The fibrinogen gamma (FGG) 10034C > T polymorphism is associated with venous thrombosis

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Abstract

Introduction: Thrombin-induced conversion of fibrinogen to fibrin plays an essential role in hemostasis and results in the stabilization of thrombi. Elevated plasma fibrinogen levels have been associated with both increased plasma viscosity and platelet aggregability. Recently, a haplotype-tagging single nucleotide polymorphism characterized by a C to T substitution at nucleotide 10034 of the fibrinogen gamma gene (FGG 10034C > T, rs2066865), has been proposed as a novel risk factor for deep venous thrombosis (DVT). Aim of the present study was to provide further data on the role of the FGG 10034C > T polymorphism for DVT.

Materials and methods: FGG genotypes were determined by 5'-exonuclease assay (TaqMan) in 358 patients with documented DVT and a total of 783 control subjects.

Results: In a multivariate analysis adjusting for age, sex, presence of factor V Leiden and carriage of prothrombin 20210A, homozygosity for the FGG 10034 TT genotype yielded an odds ratio of 2.01 (95% CI 1.23 – 3.31; p = 0.006) for DVT.

Conclusions: Our data confirm the primary finding that the FGG 10034C > T polymorphism is associated with DVT risk.

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Risk factor

Introduction

Deep venous thrombosis (DVT) is a multifactorial disease with both environmental and genetic factors.
contributing to its development [1,2,]. Elevated plasma fibrinogen concentrations have previously been associated with an increased risk for DVT [3,4]. Fibrinogen is a 340 kDa plasma glycoprotein and consists of three pairs of non-identical polypeptide chains (Aα, Bβ, and γ), which are each encoded by a different gene (fibrinogen alpha [FGA], fibrinogen beta [FGB] and fibrinogen gamma [FGG]) [5–8]. Thrombin-induced conversion of fibrinogen to fibrin plays an essential role in hemostasis and results in stabilization of the thrombus. Furthermore, elevated plasma fibrinogen levels have been associated with both increased plasma viscosity and platelet aggregability [9,10].

A haplotype-tagging single nucleotide polymorphism (htSNP) characterized by a C to T substitution at nucleotide 10034 of the FGG gene (FGG 10034C>T, rs2066865), has recently been proposed as a novel risk factor for DVT in the Leiden Thrombophilia Study [11]. The purpose of the present study was re-analyzed by the previously described association between the FGG 10034C>T polymorphism and DVT in an Austrian population.

**Materials and methods**

Three hundred and fifty eight subjects with a documented episode of DVT of the lower extremities, admitted to the Department of Internal Medicine, Medical University Graz, between December 1997 and June 2002, were enrolled as the patient group [12]. DVT was diagnosed by ultrasonography and/or venography. Pulmonary embolism (PE) was diagnosed by ventilation–perfusion scintigraphy and/or CT pulmonary angiography. Patients with isolated PE without diagnosis of DVT were not eligible.

A control group (in-house control group) of similar age and sex distributions was selected from patients of the same department (n = 354). Subjects were eligible as controls if they were without a history of venous (DVT, pulmonary embolism, primary varicosis) or arterial disease (coronary heart disease, cerebrovascular disease, peripheral arterial occlusive disease).

As an additional population-based control group, 429 voluntary participants from a local population-based health screening study were included. Data on previous or current venous thromboembolic disease (DVT or pulmonary embolism) were retrieved in a face-to-face interview and subjects with a history of venous thromboembolic disease were not enrolled as controls in the present study.

The study was approved by the local ethics committee and all individuals participating in the study gave their informed consent. All subjects were Austrian and of Caucasian ethnicity.

Isolation of genomic DNA and determination of prothrombin (F2 20210G>A) and factor V Leiden (F5 R506Q) genotypes were carried out as described previously [13]. The FGG 10034C>T polymorphism was determined by a TaqMan™ fluorogenic 5’-exonuclease assay (Applied Biosystems, Austria) using

| Table 1 Characteristics of DVT patients and controls |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| In-house controls | Population-based controls | DVT patients | p (patients vs. in-house controls) | p (patients vs. population-based controls) | p (patients vs. all controls) |
| n | 354 | 429 | 358 | – | – | – |
| Age, years | 54.0 ± 19.4 | 57.0 ± 13.4 | 53.3 ± 19.6 | 0.63 | 0.002 | 0.086 |
| Male sex, n (%) | 146 (41.2) | 210 (49.0) | 154 (43.0) | 0.63 | 0.093 | 0.44 |
| Factor V Leiden, n (%)* | 21 (6.0) | 25 (5.8) | 78 (21.8) | 0.001 | 0.001 | 0.001 |
| Prothrombin 20210A, n (%)b | 9 (2.6%)b | 13 (3.0) | 27 (7.5%) | 0.003 | 0.004 | 0.001 |
| Age at first thrombosis, years | – | – | 46.5 ± 19.2c | – | – | – |
| Pulmonary embolism | – | – | – | – | – | – |
| FGG 10034C>T CC | 206 (60.2) | 255 (59.4) | 180 (52.8) | – | – | – |
| CT | 118 (34.5) | 151 (35.2) | 129 (37.8) | 0.17 | 0.22 | 0.14 |
| TT | 18 (5.3) | 23 (5.4) | 32 (9.4) | 0.021 | 0.018 | 0.005 |
| T allele frequency | 0.225 | 0.230 | 0.283 | 0.014 | 0.015 | 0.005 |

*p values for FGG genotypes were determined by χ² test using the CC genotype group as reference.

*b 72 heterozygous and 6 homozygous carriers of factor V Leiden. Factor V Leiden genotypes were missing in three in-house controls and one patient.

b Prothrombin genotypes were missing in three controls.

*c Fifty (14%) patients were unable to specify their age at the first thromboembolic event.
Applera’s Assays-by-Design custom service. Sequences of primers and probes were as follows: forward primer (5′-ACATGCATTTCAATAAACCTTTTGTTTCCT-3′), reverse primer (5′-GGTAAATTGGCAAAAAGTGGTGGT-3′), C-probe (5′-VIC-TTTTAATGGTCAATAAAGGTACCA-NFQ-3′), and T-probe (5′-FAM-ATGGTCAATAAAGATACCA-NFQ-3′).

Statistical analysis was done using SPSS 14.0 for Windows. Metric values were analyzed by Student’s t-test and presented as mean± standard deviation. Categorical values were compared by χ² test. Odds ratios (OR) and 95% confidence intervals (CI) were determined by logistic regression analysis. The criterion for statistical significance was p<0.05.

Results

Characteristics of study subjects are summarized in Table 1. DVT was diagnosed as first event in 299 (83.5%) patients and recurrent event in 59 (16.5%) patients. FGG genotypes were determined successfully in 341 (95.3%) patients with DVT, 342 (96.6%) in-house controls and all population-based controls and did not deviate from the Hardy–Weinberg equilibrium among either group. Genotype distributions are summarized in Table 1.

Genotype frequencies were almost identical among in-house controls and population-based controls and significantly different from the patient group. For further comparison, in-house controls and population-based controls were combined. Homozygotes for the FGG 10034 TT genotype were significantly more prevalent in patients with DVT than among control subjects. Presence of the homozygous FGG 10034 TT genotype was associated with an OR of 1.84 (95% CI 1.14–2.98; p=0.013) for DVT. In a multivariate analysis adjusting for age, sex, presence of factor V Leiden and carriage of prothrombin 20210A, homozygosity for the FGG 10034 TT genotype yielded an OR of 2.01 (95% CI 1.23–3.31; p=0.006) for DVT.

Assuming a codominant effect of the FGG polymorphism (TT versus CT versus CC), the OR of one additional T allele for DVT was 1.33 (95% CI 1.09–1.63; p=0.006). This association remained similar in a multivariate analysis including sex, age, presence of factor V Leiden and carriage of prothrombin 20210A as potential confounders (OR 1.35; 95% CI 1.09–1.66; p=0.005). Age at first thrombosis tended to be lower in carriers of the FGG TT genotype (41±21 years) compared to CC (47±19) or CT (47±19 years) genotypes, but this difference was not statistically significant (p=0.086). Among patients, pulmonary embolism was found with similar frequencies among FGG genotype groups (CC: 43.3%; CT: 48.1%; TT: 40.6%; p=0.63).

Data from the primary report on the FGG polymorphism [11] and data from the present study are compared in Fig. 1. The association of the FGG 10034C>T polymorphism with DVT was similar in both studies.

Discussion

In the present study, homozygous carriers of the FGG 10034T-allele were found more often in patients with DVT than among control subjects without thrombosis. Our result confirms the primary finding of Uitte de Willige and coworkers, who identified this genotype as a novel risk factor for DVT [11] (Fig. 1).
The precise mechanism by which this polymorphism affects susceptibility to DVT has only partially been determined. According to SeattleSNPs (http://pga.gs.washington.edu), the genetic diversity of FGG gene can largely be explained by five common haplotypes [11]. One of these haplotypes, FGG H2, contains the rare alleles of three htSNPs, one in intron 8 (7874G>A), one in intron 9 (9615C>T) and one downstream from the 3′ untranslated region (10034C>T). This haplotype has been associated with reduced fibrinogen γ′ levels. The 10034C>T polymorphism is located in a CstF (Cleavage stimulatory Factor) consensus 2a sequence close to polyadenylation site (nt 9997–10002) of the fibrinogen γA specific exon 10 and is therefore the most likely functional cause for the reduced production of fibrinogen γ′. Fibrinogen γ′ originates from alternative mRNA processing of the fibrinogen gamma chain, and includes a unique binding site for factor XIII B, leading to increased resistance to fibrinolysis [14,15]. Fibrinogen γ′ also contains a high-affinity, nonsubstrate thrombin-binding site, which acts as a thrombin inhibitor and might explain the association between low plasma fibrinogen γ′ concentrations and increased risk for DVT [11].

Some limitations of the present study should be taken into account. First, data on some risk factors for DVT, such as body–mass-index or intake of contraceptives, were not available for study participants. Second, as this study was designed to investigate the role of genetic polymorphisms as potential risk factors for DVT, fibrinogen γ′ levels were not determined in the present study. Prospective studies focusing on the role of fibrinogen γ′ as a risk factor for DVT are therefore clearly warranted.

The FGG 10034 TT genotype increased the individual risk for DVT only moderately and had less effect on the DVT risk than factor V Leiden. It remains to be determined whether routine testing for the FGG 10034C>T polymorphism is useful for the estimation of individual thrombosis risk.

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