Fibrinogen Polymorphisms Taq I, Hae III and Bcl I Are Not Associated with a Higher Risk of Deep Vein Thrombosis

Mojca Božič¹ Nataša Teran² Borut Peterlin² Mojca Stegnar¹
Departments of ¹Vascular Diseases and ²Obstetrics and Gynaecology, University Medical Centre, Ljubljana, Slovenia

Key Words
Fibrinogen · Venous thrombosis · Polymorphism (genetics) · Aetiology of DVT

Abstract
High fibrinogen is recognised as a risk factor for atherosclerosis. It seems that high fibrinogen is also a risk factor for deep vein thrombosis (DVT). It has been shown that certain polymorphisms in fibrinogen genes can influence the fibrinogen level. In this study, fibrinogen levels and the frequency of the polymorphisms Taq I, Hae III and Bcl I were studied in 114 patients with DVT and 244 healthy subjects. In non-smokers, fibrinogen levels above 5 g/l were associated with an increased risk of DVT (odds ratio 3.3, 95% confidence interval 1.6–7.0). The frequencies of common alleles were similar in patients and healthy subjects for all polymorphisms. An association between fibrinogen levels and the polymorphisms Taq I, Hae III and Bcl I was found in healthy subjects, but not in the patients. It was concluded from these data that the polymorphisms Taq I, Hae III and Bcl I are not major risk factors for DVT.

Introduction
Increased plasma fibrinogen has been repeatedly recognised as a risk factor for myocardial infarction and stroke [1–4]. For deep vein thrombosis (DVT), similar fibrinogen levels have been reported for patients and healthy subjects [5, 6]. However, in one study, a fibrinogen level above 5 g/l was associated with a four-fold risk of DVT [5].

Plasma fibrinogen is influenced by many factors, such as age, gender, smoking, physical activity, obesity, diabetes and hyperlipidaemia [7–9]. In addition, fibrinogen is an acute-phase protein [10]. However, these factors cannot explain fully the variation in fibrinogen levels among individuals. It has been shown that several polymorphisms in genes for fibrinogen polypeptide chains α, β and γ might play a role in the determination of the plasma fibrinogen concentration. Several restriction fragment length polymorphic sites have been found in the fibrinogen gene cluster [11]. The polymorphic Taq I and Bcl I sites have been mapped to the 3' end of the α- and β-fibrinogen genes, respectively [11, 12], and the polymorphic Hae III site to the 5' flanking region of the β-fibrinogen gene [13]. These polymorphisms have been shown to be associated with fibrinogen levels in healthy subjects in some [13–15], but not in other studies [16, 17]. In patients with coronary heart disease, these polymorphisms were
significantly associated with fibrinogen concentration [18, 19]. In patients with DVT, only the influence of the 
Hae III polymorphism has been studied, and the results 
were conflicting; one study found a significant association 
between Hae III and fibrinogen levels [6], but in another 
study, no such association was observed [5]. If we specu-
late that certain genotypes elevate levels of fibrinogen and 
that high fibrinogen plays a causal role in the develop-
ment of the disease, then these unfavourable genotypes 
should be more frequent in patients than in healthy sub-
jects. In the Leiden Study of Myocardial Infarction [19], a 
similar prevalence of the three polymorphisms was ob-
erved among patients with myocardial infarction and 
healthy subjects, and so the hypothesis about the causal 
role of fibrinogen in the aetiology of myocardial infarction 
was rejected.

To establish whether the fibrinogen polymorphisms 
Taq I, Hae III and Bcl I represent risk factors for DVT, the 
frequency of these polymorphisms and their association 
with plasma fibrinogen levels were studied in patients 
with DVT and healthy subjects.

**Patients and Methods**

**Patients and Healthy Subjects**

Subjects participated in the study after they had given their full 
informed consent. All subjects originated from Central Europe and 
were not related to each other. None of the women included in the 
study was taking oral contraceptives or hormonal replacement ther-
apy. The study was approved by the State Ethical Committee.

Subjects with a history of DVT were recruited from consecutive 
patients treated at the Department of Angiology (University Medical 
Centre) in Ljubljana, in the period from January 1992 to April 1996. 
One hundred and fourteen patients 19–60 years old (44 women and 
70 men) were evaluated at least 3 months (on average 16 ± 9 
months, mean ± standard deviation) after objectively confirmed 
acute DVT. The control group consisted of 244 apparently healthy 
unrelated volunteers (134 women and 110 men) with no history of 
DVT or pulmonary embolism. All subjects were asked about their 
smoking habits and then classified as current smokers or non-smok-
ers. Ex-smokers were considered as non-smokers if they had stopped 
smoking at least 5 years previous to this study [9]. At the time of 
sampling, patients and healthy subjects with an obvious inflammato-
ry condition or a recent febrile episode were excluded.

**Blood Collection and Determination of Fibrinogen and Lipids**

From each patient and healthy subject, one blood sample 
collected between 7.00 and 9.00 a.m. after an overnight fast and 20 min 
of rest was obtained. Venous blood was drawn from the antecubital 
vein and collected into three Vacutainer® tubes (Becton Dickinson), 
the first without an anticoagulant, the second containing 0.11 mol/l 
sodium citrate and the third containing 0.17 mol/l K3-EDTA. Serum 
was harvested after 1 h and analysed the same day for total cholester-
ol and high-density lipoprotein cholesterol with an Ektachem 250 
(Kodak) biochemical analyser. Low-density lipoprotein (LDL) cho-
lesterol was calculated with the Friedewald formula [20]. Platelet-
poor plasma was prepared from citrated blood by centrifugation at 
2,000 × g and 4°C for 30 min, snap frozen and stored at –70°C until 
analysis. In this plasma, fibrinogen levels were determined by a mod-
ified Clauss method using Multifibren® U (Dade Behring). From the 
blood collected into EDTA, genomic DNA was isolated using a com-
mercially available genomic DNA purification kit (Wizard™, 
Promega, USA) or manually by a salting-out procedure [21].

**Analysis of Genetic Polymorphisms**

We identified the Taq I polymorphism (table 1) as a 28-bp deletion 
(BK001430) of a duplication of nucleotides 8884–8911 (Gen-
Bank accession number AF361104), which eliminated a Taq I recog-
nition sequence. For the detection of this polymorphism, a new set of 
primers was designed: forward 5'-ATG AAA ATT AGG CCC C-
HEX, and reverse 5'-ATG TCT CAG GTA CAT TTA GC. With 
these primers, a PCR fragment of 184 and 212 bp, depending on the 
presence or the absence of the deletion, was amplified using Taq 
polymerase (Promega). Genomic DNA was denatured at 95°C for 
5 min, and the 36 subsequent cycles were performed at 95°C for 
1 min, 55°C for 1 min and 72°C for 1 min. The amplicon size was 
determined by GeneScan (Aby Prism 310, Applied Biosystems). The 
common allele (184 bp) was coded as T1 and the rare allele (212 bp) 
as T2. The Hae III polymorphism (table 1) was detected by real-time 
PCR on a LightCycler (Roche) using hybridisation probes. The prim-
ers and hybridisation probes were designed by Tib Molbiol (Berlin, 
Germany) as follows: forward 5'-GAT GTG TAT TTT TCA TAG 
AAT AGG GTA, reverse 5'-ATT TGA CCT ACT CAC AAG GCA 
A, Sensor (A)mt 5'-CAT TAC TAT GGA TTT TAA TAG CCC CT 
and Anchor 5'-LC Red640-TGA AAT AGA ATT ATG TCA TTG 
TCA GAA AAC. The common allele was coded as H1 and the rare 
allele as H2. The Bcl I polymorphism (table 1) was genotyped after 
amplification of the relevant DNA region by PCR and digestion with 
the Bcl I endonuclease as described previously [11]. The common 
allele was coded as B1 and the rare allele as B2.

---

**Table 1. Polymorphism notations**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene symbol</th>
<th>Polymorphism site</th>
<th>SNP annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, α chain</td>
<td>FGA</td>
<td>8884–8911del</td>
<td>Taq I</td>
</tr>
<tr>
<td>Fibrinogen, β chain</td>
<td>FGB</td>
<td>-455G&gt;A</td>
<td>Hae III</td>
</tr>
<tr>
<td>Fibrinogen, β chain</td>
<td>FGB</td>
<td>3’ untranslated region</td>
<td>Bcl I</td>
</tr>
</tbody>
</table>

SNP = Single nucleotide polymorphism.
**Statistical Analysis**

For statistical analysis, Statistica 4.5 software (StatSoft Inc., Tulsa, Okla., USA) was utilised. Allele frequencies in patients and healthy subjects were compared by $\chi^2$ analysis. A $\chi^2$ test was used to compare the observed numbers of each genotype with those expected for a population in Hardy-Weinberg equilibrium. A two-by-two table and $\chi^2$ test were used to calculate linkage disequilibrium, and Arlequin 2.000 (Genetics and Biometry Laboratory, Geneva, Switzerland) statistical software was utilised to calculate its coefficients $D$ and $D'$. An odds ratio with 95% confidence interval (CI) was calculated as a measure of relative risk. Allele frequencies are presented with 95% CIs. Fibrinogen levels are given as means and standard deviations, and differences between groups were tested with ANOVA.

**Results**

One hundred and fourteen patients and 244 healthy subjects were included in the study. The data on patients and healthy subjects are given in table 2. Fibrinogen levels adjusted for age ($\beta = 0.06$), gender ($\beta = -0.01$) and LDL ($\beta = 0.14$, $p$ for multiple regression $< 0.03$) were similar in patients and healthy subjects (table 2). Fibrinogen levels above 5 g/l were associated with an increased risk of DVT only in non-smokers (odds ratio 3.3, 95% CI 1.6–7.0).

The frequencies of the common alleles T1 (Taq I polymorphism), H1 (Hae III polymorphism) and B1 (Bcl I polymorphism) were similar in patients and healthy subjects (table 3). The distribution of genotypes was as expected for a population in Hardy-Weinberg equilibrium. The Hae III polymorphism was in linkage disequilibrium with the Bcl I polymorphism ($p < 0.001$, $D = 0.16$, $D' = 0.95$). Subjects with the rare T2 allele of the Taq I polymorphism, the H2 allele of the Hae III polymorphism or the B2 allele of the Bcl I polymorphism did not have a higher risk of DVT, with an odds ratio close to 1.

**Table 2.** Demographic data and risk factors of patients with DVT and healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Healthy subjects</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>42 ± 10</td>
<td>47 ± 17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Women/men</td>
<td>45/70</td>
<td>134/110</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smokers/non-smokers</td>
<td>41/73</td>
<td>92/152</td>
<td>0.75</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.8 ± 4.0</td>
<td>25.2 ± 3.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>6.2 ± 1.3</td>
<td>5.3 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>4.1 ± 1.0</td>
<td>3.3 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen, g/l</td>
<td>2.87 ± 0.82</td>
<td>2.82 ± 0.81</td>
<td>0.74</td>
</tr>
</tbody>
</table>

BMI = Body mass index.

**Table 3.** Frequencies of the common alleles of the Taq I, Hae III and Bcl I polymorphisms among patients and healthy subjects and the risk of DVT for heterozygous and homozygous carriers of the rare alleles

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients</th>
<th>Healthy subjects</th>
<th>Odds ratio (95% CI)</th>
<th>Population odds ratio detectable at $\alpha = 0.05$ with 80% power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq I polymorphism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1T1</td>
<td>62 (54.4)</td>
<td>130 (53.3)</td>
<td>0.95 (0.6–1.5)</td>
<td>&lt;0.50 or &gt;1.94</td>
</tr>
<tr>
<td>T1T2</td>
<td>44 (38.6)</td>
<td>97 (39.7)</td>
<td>0.99 (0.4–2.4)</td>
<td>&lt;0.09 or &gt;2.85</td>
</tr>
<tr>
<td>T2T2</td>
<td>8 (7.0)</td>
<td>17 (7.0)</td>
<td>0.88 (0.5–1.4)</td>
<td>&lt;0.48 or &gt;1.94</td>
</tr>
<tr>
<td>Allele frequency T1 (95% CI)</td>
<td>0.74 (0.66–0.82)</td>
<td>0.73 (0.67–0.79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hae III polymorphism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1H1</td>
<td>64 (56.1)</td>
<td>127 (52.0)</td>
<td>0.90 (0.6–1.4)</td>
<td>&lt;0.50 or &gt;1.94</td>
</tr>
<tr>
<td>H1H2</td>
<td>43 (37.7)</td>
<td>95 (39.0)</td>
<td>0.63 (0.3–1.6)</td>
<td>&lt;0.17 or &gt;2.64</td>
</tr>
<tr>
<td>H2H2</td>
<td>7 (6.1)</td>
<td>22 (9.0)</td>
<td>0.88 (0.5–1.4)</td>
<td>&lt;0.48 or &gt;1.94</td>
</tr>
<tr>
<td>Allele frequency H1 (95% CI)</td>
<td>0.75 (0.67–0.83)</td>
<td>0.72 (0.66–0.78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl I polymorphism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1B1</td>
<td>71 (62.3)</td>
<td>146 (59.8)</td>
<td>1.03 (0.4–2.5)</td>
<td>&lt;0.08 or 2.85</td>
</tr>
<tr>
<td>B1B2</td>
<td>35 (30.7)</td>
<td>82 (33.6)</td>
<td>0.88 (0.5–1.4)</td>
<td>&lt;0.48 or &gt;1.94</td>
</tr>
<tr>
<td>B2B2</td>
<td>8 (7.0)</td>
<td>16 (6.6)</td>
<td>1.03 (0.4–2.5)</td>
<td>&lt;0.08 or 2.85</td>
</tr>
<tr>
<td>Allele frequency B1 (95% CI)</td>
<td>0.78 (0.70–0.86)</td>
<td>0.77 (0.72–0.82)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses represent percentages, except where otherwise indicated.
Among healthy subjects, an association between fibrinogen and the Taq I polymorphism was noted (p = 0.06), but no influence of the Hae III and Bcl I polymorphisms on fibrinogen was observed (table 4). If only non-smoking healthy subjects were included, a trend towards lower fibrinogen associated with the T2 allele was apparent, but was not statistically significant, while the difference in fibrinogen for the different genotypes of the Hae III and Bcl I polymorphisms became significant (table 4).

No influence of the different genotypes of the Taq I, Hae III and Bcl I polymorphisms on fibrinogen was observed in patients with DVT, although in non-smoking patients, a trend toward lower fibrinogen associated with the T2 allele of the Taq I polymorphism was noted (table 5).

Table 4. Fibrinogen levels in 244 healthy subjects according to genotypes of the different polymorphisms

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All healthy subjects (n = 244)</th>
<th>Non-smoking healthy subjects (n = 152)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>fibrinogen level (g/l)</td>
</tr>
<tr>
<td>Taq I polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1T1</td>
<td>130</td>
<td>2.88 ± 0.84</td>
</tr>
<tr>
<td>T1T2</td>
<td>97</td>
<td>2.80 ± 0.74</td>
</tr>
<tr>
<td>T2T2</td>
<td>17</td>
<td>2.42 ± 0.83</td>
</tr>
<tr>
<td>Hae III polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1H1</td>
<td>127</td>
<td>2.77 ± 0.82</td>
</tr>
<tr>
<td>H1H2</td>
<td>95</td>
<td>2.95 ± 0.81</td>
</tr>
<tr>
<td>H2H2</td>
<td>22</td>
<td>2.63 ± 0.67</td>
</tr>
<tr>
<td>Bcl I polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1B1</td>
<td>146</td>
<td>2.76 ± 0.84</td>
</tr>
<tr>
<td>B1B2</td>
<td>82</td>
<td>2.96 ± 0.77</td>
</tr>
<tr>
<td>B2B2</td>
<td>16</td>
<td>2.69 ± 0.73</td>
</tr>
</tbody>
</table>

Fibrinogen levels are shown as mean ± standard deviation. The p values denote differences between groups (ANOVA).

Table 5. Fibrinogen levels in 114 patients with DVT according to genotypes of the different polymorphisms

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All patients (n = 114)</th>
<th>Non-smoking patients (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>fibrinogen level (g/l)</td>
</tr>
<tr>
<td>Taq I polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1T1</td>
<td>59</td>
<td>2.84 ± 0.79</td>
</tr>
<tr>
<td>T1T2</td>
<td>44</td>
<td>2.85 ± 0.80</td>
</tr>
<tr>
<td>T2T2</td>
<td>8</td>
<td>3.29 ± 1.06</td>
</tr>
<tr>
<td>Hae III polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1H1</td>
<td>64</td>
<td>2.91 ± 0.93</td>
</tr>
<tr>
<td>H1H2</td>
<td>42</td>
<td>2.82 ± 0.70</td>
</tr>
<tr>
<td>H2H2</td>
<td>7</td>
<td>2.80 ± 0.25</td>
</tr>
<tr>
<td>Bcl I polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1B1</td>
<td>70</td>
<td>2.88 ± 0.90</td>
</tr>
<tr>
<td>B1B2</td>
<td>34</td>
<td>2.88 ± 0.73</td>
</tr>
<tr>
<td>B2B2</td>
<td>8</td>
<td>2.83 ± 0.34</td>
</tr>
</tbody>
</table>

Fibrinogen levels are shown as mean ± standard deviation. The p values denote differences between groups (ANOVA).
Discussion

In this case-control study, the fibrinogen polymorphisms *Taq* I in α-fibrinogen and *Hae* III and *Bcl* I in β-fibrinogen did not represent major risk factors for DVT, although an influence of these polymorphisms on fibrinogen concentration was observed in healthy subjects.

The frequencies of the common alleles of the *Taq* I, *Hae* III and *Bcl* I polymorphisms in healthy subjects found in this study were similar to those previously reported [14, 22]. Furthermore, patients with DVT had similar allele frequencies to healthy subjects. Odds ratios for all genotypes were close to 1, and therefore, these polymorphisms were not associated with a higher risk of DVT. However, larger studies are needed that could detect smaller differences in odds ratios than was possible with our sample size.

Fibrinogen levels adjusted for age, gender and LDL cholesterol were similar in patients and in healthy subjects. Despite this, a statistically significant increase in thrombotic risk (odds ratio 3.3, 95% CI 1.6–7.0) was observed in non-smokers with fibrinogen levels above 5 g/l. If smokers were included, the significance was lost due to high fibrinogen levels in some healthy smokers. In the Leiden Thrombophilia Study, a four-fold increase in thrombotic risk was observed with fibrinogen levels above 5 g/l [5].

The *Taq* I polymorphism was associated with lower fibrinogen among healthy subjects. Although this association was not significant, a similar trend has been observed in previous studies in healthy subjects [15, 17] and in patients with cardiovascular disease [18, 19]. In our group of patients with DVT, this non-significant trend was observed only in non-smokers.

The *Hae* III polymorphism was associated with fibrinogen levels in healthy non-smokers, with the highest fibrinogen levels in heterozygotes, but no association was found in patients with DVT. For patients with DVT, our results confirmed the results of the Leiden Thrombophilia Study [5], in which fibrinogen was measured several months after the thromboembolic event, similarly to our study. In contrast, a significant association between the *Hae* III polymorphism and fibrinogen levels was reported by Renner et al. [6], but in their study, fibrinogen levels were measured during the acute phase of DVT. We have no clear explanation for why the mean fibrinogen level was the highest in heterozygote healthy non-smokers compared to homozygotes with or without the polymorphism.

A borderline association between the *Bcl* I polymorphism and fibrinogen levels was found in healthy non-smokers, but not in patients with DVT. A significant influence of this polymorphism on fibrinogen level was described in patients with coronary artery disease [18, 23, 24], but to our knowledge, this association has not yet been investigated in patients with DVT. For healthy subjects, the absence of this association was reported in two studies [16, 17], but both studies included non-smokers as well as smokers.

It is quite likely that besides age, gender, smoking, physical activity, obesity, diabetes and hyperlipidaemia [7–9], other factors influence the level of fibrinogen. It has been shown that fibrinogen shows seasonal variation, being significantly increased in the cold months [25–27]. Therefore, sampling of blood in different seasons might increase the variation in fibrinogen and mask the effect of genetic polymorphisms. Moreover, the effect of genetic polymorphisms on fibrinogen levels was observed in the acute stage of DVT [6], but not in the chronic stage of the disease [5], might suggest that gene-environment interactions have different levels of importance in different subject groups.

In conclusion, the fibrinogen polymorphisms *Taq* I, *Hae* III and *Bcl* I were equally distributed among patients with DVT and healthy subjects. Although these polymorphisms to some extent modulated plasma fibrinogen levels in healthy subjects, no such observation was evident for patients with DVT. These data suggest that the fibrinogen polymorphisms *Taq* I, *Hae* III and *Bcl* I are not major risk factors for DVT; however, further studies on larger groups of patients with DVT are needed.

Acknowledgements

This study was supported by the Slovenian Ministry of Education, Science and Sport (grant No. J3-3412). We are grateful to Mr. Gaj Vidmar from the Institute of Biomedical Informatics, Ljubljana, Slovenia, for statistical advice.
Fibrinogen Polymorphisms and DVT

Pathophysiol Haemost Thromb 2003;33:164–169

References


11. Thomas A, Lamhlu M, Humphries SE, Green FR: Linkage disequilibrium across the fibrinogen locus as shown by five genetic polymorphisms, G/A-455 (HaeIII), C/T-148 (HindIII/Alul), Y/G+1689 (Avall), and BclI (alpha-fibrinogen), and their detection by PCR. Hum Mutat 1994;3:79–81.


