bjh short report

# The effect of genetic variants in the thrombin activatable fibrinolysis inhibitor (TAFI) gene on TAFI-antigen levels, clot lysis time and the risk of venous thrombosis

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## Summary

Thrombin activatable fibrinolysis inhibitor (TAFI) is an important inhibitor of fibrinolysis. High TAFI antigen levels are associated with an increased risk of deep venous thrombosis (DVT). Because TAFI levels are partly determined genetically, we assessed the association between three TAFI gene polymorphisms (−438 G/A, 505 A/G and 1040 C/T), TAFI antigen levels and clot lysis times and the risk of DVT. Carriers of the 505G allele, which is associated with lower TAFI antigen levels than the 505A allele, showed an increased risk of DVT. This indicates that the relationship between TAFI and venous thrombosis is more complex than previously suggested.

**Keywords:** genes, venous thrombosis, coagulation, thrombin activatable fibrinolysis inhibitor, fibrinolysis.

Thrombin activatable fibrinolysis inhibitor (TAFI) is an important inhibitor of fibrinolysis, which acts by inhibiting the assembly of fibrinolytic factors on the fibrin surface (Bajzar *et al*, 1996). Recently, it was shown that elevated TAFI antigen levels are a mild risk factor for the occurrence of deep venous thrombosis (DVT) (van Tilburg *et al*, 2000). TAFI levels increase with age, mainly in women, and are elevated in oral contraceptive users (van Tilburg *et al*, 2000).

Thrombin activatable fibrinolysis inhibitor levels are also genetically determined and the −438 G/A *TAFI* single nucleotide polymorphism (SNP) in the promoter region (Henry *et al*, 2001) and the 505 G/A SNP (Henry *et al*, 2001) and 1040 C/T SNP in the coding region (Brouwers *et al*, 2001) are associated with TAFI plasma antigen levels. The −438 G/A polymorphism was reported to be associated with an increased risk of developing venous thrombosis (Franco *et al*, 2001; Zidane *et al*, 2003).

Recently, the effect of *TAFI* polymorphisms on TAFI antigen levels has been debated since variable antibody reactivity towards TAFI isoforms (in particular the 1040C/T polymorphism) leads to artefacts in TAFI antigen levels (Guimaraes *et al*, 2004).

We analysed data from the Leiden Thrombophilia Study (LETS) to assess the risk of developing DVT associated with three SNPs of the *TAFI* gene [−438 G/A (rs no. 2146881), 505 A/G (rs no. 3742264) and 1040 C/T (rs no. 1926447)] in 471 patients with a first DVT, aged 18–70 years (patients with cancer excluded) and 472 sex- and age-matched control subjects. The risk of venous thrombosis associated with each polymorphism was expressed as an odds ratio (OR) with a corresponding 95% confidence interval (95% CI). In addition, the *TAFI* multilocus haplotype effects on TAFI antigen levels were estimated using weighted linear regression as described by Tanck *et al* (2003). After modification of the method, (weighted) logistic regression was used to investigate the association between *TAFI* haplotypes and the risk of thrombosis. Furthermore, the effect of the three SNPs on the TAFI antigen levels and clot lysis time was assessed in the control group. TAFI antigen levels were measured by the Laurell method, which is insensitive to *TAFI* genotype artefacts (van

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Tilburg *et al*, 2000; Guimaraes *et al*, 2004). Moreover, TAFI antigen levels may not represent the functional activity of TAFI, which is measured by the rate of cleavage of a small substrate after activation with thrombin thrombomodulin (Zorio *et al*, 2003). Mosnier *et al* (1998) described a plasma based clot lysis assay, which was initially developed to study TAFI-related processes. It was shown that clot lysis times were associated with both TAFI antigen and activity levels in a group of 20 healthy volunteers. However, recently we showed in a much larger group of healthy subjects ($n = 469$), which is the control group of the study we report on here, that the association between the clot lysis time and TAFI antigen levels was, at most, very weak (0·188 increase in clot lysis time (in min) per 1 U/dl increase in TAFI, after age-adjustment) (Lisman *et al*, 2005).

In the total study population, 403 (43%) were men, 540 (57%) women and the mean age in patients and control subjects was 45·0 years (range 15–69 years) and 44·7 years (15–72 years), respectively. The distribution of the three *TAFI* SNPs in the control subjects did not deviate from Hardy–Weinberg equilibrium.

All three SNP were associated with TAFI antigen levels, which was more pronounced in homozygotes than in heterozygotes (Table I). For the −G438A and C1040T variants, the rare alleles were associated with lower levels than common alleles, while for the G505A the rare allele was associated with higher TAFI levels than the common allele. The effect on TAFI levels was most striking for the 505 genotypes, with 17% higher TAFI levels in carriers of 505AA than 505GG. These findings are in agreement with earlier observations (Brouwers *et al*, 2001, 2003).

These associations between genotypes and levels would predict an increased risk of thrombosis for the 505A, −438G and 1040C allele. A clear and graded relationship between genotype and risk of thrombosis was, however, only observed for the 505A genotype, with lower risks for the rare A-allele. Because there is a high level of linkage disequilibrium between these *TAFI* polymorphisms, haplotype analysis was necessary to analyse the effects of a single polymorphism while excluding the effects of associated polymorphisms. Haplotype analysis confirmed the association of the G505A polymorphism and risk of venous thrombosis. The 505G allele was associated with a mildly increased risk of venous thrombosis [OR 1·3 (1·0–1·6); Fig 1B]. The 505G allele was associated with reduced TAFI levels, both by the single genotype and by the haplotype analysis (Fig 1A). The analysis, therefore, suggested that a direct, functional role is more likely for the G505A polymorphism than for the other studied *TAFI* polymorphisms. We have now found a reduced risk of DVT with the allele that is associated with higher TAFI levels and lower clot lysis times, which was in contrast to the hypothesis on which we based on our previous findings (van Tilburg *et al*, 2000). A possible explanation, besides chance findings, is that moderately elevated TAFI antigen levels do not alter the risk of venous thrombosis. Previously, we only found an increased risk when TAFI antigen levels exceeded 122 U/dl (van Tilburg *et al*, 2000), whereas none of the genotypes we studied were associated with such high levels of TAFI antigen. However, it may also be that the elevated levels of TAFI we previously observed in patients who had suffered venous thrombosis compared with healthy controls were a consequence rather than a cause of thrombosis.

All three SNPs were associated with TAFI antigen levels, but only a relationship between *TAFI* 505A polymorphism and clot lysis times was observed (Table I). We have recently shown in these same individuals that TAFI antigen levels and clot lysis times were only weakly associated (Lisman *et al*, 2005). The present study showed an increased risk of DVT in carriers of

**Table I.** The risk of developing a venous thrombotic event and the effect of SNPs on TAFI antigen levels and clot lysis time.

| SNP | Cases (%) ($n = 471$) | Controls (%) ($n = 472$) | OR (95% CI), mean (95% CI) | TAFI Ag (U/dl)*, mean (95% CI) | CLT (min)* mean (95% CI) |
|---|---|---|---|---|---|
| −438 | | | | | |
| GG | 240 (51·0) | 257 (54·4) | 1 | 110 (108–111) | 59·6 (58·4–60·8) |
| AG | 202 (42·9) | 185 (39·2) | 1·17 (0·89–1·53) | 104 (102–106) | 63·0 (61·3–64·7) |
| AA | 29 (6·1) | 30 (6·4) | 1·04 (0·60–1·78) | 96 (92–100) | 61·5 (56·0–67·1) |
| 505 | | | | | |
| GG | 236 (50·1) | 211 (44·7) | 1 | 102 (101–103) | 61·6 (59·9–63·2) |
| AG | 198 (42·0) | 210 (44·5) | 0·84 (0·64–1·10) | 108 (107–110) | 60·7 (59·4–62·0) |
| AA | 37 (7·9) | 51 (10·8) | 0·65 (0·41–1·03) | 119 (115–122) | 60·3 (56·9–63·6) |
| 1040 | | | | | |
| CC | 219 (46·5) | 215 (45·6) | 1 | 110 (108–111) | 59·1 (57·8–60·3) |
| CT | 218 (46·3) | 212 (44·9) | 1·01 (0·77–1·32) | 105 (104–107) | 62·8 (61·2–64·3) |
| TT | 34 (7·2) | 45 (9·5) | 0·74 (0·46–1·20) | 98 (95–102) | 62·3 (58·0–66·6) |

CLT, clot lysis time.

Linear regression analysis showed a mean decrease of TAFI of 6·3 U/dl (95% CI 4·5–8·0) per additional A-allele at −438, a mean increase of 7·6 U/dl (95% CI 6·1–9·2) per additional A-allele at 505, and a decrease of 5·2 U/dl (95% CI 3·5–6·8) at 1040.

*TAFI and CLT levels in control subjects.

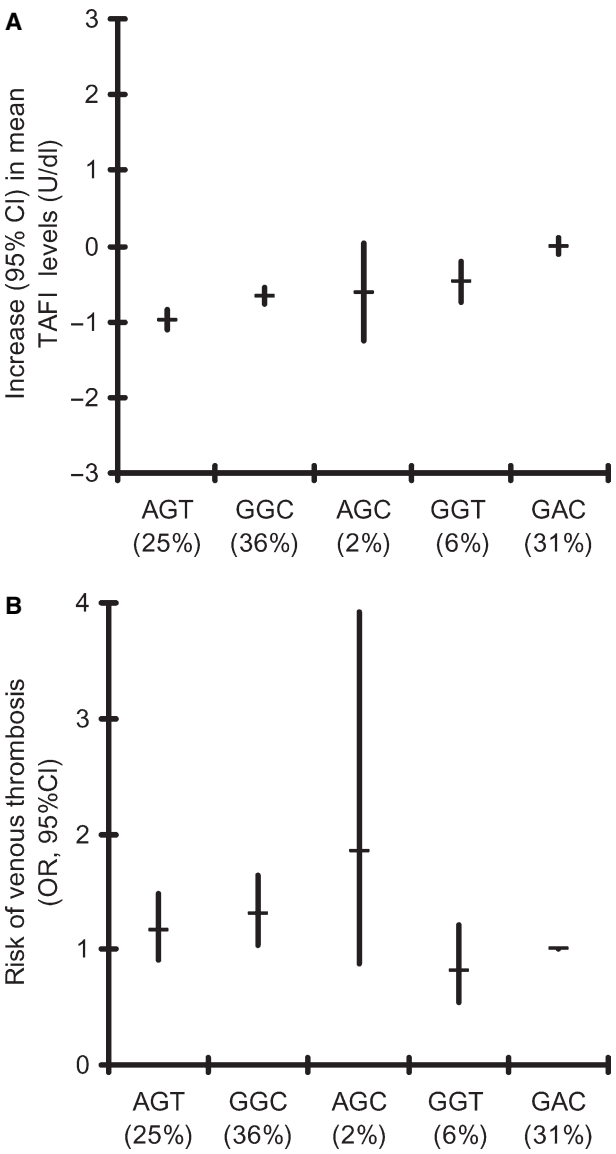


**Fig 1.** (A) The effect of TAFI haplotypes on plasma TAFI antigen levels in the LETS study population (levels are expressed as mean and 95% CI). (B) The effect of TAFI haplotypes on risk of venous thrombosis in patients vs controls expressed as odds ratios. The alleles in the haplotype are given in the following polymorphism order: −438G/A. 505G/A (Ala147Thr) and 1040C/T (Thr325Ile). GAC as reference.

the 505G allele. The 505 G-allele is associated with low TAFI antigen levels, which indicates that there is complex relationship between TAFI and the risk of venous thrombosis.

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