polymorphisms in the coding region of the factor XIII gene which are not associated with factor XIII deficiency (Mikkola *et al*, 1994; Suzuki *et al*, 1996). Factor XIII 34Leu has been associated with increased factor XIII activity (Kangsadalampai & Board, 1998; Kohler *et al*, 1998b; Anwar *et al*, 1999) but not with higher levels of A and B subunit antigen (Kohler *et al*, 1998b; Balogh *et al*, 2000). After full activation, however, the specific cross-linking activity does not differ (Ariëns *et al*, 2000; Wartiovaara *et al*, 2000), suggesting that the polymorphism affects activation rates rather than cross-linking activity itself.

© 2002 Blackwell Publishing Ltd

Recent evidence indicates that the catalytic efficiency of thrombin-induced cleavage of factor XIII 34Leu is two to threefold higher than that of factor XIII 34Val (Ariëns *et al*, 2000; Wartiovaara *et al*, 2000). The accelerated activation of factor XIII 34Leu has been shown to alter the structure of the cross-linked fibrin. Early covalent cross-linking by factor XIII 34Leu inhibits lateral aggregation of the fibrin fibres, whereas delayed cross-linking by factor XIII 34Val will allow for more lateral aggregation (Ariëns *et al*, 2000). A fibrin clot cross-linked by factor XIII 34Leu is characterized by reduced pore size and fibres with a reduced mass–length ratio. These changes in clot structure may affect the risk of thrombosis and haemorrhagic events.

At present, little is known about the effect of the laboratory phenotype associated with this polymorphism on the risk of venous thrombosis. In order to understand if and how the factor XIII Val34Leu polymorphism affects the risk of deep venous thrombosis, it is important to establish the relationship between this polymorphism and factor XIII protein levels or activity and their effect on thrombotic risk. The aim of this study was therefore to assess the relationship between factor XIII Val34Leu, factor XIII A and B subunit levels, factor XIII activity and D-dimer levels in a large case–control study, and to assess the effect of this genotype and the laboratory phenotypes on venous thrombotic risk.

MATERIALS AND METHODS

Study design. This study was part of a large population-based case–control study on risk factors for venous thrombosis, the Leiden Thrombophilia study (LETS). The design of this study has been described in detail previously (Koster *et al*, 1993; van der Meer *et al*, 1997). In brief, we included 474 consecutive patients younger than 70 years, with a first objectively diagnosed episode of deep venous thrombosis that occurred in the period between 1 January 1988 and 31 December 1992. Subjects were selected from the files of the anticoagulation clinics in Leiden, Amsterdam and Rotterdam. We also included 474 population control subjects who were friends or partners of the patients. The control subjects were of the same sex and approximately the same age (± 5 years) as the patients. Blood was collected from the antecubital vein into Sarstedt Monovette® tubes, in 0·1 volume 0·106 mol/l trisodium citrate. Plasma was prepared by centrifugation for 10 min at 2000 *g* at room temperature and stored at −70°C until used. High-molecular-weight DNA was isolated from leucocytes and stored at 4°C.

Factor XIII activity and subunit levels. The factor XIII activity and factor XIII A and B subunit antigen were measured as described previously (Ariëns *et al*, 1999). Factor XIII activity was measured with a microtitre assay using fibrinogen and 5-(biotinamido) pentylamine as substrates, adapted from a method previously described by Song *et al* (1994). The assay is based on the measurement of the amount of biotinylated pentylamine incorporated by factor XIII into fibrinogen-coated microtitre plates. The reaction was started by the addition of thrombin, calcium and 5-(biotinamido) pentylamine. After fixed time intervals (3 and 10 min), reactions were stopped by the addition of EDTA and wells were stained for immobilized biotin. This assay is sensitive both to changes in activation rate and to changes in specific activity of the transglutaminase formed during activation. It measures the increase of factor XIIIa cross-linking activity within a certain time interval after thrombin addition and therefore is not an activity test in the strictest sense. However, very similar or identical assays have been reported previously as factor XIII activity (Kangsadalampai & Board, 1998; Kohler *et al*, 1998b; Anwar *et al*, 1999) and we have followed this nomenclature.

Factor XIII A and B subunit levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA) (Ariëns *et al*, 1999). Both assays used the same polyclonal antibody against zymogen factor XIII tetramer as capture antibody. Each ELISA was then further developed with a polyclonal antibody specific for either the A or the B subunit (Diagnostica Stago Asnieres, France). The assays were specific for the A and B subunit; there was no cross-reactivity with the other subunit as tested with purified recombinant factor XIII subunits. The B subunit assay appeared equally sensitive to both free B subunits as well as to A_2B_2-subunit complex, as demonstrated by identical dose–response curves for pooled normal plasma and B antigen of a patient homozygous for a severe factor XIII A deficiency (kindly provided by Dr P. M. Mannucci). All results are expressed as the concentration in U/dl, where 1 U represents the amount of the analyte in 1 ml pooled normal plasma. For one patient, no information on factor XIII activity or the A and B subunit concentration was available.

D-dimer levels were measured by ELISA using two monoclonal antibodies against non-overlapping antigenic determinants (Declerck *et al*, 1987). D-dimer levels were expressed in ng/ml. For 10 patients and seven controls, no information was available on D-dimer levels.

Factor XIII A_2B_2-tetramer levels and dissociation index. A novel sandwich ELISA was developed to measure levels of the A_2B_2-subunit complex before and after partial activation with thrombin (Komanasin *et al*, 1999). The sugar moiety of a polyclonal anti-FXIII A IgG (Diagnostica Stago) was labelled with biotin [Biotin-ϵ-amino-caproyl hydrazide (BACH); Calbiochem, La Jolla, CA, USA], according to the method of O'Shannessy (1990). Microtitre plates (Nunc Maxisorp) were coated with anti-FXIII B IgG (also from Diagnostica Stago) diluted 1/600 in 50 mmol/l sodium carbonate pH 9·6. Non-specific binding sites were blocked with 150 μl 50 mmol/l Tris-HCl, 150 mmol/l NaCl, 1% bovine serum albumin (BSA), 0·01% NaN_3, pH 7·5. Plasma samples were diluted 1/400 in 50 mmol/l Tris-HCl, 150 mmol/l NaCl, 0·3% NaN_3, 0·3% BSA, pH 7·5, and a total of 100 μl was loaded into the wells in duplicate. After washing four times with 200 μl 50 mmol/l Tris-HCl, 150 mmol/l NaCl, 0·01% NaN_3, 0·1% Tween-20, pH 7·5, 100 μl of biotinylated anti-FXIII A IgG was added (1/500 dilution). Biotinylated antibody sandwiches were reacted with 100 μl of 1/500 streptavidin conjugated with alkaline

phosphatase (Sigma Chemicals, St Louis, USA) and bound conjugate was detected by addition of 100 μl of p-nitrophenol phosphate (Sigma Chemicals). Within 15 min of colour development, the reaction was stopped with 100 μl 4 mmol/l NaOH per well and absorbance was read at 405 and 550 nm. Levels of FXIII A_2B_2 complex were calculated from a standard curve of pooled normal plasma and expressed in U/dl. Intra-assay coefficient of variation (CV) was 5·2% ($n = 20$) and interassay CV was 6·8% ($n = 14$). The factor XIII A_2B_2-tetramer ELISA can be used to measure dissociation of the subunits and hence activation. Measurement of the complex before and after activation provided information on the percentage of the A_2B_2-subunit complex that was dissociated under influence of thrombin and calcium ions. Dissociation of the factor XIII A_2B_2 tetramer was analysed under controlled *in vitro* conditions that lead to, on average, 65% activation of factor XIII. Plasma samples were diluted 1:10 in 50 mmol/l Tris-HCl, 150 mmol/l NaCl, 0·3% NaN_3, 0·3% BSA, pH 7·5 containing 0·4% Gly-Pro-Arg-Pro-amide (Sigma Chemicals) to prevent inopportune polymerization of fibrin. Five microlitres of a mixture of 5 U/ml bovine thrombin (Sigma) and 500 mmol/l $CaCl_2$ was added to 50 μl of diluted plasma. The mixtures were incubated at room temperature for 30 min and the dissociation reaction was stopped with 10 volumes of 0·1 mol/l trisodium citrate. Samples were further diluted 1:4 and the remaining A_2B_2 complex was measured by ELISA as described above.

No data on the dissociation index were available for 10 individuals (three patients and seven controls).

Determination of genotypes. Factor XIII Val34Leu genotypes were identified using an allele-specific polymerase chain reaction (PCR) based on a method described by Saiki *et al* (1988). Two external primers (primer 1: forward primer, 5′-ATG TCA GAA ACT TCC AGG AC-3′; primer 2: reverse primer, 5′-CTG GAC CCA GAG TGG TGG-3′) and two internal forward (nested) primers (primer 3: forward primer G, 5′-CTG CCC ACA GTG GAG CTT CAG GCC G-3′; primer 4: forward primer T, 5′-CTG CCC ACA GTG GAG CTT CAG GCC T-3′) were designed. The two internal primers only differ in the last nucleotide, i.e. the G/T base variation (FXIII Val34Leu).

The 169 bp DNA fragment produced by the external primers serves as a template for either primer 3 or primer 4 and as an internal control of the PCR reaction. Use of either primer 3 or 4 as the third primer in a PCR resulted in the production of a 91 bp fragment when, respectively, a G or a T allele was present.

Amplification was carried out using 50 μl of sample in a programmable Thermal controller (PTC-100™; MJ Research, Watertown, MA, USA). Each sample contained 100–500 ng genomic DNA, 1·5 mmol/l of each dNTP, 67 mmol/l Tris-HCl pH 8·8, 16·6 mmol/l ammonium sulphate, 6·7 mmol/l MgCl, 10 mmol/l 2-mercaptoethanol, 100 μg/ml bovine serum albumin, 10% dimethylsulphoxide, 1·25 units of Amplitaq DNA polymerase and 325 ng of each primer for genotype determination. The solution was overlaid with mineral oil. A first denaturation step at 95°C for 4 min was followed by 34 cycles of 1 min of denaturation at 95°C, 1 min annealing at 61°C and 2 min extension at 67°C. Five microlitres of each amplification sample was loaded on a 2% agarose gel which was ethidium bromide stained. The factor XIII Val34Leu polymorphism was reported as Val/Val (G/G), Val/Leu (G/T) or Leu/Leu (T/T). For three patients, no information on factor XIII genotype was available.

Statistical analysis. Determinants of factor XIII activity and A and B subunit levels were evaluated by comparing means between groups, including only control subjects as reflecting the general population.

We investigated whether carrying the factor XIII 34Leu allele was associated with a decreased venous thrombotic risk by calculating odds ratios (OR) and their 95% confidence intervals (95% CI). To calculate the risk of venous thrombosis associated with increased factor XIII activity or increased subunit levels, we used the 90th (P90) percentile measured in the control subjects as a cut-off point. The P90 of factor XIII activity was 158·9 U/dl, of the A subunit level P90 was 137·0 U/dl and of the B subunit level P90 was 125·0 U/dl.

We compared factor XIII activity with the results of the dissociation index in the control subjects, to provide information on the activation patterns of the different Val34Leu genotypes.

As D-dimer, a fibrin degradation product, indicates the activation of both the fibrinolytic as well as the coagulation system, we included D-dimer levels in the analysis to assess the overall effect of the factor XIII genotype on fibrin formation and dissociation.

RESULTS

The mean age of the patients and the controls at the time of the thrombosis was 45 years (range patients 15–69, controls 15–72). Among both patients and controls, 57% were women. The frequency of the Leu allele was 0·22 in the patients and 0·24 in the control subjects. The distribution of the factor XIII Val34Leu in the control subjects was according to the Hardy–Weinberg equilibrium.

Factor XIII activity and subunit levels

In Table I, the results of the factor XIII activity and A and B subunit levels, measured in the healthy control subjects, are shown by genotype, age, sex and oral contraceptive use. Factor XIII activity was positively associated with the Leu allele and increasing age, but not with oral contraceptive use and sex [linear regression coefficients with factor XIII activity as dependent variable: FXIII genotype (0 = Val/Val, 1 = Val/Leu, 2 = Leu/Leu) β: 33·9 (95%CI: 29·9–37·8), Age (per 10 years increase) β: 2·8 (95%CI: 0·6–5·0), oral contraceptive use (0 = no, 1 = yes) β: 10·5 (95%CI: −0·9–21·9), sex (0 = female, 1 = male) β: 1·1 (95%CI: −5·0–7·2)]. The effect of the genotype was pronounced, with more than 60% higher levels for the Leu/Leu genotype (mean: 158·0 U/dl) compared with the Val/Val genotype (mean: 95·0 U/dl). The A and B subunit levels were positively associated with increasing age, but not with sex, genotype or oral contraceptive use [linear regression coefficients with

Table I Mean (SD) of factor XIII activity and factor XIII A and B subunit levels (U/dl) measured in 474 healthy control subjects

		Activity	A subunit	B subunit
Total control group		111 8 (33 4)	107 6 (23 3)	100 1 (18 5)
Genotype				
GG (Val/Val)	(*n* = 273)	95 0 (23 6)	109 7 (23 8)	100 3 (18 6)
GT (Val/Leu)	(*n* = 174)	131 1 (29 9)	104 1 (20 7)	99 6 (18 1)
TT (Leu/Leu)	(*n* = 27)	158 0 (29 6)	108 5 (30 9)	101 9 (21 2)
Sex				
Male	(*n* = 202)	112 5 (31 9)	108 7 (23 0)	101 9 (19 4)
Female	(*n* = 272)	111 4 (34 5)	106 8 (23 5)	98 8 (17 8)
Age (years)				
< 30	(*n* = 77)	102 2 (30 2)	95 6 (21 0)	92 0 (16 0)
30–50	(*n* = 224)	113 1 (34 1)	107 8 (23 8)	98 4 (18 0)
≥ 50	(*n* = 173)	114 5 (33 3)	112 6 (21 6)	106 0 (18 5)
Oral contraceptive use*				
Yes	(*n* = 54)	116 6 (34 6)	100 8 (22 2)	96 8 (15 5)
No	(*n* = 99)	106 0 (33 9)	103 0 (24 6)	93 4 (17 0)

*At the time of the venepuncture (women aged 15–49 years who were not pregnant not within 30 d postpartum did not have a recent miscarriage and did not use depot contraceptives)

factor XIII A subunits as dependent variable age (per 10 years increase) β 3 9 (95%CI 2 4–5 4), sex β 1 9 (95%CI −2 3–6 2), FXIII Val34Leu −3 2 (95%CI −6 6–0 3), oral contraceptives β −2 2 (95%CI −10 2–5 7), linear regression coefficients with factor XIII B subunits as dependent variable age (per 10 years increase) β 3 7 (95%CI 2 5–4 8), sex β 3 2 (95%CI −0 2–6 5), FXIII Val34Leu 0 0 (95%CI −2 7–2 8), oral contraceptives β 3 4 (95%CI −2 1–8 9)]

Risk of deep venous thrombosis

Table II shows the risk of venous thrombosis for individuals who carried the factor XIII 34Leu allele compared with individuals homozygous for the 34Val allele The risk of deep venous thrombosis was reduced by 10% for heterozygous carriers of the factor XIII 34Leu allele [OR 0 9 (95% CI 0 7–1 2)] and by 30% for individuals homozygous for the 34Leu allele [OR 0 7 (95% CI 0 4–1 3)] This effect stood out more clearly with increasing age and was completely restricted to men

Using the 90th percentile measured in the control subjects as a cut-off point for factor XIII activity and A and B subunit levels, we calculated the risk of deep venous thrombosis associated with increased factor XIII activity and increased subunit levels Individuals with high factor XIII activity had a slightly lower risk of thrombosis compared with individuals with factor XIII activity below the cut-off value [factor XIII activity > P90 OR 0 8 (95% CI 0 5–1 3), factor XIII activity > P95 OR 0 3 (95% CI 0 1–0 7)] Increased A and B subunit levels (> P90) were associated with a weak increase in thrombotic risk [$OR_{A\ subunit}$ 1 4 (95%CI 0 9–2 2), $OR_{B\ subunit}$ 1 6 (95%CI 1 1–2 4)]

Table III shows the associations of the factor XIII genotypes and factor XIII activity with the A_2B_2-dissociation index Individuals with the factor XIII 34Leu allele had a higher dissociation index i e in these individuals

Table II Risk of deep venous thrombosis (OR 95% CI) for factor XIII 34Leu carriers

		GT (Val/Leu)*	TT (Leu/Leu)*	GT + TT*
Total study population	(*n* = 945)	0 9 (0 7–1 2)	0 7 (0 4–1 3)	0 9 (0 7–1 1)
Sex				
Male	(*n* = 404)	0 8 (0 5–1 2)	0 4 (0 2–1 2)	0 7 (0 5–1 1)
Female	(*n* = 541)	1 0 (0 7–1 4)	0 9 (0 4–2 0)	1 0 (0 7–1 4)
Age (years)				
< 30	(*n* = 159)	1 3 (0 7–2 5)	1 0 (0 2–5 3)	1 2 (0 7–2 3)
30–50	(*n* = 442)	0 9 (0 6–1 3)	0 8 (0 3–1 8)	0 9 (0 6–1 3)
≥ 50	(*n* = 344)	0 8 (0 5–1 2)	0 6 (0 2–1 5)	0 7 (0 5–1 1)

*All ORs calculated with factor XIII Val/Val (wild type) as a reference category

Table III Mean (SD) of levels of the factor XIII A_2B_2-subunit complex before and after thrombin activation and the factor XIII dissociation index measured in 467 healthy control subjects (no plasma samples available for seven control subjects)

		A_2B_2-subunit complex before thrombin activation (U/dl)	A_2B_2-subunit complex after thrombin activation (U/dl)	Factor XIII dissociation index [(before–after)/before] (% dissociated after thrombin activation)
Genotype				
GG (Val/Val)	(n = 271)	116 4 (35 4)	50 2 (18 7)	55 7 (13 1)
GT (Val/Leu)	(n = 169)	108 3 (30 5)	34 5 (12 2)	67 1 (10 6)
TT (Leu/Leu)	(n = 27)	111 8 (41 0)	26 0 (9 9)	74 7 (9 5)
FXIII activity				
< 100 0	(n = 194)	106 4 (32 3)	46 4 (17 3)	55 4 (12 9)
100 0–125 0	(n = 131)	113 7 (35 2)	44 8 (20 0)	60 4 (13 2)
> 125 0	(n = 142)	122 1 (34 1)	37 2 (16 5)	69 0 (10 9)

more A_2B_2-subunit complex was dissociated under controlled *in vitro* activation conditions (see *Materials and methods*) Higher factor XIII activity was associated with an increased dissociation index (linear regression coefficient with factor XIII activity as dependent variable β 1 1, 95% CI 0 9–1 3)

Individuals homozygous Leu/Leu had slightly lower D-dimer levels compared with individuals with the wild-type genotype Median D-dimer levels measured in the control group were GG 75 3 (range 4 0–1608 9), GT 75 8 (range 16 5–1229 5), TT 69 5 (range 22 3–194 0)

DISCUSSION

We studied the relationship between factor XIII Val34Leu and factor XIII activity and subunit levels in a large case–control study Our aim was to classify the association between factor XIII Val34Leu and factor XIII laboratory phenotypes, and their effect on thrombotic risk This would provide more insight into the effect of this polymorphism on the risk of venous thrombosis

Whereas there was a strong association between factor XIII Val34Leu and factor XIII activity, we found no effect of factor XIII Val34Leu genotypes on A and B subunit levels These results confirmed that Val34Leu does not have an effect on circulating factor XIII subunit levels and were in agreement with earlier studies (Ariens *et al*, 2000, Balogh *et al*, 2000, Wartiovaara *et al*, 2000) that reported an increased factor XIII activation rate associated with the factor XIII 34Leu allele Apparently, under our assay conditions, the increased activation of factor XIII 34Leu contributed to the factor XIII activity Upon full activation by thrombin, however, both forms of factor XIII show equal specific cross-linking activity (Ariens *et al*, 2000, Wartiovaara *et al*, 2000) The pentylamine incorporation assay, as it is routinely used to measure factor XIII activity levels, appeared to be very sensitive to changes in the kinetics of the activation of factor XIII by thrombin and, therefore, to the Val34Leu polymorphism It was shown that increased factor XIII activity was associated with a slightly decreased risk of thrombosis [OR 0 8 (95% CI 0 5–1 3)] that was of the same magnitude as the decreased risk associated with factor XIII 34Leu allele carriers The thrombotic risk associated with elevated levels of both the A and the B subunit levels was not decreased but even slightly increased

Individuals with the factor XIII 34Leu allele had a higher factor XIII dissociation index under conditions of controlled *in vitro* activation of factor XIII These results indicate that, under these conditions, in individuals homozygous for factor XIII 34Leu more A_2B_2-tetramer was dissociated under these conditions, than in individuals homozygous for factor XIII 34Val When we analysed the A_2B_2-subunit complex before and after thrombin activation and the dissociation index in relation to the three categories of factor XIII activity (Table III), we found that higher activity was associated with a higher dissociation index These results confirmed that the increased activity in individuals homozygous for the Leu allele appeared to be caused by an increased activation rate of the factor XIII 34Leu variant by thrombin

We found a slightly decreased risk of venous thrombosis for individuals with the factor XIII 34Leu allele compared with individuals homozygous for the wild-type 34Val allele, but the effects were small, with no more than a 10% risk reduction for heterozygous factor XIII 34Leu carriers [OR 0 9 (95% CI 0 7–1 2)] The effect was more apparent in individuals homozygous for the 34Leu allele [OR 0 7 (95% CI 0 4–1 3)]

A number of studies with varying results on the effect of the factor XIII Leu allele have been reported Several studies reported a protective effect of the 34Leu allele on the risk of deep venous thrombosis (Catto *et al*, 1999, Alhenc-Gelas *et al*, 2000, Renner *et al*, 2000), while others reported no effect (Balogh *et al*, 2000, Corral *et al*, 2000, Margaglione *et al*, 2000) In a study by Franco *et al* (1999) only individuals homozygous for the factor XIII 34Leu allele had a decreased thrombotic risk

The weak nature of the protective effect of FXIII Val34Leu means that even in a large study such as ours, we cannot confidently exclude the absence of an effect and, similarly, the studies that did not see protection (Balogh *et al*, 2000, Corral *et al*, 2000, Margaglione *et al*, 2000) could not exclude a small effect Unexpectedly, the

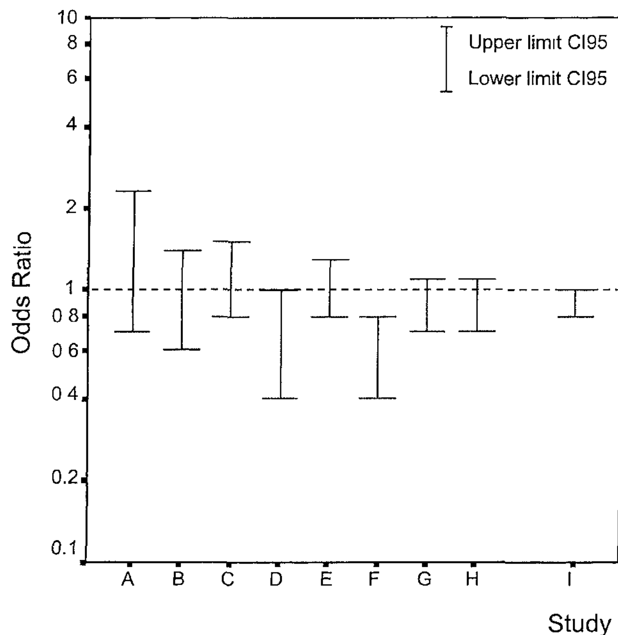

Fig 1. Comparison of odds ratios and 95% confidence intervals for the total group of carriers of the Leu allele (GT + TT) compared with the wild-type genotype (GG). Studies: (A) Corral *et al* (2000), patients $n = 97$, controls $n = 97$; (B) Franco *et al* (1999), patients $n = 189$, controls $n = 187$; (C) Balogh *et al* (2000), patients $n = 273$, controls $n = 288$; (D) Renner *et al* (2000), patients $n = 154$, controls $n = 308$; (E) Margaglione *et al* (2000), patients $n = 427$, controls n = 1045; (F) Catto *et al* (1999), patients $n = 217$, controls $n = 252$; (G) PATHROS, Alhenc-Gelas *et al* (2000), patients $n = 354$, controls $n = 1229$; (H) LETS, patients $n = 471$, controls $n = 474$; (I) Overall risk estimate of all eight studies, OR Mantel–Haenszel.

protective effect we found was restricted to men. This difference in effect of factor XIII Val34Leu between sexes remains unexplained.

As a number of studies with varying results on the effect of factor XIII Val34Leu on the risk of deep venous thrombosis have been published, we proceeded to calculate an overall risk estimate using the Mantel–Haenszel procedure (Fig 1). We performed a PubMed search on the keywords 'Val34Leu' or 'Val 34 Leu' and 'thrombosis'. In total, we found 15 articles of which nine contained data from studies on venous thrombosis. We selected only those articles in which a risk associated with the factor XIII 34Leu allele compared with the factor XIII wild-type genotype was reported or when we could calculate the risk from the data in the paper. One study only included individuals carrying the factor V Leiden mutation (Franco *et al*, 2000b) and was therefore excluded. The studies that were included for this calculation were: Catto *et al* (1999), Franco *et al* (1999), Alhenc-Gelas *et al* (2000), Balogh *et al* (2000), Corral *et al* (2000), Margaglione *et al* (2000), Renner *et al* (2000), and the results of the current study (LETS). The pooled risk estimates were similar to the results found in the current study: OR_{MH} (Val/Leu): 0·9 (95% CI: 0·8–1·0); OR_{MH} (Leu/Leu): 0·8 (95% CI: 0·6–1·0); OR_{MH} (Val/Leu + leu/Leu): 0·9 (95% CI: 0·8–1·0).

The design of the studies used in this calculation differed in some points. Some studies included both patients with deep venous thrombosis as well as patients with pulmonary embolism, while others only included patients with deep venous thrombosis. Another difference was that in some studies various patient groups with venous thrombosis were included (Catto *et al*, 1999; Franco *et al*, 1999; Alhenc-Gelas *et al*, 2000; Corral *et al*, 2000; Renner *et al*, 2000), while in other studies patients were selected on thrombophilia screening or family history (Balogh *et al*, 2000; Margaglione *et al*, 2000). Preferably, a meta-analysis includes studies with a similar design. When only the studies with various patient groups were included in the meta-analyses, the pooled effect became more pronounced: OR_{MH} (Val/Leu): 0·8 (95% CI: 0·7–1·0); OR_{MH} (Leu/Leu): 0·6 (95 CI: 0·4–0·8); OR_{MH} (Val/Leu + leu/Leu): 0·8 (95 CI: 0·7–0·9). It may be that a mild protective effect caused by the factor XIII 34Leu allele was no longer observed when other thrombotic risk factors were present in these selected patients with suspected familial thrombophilia.

In the present study, we found a weak protective effect on the risk of venous thrombosis of the factor XIIIVal34Leu polymorphism. This effect was somewhat more pronounced in the results of the meta-analysis. This effect appeared to be restricted to men. Additionally the 34Leu phenotype was associated with increased factor XIII cross-linking activity and enhanced dissociation of the factor $XIIIA_2B_2$ complex. These results support the hypothesis that a protective effect of factor XIII 34Leu is mediated through a laboratory phenotype of an increased activation rate. The risk estimates we calculated were all minor compared with other risk factors for thrombosis and evaluation of this genotype will have no relevance for clinical care.

The factor XIII 34Leu genotype was associated with slightly lower D-dimer levels. D-dimer is a specific degradation product of cross-linked fibrin. Lower D-dimer levels could be the result of reduced fibrinolysis caused by a clot which is more lysis resistant. As the factor XIII 34Leu genotype appeared to be associated with a weak protective effect on the risk of venous thrombosis, this does not seem likely. The lower D-dimer levels associated with the factor XIII 34Leu allele could also be the result of reduced fibrin formation. The results of this study would support this view, although further *in vitro* studies are required to evaluate this point.

ACKNOWLEDGMENTS

We thank Dr T. Koster for collecting blood samples of patients and control subjects, Ank Schreijer and Ingeborg de Jonge for performing data management, and Dr M. Cushman (Departments of Medicine and Pathology, University of Vermont, VT, USA) for the measurements of D-dimer levels. We also thank all participants of the Leiden Thrombophilia Study for their co-operation. The LETS study

was financially supported by The Netherlands Heart Foundation (grant no. 89.063).

REFERENCES

Alhenc-Gelas, M., Reny, J., Aubry, M., Aiach, M. & Emmerich, J. (2000) The FXIII Val 34 Leu mutation and the risk of venous thrombosis. *Thrombosis and Haemostasis*, **84**, 1117–1118.

Anwar, R., Gallivan, L., Edmonds, S.D. & Markham, A.F. (1999) Genotype/phenotype correlations for coagulation factor XIII: specific normal polymorphisms are associated with high or low factor XIII specific activity. *Blood*, **93**, 897–905.

Ariëns, R.A., Kohler, H.P., Mansfield, M.W. & Grant, P.J. (1999) Subunit antigen and activity levels of blood coagulation factor XIII in healthy individuals. Relation to sex, age, smoking, and hypertension. *Arteriosclerosis Thrombosis and Vascular Biology*, **19**, 2012–2016.

Ariëns, R.A., Philippou, H., Nagaswami, C., Weisel, J.W., Lane, D.A. & Grant, P.J. (2000) The factor XIII Val34Leu polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. *Blood*, **96**, 988–995.

Balogh, I., Szôke, G., Kárpáti, L., Wartiovaara, U., Katona, É., Komáromi, I., Haramura, G., Pflieger, G., Mikkola, H. & Muszbek, L. (2000) Val34Leu polymorphism of plasma factor XIII: biochemistry and epidemiology in familial thrombophilia. *Blood*, **96**, 2479–2486.

Catto, A.J., Kohler, H.P., Bannan, S., Stickland, M., Carter, A. & Grant, P.J. (1998) Factor XIII Val 34 Leu: a novel association with primary intracerebral hemorrhage. *Stroke*, **29**, 813–816.

Catto, A.J., Kohler, H.P., Coore, J., Mansfield, M.W., Stickland, M.H. & Grant, P.J. (1999) Association of a polymorphism in the factor XIII gene with venous thrombosis. *Blood*, **93**, 906–908.

Corral, J., Gonzalez-Conejero, R., Iniesta, J.A., Rivera, J., Martinez, C. & Vicente, V. (2000) The FXIII Val34Leu polymorphism in venous and arterial thromboembolism. *Haematologica*, **85**, 293–297.

Declerck, P.J., Mombaerts, P., Holvoet, P., De Mol, M. & Collen, D. (1987) Fibrinolytic response and fibrin fragment D-dimer levels in patients with deep vein thrombosis. *Thrombosis and Haemostasis*, **58**, 1024–1029.

Elbaz, A., Poirier, O., Canaple, S., Chédru, F., Cambien, F. & Amarenco, P. (2000) The association between the Val34Leu polymorphism in the factor XIII gene and brain infarction. *Blood*, **95**, 586–591.

Franco, R.F., Reitsma, P.H., Lourenco, D., Maffei, F.H., Morelli, V., Tavella, M.H., Araujo, A.G., Piccinato, C.E. & Zago, M.A. (1999) Factor XIII Val34Leu is a genetic factor involved in the etiology of venous thrombosis. *Thrombosis and Haemostasis*, **81**, 676–679.

Franco, R.F., Pazin-Filho, A., Tavella, M.H., Simoes, M.V., Marin-Neto, J.A. & Zago, M.A. (2000a) Factor XIII val34leu and the risk of myocardial infarction. *Haematologica*, **85**, 67–71.

Franco, R.F., Middeldorp, S., Meinardi, J.R., van Pampus, E.C. & Reitsma, P.H. (2000b) Factor XIII Val34Leu and the risk of venous thromboembolism in factor V Leiden carriers. *British Journal of Haematology*, **111**, 118–121.

Kangsadalampai, S. & Board, P.G. (1998) The Val34Leu polymorphism in the A subunit of coagulation factor XIII contributes to the large normal range in activity and demonstrates that the activation peptide plays a role in catalytic activity. *Blood*, **92**, 2766–2770.

Kohler, H.P., Stickland, M.H., Ossei-Gerning, N., Carter, A., Mikkola, H. & Grant, P.J. (1998a) Association of a common polymorphism in the factor XIII gene with myocardial infarction. *Thrombosis and Haemostasis*, **79**, 8–13.

Kohler, H.P., Ariëns, R.A., Whitaker, P. & Grant, P.J. (1998b) A common coding polymorphism in the FXIII A-subunit gene (FXIIIVal34Leu) affects cross-linking activity. *Thrombosis and Haemostasis*, **80**, 704.

Komanasin, N., Futers, T.S., Ariëns, R.A.S. & Grant, P.J. (1999) A novel polymorphism in the factor XIII B subunit (His95Arg) relates to the dissociation of the A_2B_2 tetramer. *Thrombosis and Haemostasis*, **82**, 111a.

Koster, T., Rosendaal, F.R., de Ronde, H., Briët, E., Vandenbroucke, J.P. & Bertina, R.M. (1993) Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet*, **342**, 1503–1506.

Lane, D.A. & Grant, P.J. (2000) Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood*, **95**, 1517–1532.

Margaglione, M., Bossone, A., Brancaccio, V., Ciampa, A. & Di Minno, G. (2000) Factor XIII Val34Leu polymorphism and risk of deep vein thrombosis. *Thrombosis and Haemostasis*, **84**, 1118–1119.

van der Meer, F.J., Koster, T., Vandenbroucke, J.P., Briët, E. & Rosendaal, F.R. (1997) The Leiden Thrombophilia Study (LETS). *Thrombosis and Haemostasis*, **78**, 631–635.

Mikkola, H., Syrjala, M., Rasi, V., Vahtera, E., Hamalainen, E., Peltonen, L. & Palotie, A. (1994) Deficiency in the A-subunit of coagulation factor XIII: two novel point mutations demonstrate different effects on transcript levels. *Blood*, **84**, 517–525.

O'Shannessy, D.J. (1990) Antibodies biotinylated via sugar moieties. *Methods in Enzymology*, **184**, 162–166.

Renner, W., Köppel, H., Hoffmann, C., Schallmoser, K., Stanger, O., Toplak, H., Wascher, T.C. & Pilger, E. (2000) Prothrombin G20210A, Factor V Leiden, and Factor XIII Val34Leu: Common mutations of blood coagulation factors and deep vein thrombosis in Austria. *Thrombosis Research*, **99**, 35–39.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. & Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239**, 487–491.

Song, Y.C., Sheng, D., Taubenfeld, S.M. & Matsueda, G.R. (1994) A microtitre assay for factor XIII using fibrinogen and biotinylcadaverine as substrates. *Analytical Biochemistry*, **223**, 88–92.

Suzuki, K., Henke, J., Iwata, M., Henke, L., Tsuji, H., Fukunaga, T., Ishimoto, G., Szekelyi, M. & Ito, S. (1996) Novel polymorphisms and haplotypes in the human coagulation factor XIII A-subunit gene. *Human Genetics*, **98**, 393–395.

Wartiovaara, U., Perola, M., Mikkola, H., Tötterman, K., Savolainen, V., Penttilä, A., Grant, P.J., Tikkanen, M.J., Vartiainen, E., Karhunen, P.J., Peltonen, L. & Palotie, A. (1999) Association of FXIII Val34Leu with decreased risk of myocardial infarction in Finnish males. *Atherosclerosis*, **142**, 295–300.

Wartiovaara, U., Mikkola, H., Szôke, G., Haramura, G., Kárpáti, L., Balogh, I., Lassila, R., Muszbek, L. & Palotie, A. (2000) Effect of Val34Leu polymorphism on the activation of the coagulation factor XIII-A. *Thrombosis and Haemostasis*, **84**, 595–600.