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## The 46C→T polymorphism in the factor XII gene (*F12*) and the risk of venous thrombosis

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The precise role of factor XII (FXII) in the regulation of blood coagulation and fibrinolysis is still undefined. Activation of FXII initiates both the kinin-forming cascade and the intrinsic coagulation and fibrinolytic pathways. Subjects with severe FXII deficiency show a prolonged activated partial thromboplastin time but do not have a bleeding tendency, which suggests a minor role for FXII in the regulation of fibrin formation *in vivo*. The observation that FXII is also involved in the activation of the fibrinolytic system led to the hypothesis that partial or severe FXII deficiency might result in impaired fibrinolysis and as a consequence in a thrombotic tendency. Indeed, a high frequency (9–15%) of reduced plasma FXII levels was found among patients with (venous) thrombosis [1,2] and women with recurrent miscarriages [3], a condition often associated with a thrombophilic state. However, Koster *et al.* showed that the frequency of reduced FXII levels was as high in control subjects as in patients with venous thrombosis [4]. Other studies failed to find an association between partial, and probably also severe, factor XII deficiency and venous thrombosis in families with hereditary FXII deficiency [5–7],

indicating that heterozygous FXII deficiency in itself is not a risk factor for thrombosis.

Recently it was reported that in the families of the GAIT-study FXII levels exhibited a significant positive correlation with thrombosis, indicating that high FXII levels might enhance thrombosis risk [8]. Later the same group found evidence for a quantitative trait locus in *F12* which influenced both FXII levels and thrombosis risk [9]. A previously reported polymorphism in the 5'-untranslated region of the *F12* gene (46 C→T) [10], of which the T-allele is associated with reduced plasma FXII levels [10,11], explained part of the linkage signal [9]. In a subsequent study Tirado *et al.* reported a 3-fold increased risk of venous thrombosis for carriers of the 46TT genotype (crude OR 3.1; 95% CI 1.1–8.7) and concluded that the 46T-allele is an independent genetic risk factor for venous thrombosis in the Spanish population [12]. A previous study from Franco *et al.* reported that homozygous 46T carriers did not have an increased risk of venous thrombosis (OR 0.8, 95% CI 0.3–1.9) [13]. The reason for the apparent discrepancy between these two studies may be that both were relatively small and included only around 15–22 homozygous individuals. We studied the effect of the 46C→T polymorphism on plasma FXII levels and thrombosis risk in a large population-based case-control study on venous thrombosis (Leiden Thrombophilia Study, LETS). This study, which included 474 patients with a first deep vein thrombosis (96% in the leg, 4% in the upper extremities) and 474 control subjects, has been described previously [14]. Patients (202 men, 272 women) had a mean age of 45 years (range 14–69). In 46% of the patients the thrombosis was spontaneous, i.e. occurred in the absence of

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**Table 1** Frequency of 46C/T genotypes in patients ( $n = 471$ ) and controls ( $n = 471$ ) of the Leiden Thrombophilia Study

Genotype	Patients $n$ (%)	Controls $n$ (%)	OR	95%CI
46CC	277 (58.8)	261 (55.4)	1*	
46CT	168 (35.7)	180 (38.2)	0.88	0.67–1.15
46TT	26 (5.5)	30 (6.4)	0.82	0.47–1.42

\*Reference category.

risk factors such as surgery, hospital admissions, bed rest (> 2 weeks), pregnancy and use of oral contraceptives. Overall the risk factor profile of our patients was very similar to that of the patients included in the report by Tirado *et al.* [12], except that we included first events only. The latter may explain the lower number of individuals with a positive family history in our study (25% vs. 39% in the study reported by Tirado *et al.* [12]).

Blood was collected in 0.1 volume 0.106 mol L<sup>-1</sup> trisodium citrate. Plasma was prepared by centrifugation for 10 min at 2000 *g* at room temperature and stored at -70 °C. FXII activity was measured using a one-stage clotting assay.

High molecular weight DNA was isolated from leukocytes and stored at 4 °C. Polymerase chain reaction (PCR) was designed to amplify the region in and around exon 1. The sequences of the primers were: forward 5'-GAT AGG CAG CTG GAC CAA CG-3' (nt 21–40 [15]) and reverse 5'-TGA TAG CGA CCC CCC AGA AC-3' (nt 162–143 [15]). The amplified DNA fragments [142 base pairs (bp)] were digested with Bsa HI, which recognizes a site on the 46C fragment, and separated by electrophoresis on agarose gels. The 46C-allele is cut into fragments of 116 bp and 26 bp, while the 46T-allele is not cut.

The frequency of the 46T-allele was 0.254 in controls and 0.233 in the patients, values similar to those reported previously in Caucasian populations (0.2–0.28) [10–13]. Among controls there was an allele-specific dosage-dependent effect of the 46T-allele on plasma FXII levels [U dL<sup>-1</sup>, mean (95%CI)]: 46CC ( $n = 261$ ): 123 (120–125), 46CT ( $n = 180$ ): 89 (87–92) and 46TT ( $n = 30$ ): 57 (50–63). This confirms previous reports [11] and indicates that about 5% of the population (the carriers of the 46TT genotype) have plasma FXII levels identical to those of heterozygous carriers of a *F12* null mutation. This might explain the high frequency of FXII deficiency reported in earlier studies of uncontrolled groups of patients with thrombotic disease.

Table 1 shows the frequencies of the 46C/T genotypes in patients and controls. Odds ratios (OR) were calculated as estimates of the relative risk by an unmatched method. Ninety-five per cent confidence intervals were assessed according to Woolf [16]. (Homozygous) carriers of the 46T-allele did not have an increased risk of venous thrombosis (OR 46TT-carriers 0.82, 95% CI 0.47–1.42). Similar results were obtained for men and women, and for subjects > 45 years and ≤45 years. Our results confirm the findings of Franco *et al.* [13] and differ from those reported by Tirado *et al.* [12] In fact, the OR of 0.82 that we observed for

46TT carriers is identical to the one reported by Franco *et al.* (OR 0.8), which might even indicate a slight protective effect of the 46T allele. Such a protective effect would be in agreement with the previously reported positive correlation of FXII levels and thrombosis [8]. The reason why Tirado and coworkers found a crude OR of 3.1 for 46TT carriers is not known. Among 250 healthy individuals, 90 had the 46CT and five the 46TT genotype. This latter number is statistically the less stable one. When we apply the Hardy–Weinberg equilibrium equation to the 155 CC and 90 CT carriers, which numbers are larger and therefore more stable, we can estimate an allelic prevalence for the 46T allele of 0.225. Under this allelic prevalence, the expected number of 46TT carriers is 12.7 (five observed). In a subsequent study from the same group the prevalence of the 46T allele among 100 healthy subjects was 0.23 and the number of 46TT carriers 4 (five expected) [17], indicating that the finding of Tirado *et al.* may be false positive due to the low number of 46TT genotypes in their sample of the healthy population (see also [18]). The same considerations might apply to the recent finding from the same group that 46TT carriers have a 4-fold increased risk of ischemic stroke (crude OR of 4.7) [19]. Also in this study the number of 46TT carriers in the control population was the less stable figure (observed 3/231, expected 8/231).

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## Should we screen Eastern Mediterranean sickle beta-thalassemia patients for inherited thrombophilia?

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A hypercoagulable state in sickle cell disease and beta-thalassemia is well documented [1,2]. Factor V (FV) Leiden is the largest inherited risk factor of venous thrombosis [3]. Risks are estimated to be increased up to 50–100-fold for homozygous adults [4]. In eastern Mediterranean countries, a high prevalence of FV Leiden was reported in healthy individuals, with the highest frequency reported in Lebanon (14%) [5,6]. Reduced methylenetetrahydrofolate reductase (MTHFR) levels or activity is regarded as a risk factor for deep-vein thrombosis (DVT) [7]. Lebanon has a relatively high prevalence of mutated homozygous (T/T) and heterozygous (C/T)

C677T MTHFR genotypes (11.04% and 39.73%, respectively) [8].

One of our patients with sickle beta-zero thalassemia who presented with pain in the bilateral shoulder and left knee areas was diagnosed initially as having sickle cell crisis, but was shown to have extensive DVT by duplex scanning. Because of the high frequency of FV Leiden and MTHFR mutations in our population, these were measured and the patient was found to be homozygous for FV Leiden and heterozygous for the MTHFR mutation. After treatment with warfarin, the patient has done well.

To our knowledge, there are no reported cases of sickle beta-zero thalassemia patients with this profile of inherited thrombophilia, making our case the first. The high frequency of FV mutation in our region suggests a role for screening in patients with sickle beta-thalassemia. Studies are lacking to define a prophylactic anticoagulation approach for sickle cell patients with underlying thrombophilic tendencies. We need to define preventive guidelines of sickle beta-thalassemia with thrombophilia in the eastern Mediterranean region.

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