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Factor X Levels, Polymorphisms in the Promoter Region of Factor X, and the Risk of Venous Thrombosis

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Key words

Factor X, venous thrombosis, polymorphisms

Summary

Elevated levels of procoagulant proteins factor II, factor VIII, factor IX, factor XI and fibrinogen are associated with an increased risk of venous thrombosis. In a population-based case-control study on venous thrombosis (Leiden Thrombophilia Study, LETS) we investigated whether elevated coagulation factor X (FX) levels are a risk factor for venous thrombosis and whether FX levels are determined by polymorphisms in the promoter region of the FX gene. We found that subjects with high FX levels (above the 90th percentile, ≥ 126 U/dl) had a 1.6-fold increased risk of venous thrombosis. The highest risk (OR = 4.3, 95% confidence interval: 1.5-12) was found in the subgroup of premenopausal women who are not using oral contraceptives. However, these estimated risks disappeared after adjustment for other vitamin K-dependent coagulation factors II, VII and IX. To study the influence of genotypic variation on plasma FX levels we assessed four polymorphisms in the promoter region of the FX gene: a TTGTGA insertion between position -343A and -342G, a C/T polymorphism at position -222, a C/A polymorphism at position -220 and a C/T polymorphism at position -40. No relationship between these investigated genotypes and FX levels was observed. We conclude that high FX levels predict risk of thrombosis, but are not a risk factor for venous thrombosis when the levels of other vitamin K-dependent proteins are taken into account.

Introduction

During the past years the search for causes of deep-vein thrombosis has led to the identification of a number of new risk factors, which can be genetic or acquired (for a review see ref. 1). The development of a deep-vein thrombosis may in theory be the result of local disturbances in the hemostatic balance due to enhanced procoagulant, reduced anticoagulant or reduced fibrinolytic activity. Reduced anticoagulant activity resulting from deficiencies of antithrombin (2), protein C (3) and protein S (4) and resistance to activated protein C (5) [in the presence (6-8) or absence of factor V Leiden (9)] increases the risk of venous thrombosis. Elevated levels of procoagulant factors II (10), VIII (11), IX (12), XI (13) and fibrinogen (14) have also been shown to lead to an increased thrombosis risk.

Factor X (FX), the zymogen of a vitamin K-dependent serine protease, plays a central role in the coagulation cascade (for a review see ref. 15). It can be activated either by the intrinsic contact-activated pathway or by the extrinsic tissue factor pathway. Activated FX (FXa) in association with its cofactor activated factor V (FVa) catalyzes the conversion of prothrombin into thrombin in the presence of phospholipids and calcium ions (16-18). Thrombin in turn converts fibrinogen into insoluble fibrin. FXa is inhibited by antithrombin with which it forms a stable inactive complex. Furthermore, prothrombinase activity is regulated by activated protein C, which inactivates FVa and FVIIIa. The main inhibitor of the extrinsic pathway is the factor Xa-dependent tissue factor pathway inhibitor (TFPI). FX deficiency results in a serious bleeding disorder which points to an essential role for FX in hemostasis (15).

The gene for FX is located on chromosome 13q34-qter in close proximity to the gene for FVII and spans approximately 25 kilobases, containing 8 exons (19). The overall domain structure of FX is highly homologous to that of the other vitamin K-dependent coagulation factors. Expression of the FX gene occurs primarily in the liver (20, 21).

In this study we investigated whether elevated FX levels are associated with thrombosis risk. Furthermore, we examined four polymorphisms in the promoter region of the FX gene and their relation with FX levels, as there have been a number of reports which showed genetically determined differences in plasma levels for some components of the blood coagulation system [i.e. factor VII (22), fibrinogen (23), protein C (24), factor II (10), PAI-1 (25)]. This study was part of a large population based case-control study on venous thrombosis, the Leiden Thrombophilia Study (LETS).

Patients and Methods

Subjects

The design of our population-based case-control study (Leiden Thrombophilia Study, LETS) has been described previously (26). Patients with a first episode of deep-vein thrombosis were selected from the files of three anticoagulation clinics in the Netherlands. All patients were younger than 70 years and were not known to have a malignant disorder. Acquaintances and partners of

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the patients served as controls. The study included 474 patients and 474 age- and sex-matched healthy controls. The ratio of men to women was 1:1.3 and mean age was 47 years for both patients (range, 16 to 70 years) and controls (range, 16 to 73 years). Venepuncture took place at least six months after the thrombotic event.

Forty-eight of the patients and one of the control subjects were on long term oral anticoagulant therapy at the time of the venepuncture. For analyses concerning FX levels, these 49 subjects were excluded.

To investigate the relationship between FX levels and oral contraceptive use, an additional selection was made, as described before (27, 28). Premenopausal women aged 15-49 were selected. Women who were pregnant, within 30 days post-partum, with a recent miscarriage or who had used only depot contraceptives at the index date, were excluded.

Blood Collection

Venous blood was collected into tubes containing 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. High molecular weight DNA was isolated from leukocytes by standard methods and stored at 4° C.

Factor X Antigen Measurements

Factor X antigen (FXag) levels were measured by enzyme linked immunosorbent assay (ELISA). Plastic 96-well microtiter plates were coated with rabbit anti-human FX polyclonal antibodies (DAKO A/S, Glostrup, Denmark; 4 µg/ml, 100 µl/well) during an overnight incubation at 4° C. One hundred microliter of diluted plasma sample (dilution buffer: 50 mM TEA pH 7.5, 100 mM NaCl, 10 mM EDTA, 0.1% Tween-20) was added to the wells and plates were incubated at room temperature for 2 h. Subsequently, 100 µl of a 1:1500 dilution of peroxidase-conjugated rabbit anti-human FX (DAKO A/S, Glostrup, Denmark) was added to the wells. After 2 h incubation at room temperature, the plate was incubated with substrate buffer (0.1 M sodium acetate pH 5.5, 0.1 mg/ml tetramethyl-benzidine, H_2O_2, 100 µl/well). After 10 min the color reaction was stopped by adding 2 M H_2SO_4 (100 µl/well) and read spectrophotometrically at 450 nm. Between all incubation steps, the wells were washed 5 times with dilution buffer. A calibration curve was obtained using 1:200 to 1:12800 dilutions of pooled normal plasma. FXag level of a plasma sample was calculated as the mean result of the measurements of three different independent dilutions (1:800, 1:1600, 1:3200). FXag levels were expressed in units per deciliter (U/dl). By definition 1 ml pooled normal plasma contains 1 unit. Results were accepted when the coefficient of variation (CV) was less than 10%, otherwise the measurement was repeated. Under these conditions the interassay CV was 3.2% (n = 42) at a FXag level of about 100 U/dl and 7.0% (n = 42) at a level of about 40 U/dl. At a FXag level of about 100 U/dl the intra-assay CV was 2.7% (n = 26). The ELISA is specific for FX and no crossreactivity with other vitamin K-dependent proteins was detected (FX deficient plasma gives no signal).

Measurements of factor VII, factor II and factor IX levels have been described before (10, 12, 14). Factor II activity was measured with a chromogenic method using S-2238 (Chromogenix, Mölndal, Sweden) and *Echis carinatus* snake venom (Sigma Chemical Co, St. Louis, MO) on an ACL-200 (10). Factor VII was measured using Thromborel S reagent (Behringwerke AG, Warburg, Germany) and FVII deficient plasma (Organon Teknica, Durham, USA) (14). Factor IX was measured by an ELISA using commercial rabbit antifactor IX antibodies as capture antibodies (DAKO A/S, Glostrup, Denmark). Bound FIX was detected with non Ca^{2+}-dependent antifactor IX IgG (29) conjugated to horseradish peroxidase (12).

Genetic Analysis

To perform a genetic analysis of four polymorphisms in the promoter region of the factor X gene: a TTGTGA insertion between position -343A and -342G [numbering according to Miao et al (30)], a C/T polymorphism at position -222, a C/A polymorphism at position -220 and a C/T polymorphism at position -40 (31), we designed 4 sets of oligonucleotide primers, derived from the genomic sequence (Table 1) (30,32). Using these primers, DNA fragments were amplified by polymerase chain reaction (PCR). Amplification with primers 96-348 and 96-349 resulted in a 269 bp fragment, when the TTGTGA insertion is absent and in a 275 bp fragment, when this insertion is present. These fragments could be distinguished on gel. In heterozygotes an additional heteroduplex band was observed. Amplification with primers 96-824 and 96-360 introduced an *AlwNI* site (-CAGNNN/CTG-) in the amplified DNA fragment when a T is present at position -222. The resulting 149 bp PCR fragment was cut by *AlwNI* into fragments of 120 and 29 bp. Amplification with primers 96-364 and 96-823 introduced a *Cac8I* site (-GCN/NGC-) in the amplified DNA fragment when a C is present at position -220. In this instance the 148 bp PCR fragment was cut by *Cac8I* into fragments of 120 and 28 bp. With primers FXupstr and 96-355 a 211 bp fragment was amplified. Digestion with *AluI* resulted in fragments of 136 and 75 bp in case of a -40 C allele and in fragments of 110, 75 and 26 bp in case of a -40 T allele.

Statistical Analysis

Odds ratios (OR) were calculated in the standard unmatched fashion. Ninety-five percent confidence intervals (95% CI) were constructed according to Woolf (33). We adjusted for the matching variables age and sex as well as for putative confounders (oral contraceptive use, vitamin K-dependent clotting

Table 1 Sequence of the synthetic oligonucleotides used for amplification of parts of the promoter region of the factor X gene

Code	Sequence (5'→ 3')	Nucleotide numbering (30)	Polymorphic site detection	Restriction enzyme
FXupstr	CTGCCTCGCCAGCAGGTCT	-144 to –126	-40 (C/T)	AluI
96-355	ACTTTCCCCGAGCAGCAGGAG	+69 to +49		
96-348	TGATACCACCCAGAGAGTGGC	-526 to –506	Insertion between –343A and -342G (TTGTGA)	none
96-349	TGTGGGGACTTTCTGTTCTA	-258 to –277		
96-364	CTGAGAGTTAAATGTAAACATCTGG	-338 to –314	-220 (C/A)	Cac8I
96-823	CAGGCAGAGCTGGGCGCCAGAGAGGGCC	-191 to –218		
96-824	CGGCCCTGGTGACTGATGAGGATCAGG	-252 to -226	-222 (C/T)	AlwNI
96-360	TTAGGCCTCCTGATTGGAGCCG	-104 to –125		

The underlined nucleotides in the mutagenic primer sequences deviate from the normal sequence

factors FII, FVII and FIX) by unconditional logistic regression. FII, FVII and FIX levels were entered into the logistic model as categorized variables (dichotomized at the 90th percentile). Entering of FII, FVII and FIX levels into the logistic model as continuous variables gave similar results. The OR is used as a measure of the relative risk, which indicates the risk of developing venous thrombosis in a category of exposure relative to the reference category. An OR of 1 indicates no effect on risk, while an OR above 1 indicates an increase in risk.

Results

Variables Affecting Factor X Levels

In the healthy control group putative determinants of FX levels were investigated. The mean FX level in the 473 controls was 103.5 U/dl (range, 49 to 163). Because FX levels are known to be influenced by oral contraceptive use (34), mean FX levels were calculated for subgroups according to oral contraceptive use. In men, the mean FX level was 103.6 U/dl (95% CI: 102-106, n = 201). Similar FX levels were detected in postmenopausal women (mean 104.4 U/dl, 95% CI: 101 to 108, n = 90). Premenopausal women who were not using oral contraceptives (-OC) at venepuncture time had a mean FX level of 95.1 U/dl (95% CI: 92-98, n = 99). Highest FX levels were found in premenopausal women who were using oral contraceptives (+OC) (mean 118.0 U/dl: 95% CI: 113-123, n = 54). No relationship between age and FX levels was found.

Factor X Levels and Risk of Venous Thrombosis

The 90th percentile of FX levels in controls was 126 U/dl. Sixteen percent of the patients and, by definition, 10% of the controls had a FX level above this cut-off value, indicating a 1.6-fold increased risk of venous thrombosis for subjects with a FX level ≥ 126 U/dl compared with subjects with levels below this cut-off (crude OR = 1.6, 95% CI: 1.1-2.4, Table 2). Adjustment for age and sex did not change this OR. Adjustment for age, sex and oral contraceptive use resulted in an OR of 1.8 (95% CI: 1.2-2.7). Additional correction for other vitamin K-dependent coagulation proteins (FII, FVII and FIX, all dichotomized at the 90th percentile) reduced the OR to 1.2 (95% CI: 0.8-1.9). In this same logistic model (sex, age, oral contraceptive use and vitamin K-dependent proteins FII, FVII, FIX and FX, all dichotomized at the 90th percentile) the adjusted ORs for the other vitamin K-dependent proteins were 1.4 (95% CI: 0.9-2.2) for high FII levels (above 90th percentile, ≥ 123 U/dl), 1.3 (95% CI: 0.8-2.0) for high FVII levels (above 90th percentile, ≥ 139 U/dl) and 2.3 (95% CI: 1.5-3.5) for high FIX levels (above 90th percentile, ≥ 130 U/dl). Without adjustment for other vitamin K-dependent proteins these ORs were 1.8 (95% CI: 1.2-2.7) for high FII levels, 1.6 (95% CI: 1.1-2.5) for high FVII levels and 2.7 (95% CI: 1.8-4.1) for high FIX levels.

Comparing FX levels of patients and controls in different subgroups according to oral contraceptive use, as depicted in Fig. 1, showed a similar distribution of FX levels in patients and controls for men, postmenopausal women and premenopausal women (+OC). However, for premenopausal women (-OC) the distribution of FX levels in patients and controls was different. Premenopausal patients (-OC) tended to have higher FX levels than premenopausal controls (-OC), who had the lowest FX levels. For that reason the thrombosis risk associated with a FX level above the 90th percentile was highest (crude OR = 4.3, 95% CI: 1.5-12, Table 2) in this subgroup. After correction for age and vitamin K-dependent proteins (FII, FVII, FIX) this OR reduced to 1.6 (95% CI: 0.4-5.6). For men the crude OR was 1.9 (95% CI: 1.0-3.8) and after correction for age and the vitamin K-dependent proteins 1.4 (95% CI: 0.7-2.9). Lowest risks were found in postmenopausal women (crude OR = 1.6, 95% CI: 0.5-4.8) and in premenopausal women (+OC) (crude OR = 1.1, 95% CI: 0.4-2.8).

Table 3 shows the ORs after stratification of the FX levels into four groups. Subjects with FX levels above 140 U/dl had a 2.1-fold increased risk compared with those with a FX level below 100 U/dl. After adjustment for age, sex and oral contraceptive use this OR became 2.7 (95% CI: 1.3-5.6). Additional correction for vitamin K-dependent proteins (FII, FVII, FIX) resulted in an OR of 1.6 (95% CI: 0.7-3.5).

The -222 C/T, -220 C/A and TTGTGA Insertion Promoter Polymorphisms

Exon 1 to 8 and their flanking regions and the first 506 nucleotides of the 5'-flanking region of the FX gene [numbering according to Miao et al. (30)] were sequenced in a previously described panel of 28 probands of thrombophilic families and 5 healthy controls (10), using primer sets derived from the FX gene sequence (35-37). Three variations were detected: the -222 C/T variation, the -220 C/A variation and a

Table 2 Thrombosis risk for 90th percentile cut-off (126 U/dl) of factor X

	FX (U/dl)	Patients	Controls	OR_{crude}	95% CI
All	< 126	359	424	1.6	1.1 - 2.4
(n=899)	≥ 126	67	49		
Men	< 126	149	186	1.9	1.0 - 3.8
(n=373)	≥ 126	23	15		
Premenopausal women -OC*	< 126	34	92	4.3	1.5 - 12
(n=144)	≥ 126	11	7		
Premenopausal women +OC*	< 126	20	37	1.1	0.4 - 2.8
(n=84)	≥ 126	10	17		
Postmenopausal women -OC	< 126	52	83	1.6	0.5 - 4.8
(n=149)	≥ 126	7	7		

* Oral contraceptive use for premenopausal patients both at thrombosis date and at venepuncture (28)

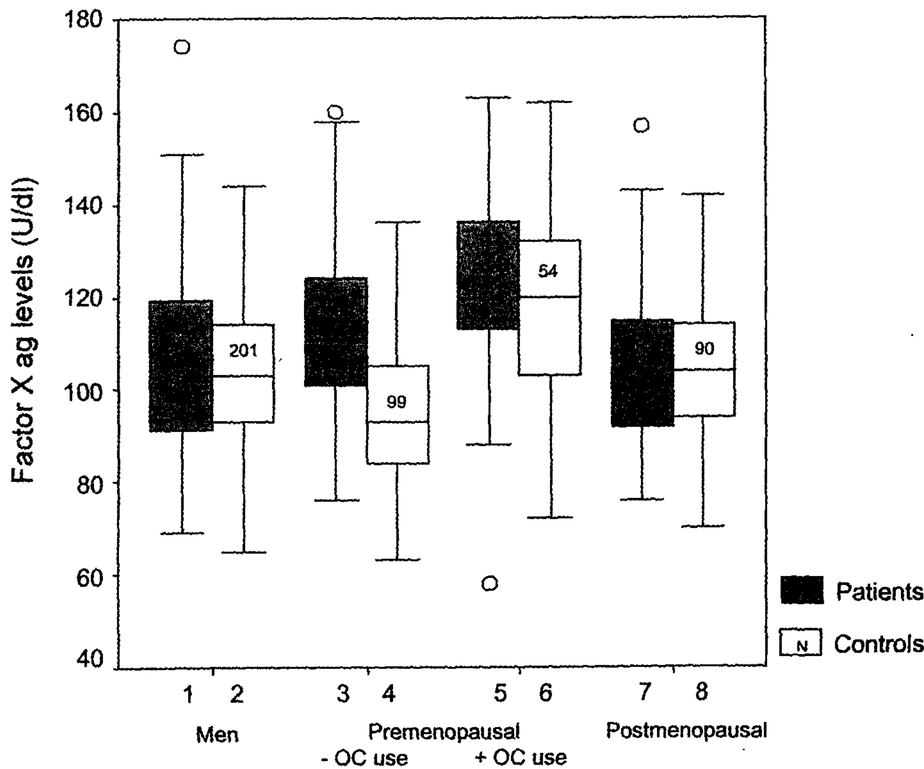

Fig. 1 Boxplot of factor X antigen levels in men and pre- and postmenopausal women. Factor X antigen levels are shown (median, interquartile range and range) for the different subgroups (numbers are depicted in the boxes): men (1 and 2); premenopausal women not using oral contraceptives (-OC, 3 and 4), premenopausal women using oral contraceptives (+OC, 5 and 6) and postmenopausal women not using oral contraceptives (7 and 8). Oral contraceptive use (or non-use) in patients refers to use both at the time of thrombosis and at the time of the venepuncture

Table 3 Thrombosis risk for strata of factor X

FX (U/dl)	Patients (%)	Controls (%)	OR_{crude} (95% CI)	$OR_{adjusted}$ (95% CI)†
< 100	173 (41)	201 (43)	1*	1*
100 – 120	153 (36)	195 (41)	0.9 (0.7 – 1.2)	0.9 (0.7 – 1.2)
120 – 140	77 (18)	64 (13)	1.4 (0.9 – 2.1)	1.1 (0.7 – 1.7)
> 140	23 (5)	13 (3)	2.1 (1.0 – 4.2)	1.6 (0.7 – 3.5)

* reference category
† OR adjusted for age, sex, oral contraceptive use and vitamin K-dependent coagulation proteins (FII, FVII, FIX)

Table 4 Factor X promoter polymorphisms

[-222 / -220 / Insertion]*	Patients (%) N=471	Controls (%) N=474	Mean FXag (95% CI) in U/dl in controls
CT	240 (51)	231 (49)	104.2 (102 – 107)†
CC / AA / + +	88 (19)	90 (19)	101.6 (97.7 – 105)
CC / CA / + +	47 (10)	47 (10)	106.3 (101 – 112)
CC / CA / + -		1 (0.2)	90
CC / CC / + +	2 (0.4)	5 (1)	96
CC / CC / + -		1 (0.2)	117
TT / CC / + +		1 (0.2)	115
TT / CC / + -	6 (1)	2 (0.4)	108
TT / CC / - -	88 (19)	96 (20)	102.4 (99.2 – 105)

* -220 C/A and TTGTGA insertion between –343A and –342G were only determined in homozygous –222 CC and TT carriers
† n=230

TTGTGA insertion between -343A and -342G. These three variations can also be detected by comparing the FX promoter sequences which were reported by Miao et al. (30) and Huang et al. (32). In a group of 97 healthy volunteers (48 men and 49 women of whom 19 were using oral contraceptives at venepuncture time) we looked at these three polymorphisms and their relationship with FX levels. Analyses suggested that carriers of the [TT/CC/- -] (-222C/T, -220C/A and no insertion following -343A, respectively) genotype had higher FX levels (mean 109.4, 95% CI: 102-116, n = 22) than carriers of the [CC/AA/+ +] genotype (mean 94.4, 95% CI: 87-102, n = 14), also after exclusion of oral contraceptive users. Because of the observed difference in FX levels between the two extreme haplotypes in this small study we chose the following approach for screening of the polymorphisms in LETS: first, all subjects were screened for the -222 C/T polymorphism in the FX promoter region (Table 4). Subsequently, all homozygous -222 CC and TT carriers were screened for the -220 C/A and TTGTGA insertion between -343A and -342G. The three polymorphisms showed a high degree of linkage disequilibrium: a -220 A allele was always accompanied by the insertion and a C allele at -222 A. The relationship between the -222 C/T polymorphism and FX levels was assessed in the healthy control subjects. Mean FX levels were 102.9 U/dl (95% CI: 100-106, n = 144) for homozygous -222 CC carriers, 104.2 U/dl (95% CI: 102-106, n = 230) for heterozygous -222 CT carriers and 102.6 U/dl (95% CI: 99.6-106, n = 99) for homozygous -222 TT carriers. Comparing of the FX levels of the two extreme genotypes [CC/AA/+ +] and [TT/CC/- -] did also not reveal any difference in the total control group and in the different subgroups (men, postmenopausal women, and premenopausal women -OC or +OC).

The -40 C/T Promoter Polymorphism

Recently, an additional polymorphism (-40 C/T) in the promoter region of the FX gene was reported (31). The T allele was reported to be associated with an increased risk of coronary artery disease. All LETS subjects were screened for this polymorphism. Two out of 471 patients were homozygous for the T allele and 36 were heterozygous (allele frequency 4.2%). Forty-five of 472 controls were heterozygous for the T allele and none of the controls was homozygous for the T allele (allele frequency 4.8%). The odds ratio (OR) for subjects carrying the T allele (in heterozygous or homozygous form) was 0.8 (95% CI: 0.5-1.3). In healthy controls we investigated the relationship between the -40 C/T polymorphism and FX levels. Mean FX levels were 103.6 U/dl (95% CI: 102-105) for homozygous wildtype -40 C carriers and 102.5 U/dl (95% CI: 97.4-108) for the heterozygous -40 CT carriers. Analyzing mean FX levels for the two genotypes in the various subgroups (men, postmenopausal women and premenopausal women -OC or +OC) also did not reveal any difference in FX level. The 2 postmenopausal female patients who were carrying the -40 T allele in homozygous form had FX levels of 84 and 92 U/dl.

Discussion

Our study shows that among healthy controls FX plasma levels are lower in premenopausal women who are not using oral contraceptives (-OC) than in men and postmenopausal women. These results correspond with previous findings of Henkens et al. (38). Highest FX levels were found in premenopausal women who were using oral contraceptives (+OC). An increase of FX levels and other procoagulant proteins during oral contraceptive use has been reported before (34, 39). We did not find an effect of age on FX levels.

The crude OR, calculated as an estimation of the relative risk of venous thrombosis, for subjects with high FX levels (above the 90th percentile, ≥ 126 U/dl) compared with subjects with low FX levels (< 126) was 1.6 with the highest risk found in the subgroup of premenopausal women (-OC) (crude OR = 4.3). This tendency of higher FX levels in premenopausal patients (-OC, group 3) than in premenopausal controls (group 4) is visualized in Fig. 1. Comparing healthy premenopausal control women (groups 4 and 6) shows that FX levels rise strongly during oral contraceptive use, as was already described above. On the other hand, premenopausal patients (+OC, group 5) have only slightly higher FX levels compared to premenopausal patients (-OC, group 3). So, it seems that in women who already have high FX levels, oral contraceptive use does not increase these levels further. Premenopausal patients and controls who are using oral contraceptives (groups 5 and 6) have therefore similar FX levels and no thrombosis risk associated with increased FX levels is found in this particular subgroup. A similar observation was made for factor IX (12). High FIX levels were shown to be a common risk factor for venous thrombosis with the highest risk found in premenopausal women (-OC). However, high FIX levels remained a risk factor after adjustment for possible confounders (oral contraceptive use and vitamin K-dependent clotting proteins FII, FVII and FX), whereas the thrombosis risk associated with high FX levels was largely reduced after correction. When the vitamin K-dependent proteins FII, FVII, FIX and FX are all included in one model only high FIX levels remain a risk factor for venous thrombosis. This indicates that the observed increased risk associated with high FX levels may be explained by high levels of FIX, which has previously been reported as risk factor for venous thrombosis (12). It is interesting that elevated levels of FIX (12) and of its cofactor FVIII (11) were both found to increase the thrombosis risk, whereas elevated levels of FX and its cofactor FV (40) were both found not to be risk factors.

Genetic variations that modulate the transcription of coagulation factor genes and thereby influence protein levels, have been reported in a number of studies. For example, a polymorphic variation in the promoter region of the protein C gene has been shown to influence transcriptional efficiency in vitro and to be correlated with plasma protein C levels (24, 41). Polymorphisms in the promoter region of the genes for PAI-1 (25), β-fibrinogen (23) and FVII (22) and a variation in the 3'-untranslated region of the prothrombin gene (10) have also been reported to be correlated with protein levels. We investigated four polymorphisms in the promoter region of the FX gene. For one of these polymorphisms (-40 C/T) it was recently reported that the T allele was associated with an increased risk of coronary artery disease (T allele frequency 4.4% in patients and 1.2% in controls) (31). The polymorphic site at position -40 is located nearby a previously described binding site for transcription factors HNF-4 and Sp-1 (nt -73 to -44) in the FX promoter region (21, 30). A nucleotide variation on adjacent position -40 may interfere with binding of a transcription factor and thereby influence gene expression. However, in LETS we found no association between this polymorphism and FX levels and also no association of the -40 T allele with thrombosis risk.

In addition to the -40 C/T polymorphism we screened for three other promoter polymorphisms (-222 C/T, -220 C/A and a TTGTGA insertion between -343A and -342G). A small pilot study suggested that carriers of two extreme genotypes [TT/CC/- -] and [CC/AA/+ +] had different mean FX levels. Therefore we first screened all subjects for the -222 C/T polymorphism and next screened all homozygous -220 CC and TT carriers for the other two variations assuming that homozygous carriers of the -220 polymorphism would have the most extreme effects. However, in this present large study the previously

observed association between the different genotypes and FX levels could not be confirmed.

In conclusion, we showed that high FX levels are not a risk factor for venous thrombosis when other vitamin K-dependent clotting factors are taken into account. Furthermore, no association between polymorphisms in the promoter of the FX gene and FX levels was observed.

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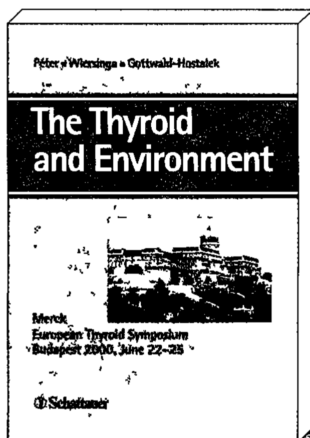

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