Coagulation Factors II, V, VII, and X, Prothrombin Gene 20210G→A Transition, and Factor V Leiden in Coronary Artery Disease

High Factor V Clotting Activity Is an Independent Risk Factor for Myocardial Infarction


Abstract—Increased levels of hemostatic factors and genetic mutations of proteins involved in coagulation may play a role in the pathogenesis of coronary artery disease. We investigated clotting activity of factors II (FII:C), V (FV:C), VII (FVII:C), and X (FX:C), the prothrombin gene 20210G→A transition, and the factor V Leiden mutation in 200 survivors of myocardial infarction and in 100 healthy controls. FV:C (P<0.0001) and FVII:C (P<0.0001) were found to be independent risk factors for myocardial infarction. High FV:C or high FVII:C combined with smoking or arterial hypertension increased the relative risk for myocardial infarction up to 50-fold. One of 177 patients (0.6%) and 4 of 89 controls (4.5%) had the prothrombin 20210 AG genotype. Eleven of 177 patients (6.2%) and 6 of 89 controls (6.7%) were heterozygous for the factor V Leiden mutation. No homozygous carrier for these mutations was found. Neither the prothrombin gene 20210G→A transition (odds ratio [OR], 0.1; 95% confidence interval [CI], 0.01 to 1.1) nor the factor V Leiden mutation (OR, 1.0; 95% CI, 0.4 to 2.8) were associated with an increased relative risk for myocardial infarction. In conclusion, our data indicate that neither the prothrombin gene 20210G→A transition nor the factor V Leiden mutation are risk factors for myocardial infarction. High FVII:C was confirmed to be an independent risk factor for myocardial infarction. Moreover, we describe for the first time that high FV:C is an independent risk factor for myocardial infarction. (Arterioscler Thromb Vasc Biol. 1999;19:1020-1025.)

Key Words: coagulation factor V ■ prothrombin gene ■ factor V Leiden ■ myocardial infarction

Myocardial infarction and unstable angina pectoris are very common in Western countries. Several studies have clearly shown the pathogenetic role of local thrombotic occlusion in coronary arteries at the site of a ruptured plaque.1-3 The fact that high clotting activity of coagulation factor VII (FVII:C) and high plasma levels of fibrinogen are associated with an increased risk for coronary artery disease further corroborates the crucial role of blood coagulation in the pathogenesis of myocardial infarction.4-9 The causes of elevated FVII:C and elevated plasma levels of fibrinogen are still a matter of debate. Aside from FVII:C and fibrinogen, other hemostatic factors are under investigation for their possible role as risk factors for coronary artery disease.6,8,10,11

Recently, a novel inherited risk factor for venous thrombosis was identified.12 A G→A transition at nucleotide 20210 in the 3’ untranslated region of the prothrombin gene was associated with a higher prothrombin clotting activity and a 2.7-fold increased risk for venous thrombosis. Other groups reported similar findings.13-20 The role of the prothrombin gene 20210A variant in arterial disease is not established yet. Several investigators reported a significantly increased prevalence of 1.8% to 12.5% of the prothrombin gene 20210A variant in patients with arterial disease (coronary artery disease and cerebrovascular disease) compared with newborns or age-matched controls,18,21-24 and a 4.0-fold increased risk for myocardial infarction in young women with the variant.25 Others found no increased prevalence of the prothrombin gene 20210A variant in patients with arterial disease compared with age- and sex-matched controls.16,20,26

A single base mutation in which adenine is substituted for guanine at nucleotide 1691 in the gene coding for coagulation factor V resulting in the amino acid substitution 506 Arg→Gln is the cause of activated protein C (APC) resistance.27 Its relation to coronary artery disease is still controversial. Several investigators found a significant association between factor V Leiden and coronary artery disease,28-30 or
found an increased prevalence of APC resistance in stroke patients, whereas other groups reported no association of APC resistance or factor V Leiden with coronary artery disease or ischemic stroke, respectively.

In the present study we investigated in a case control design the possible association of the clotting activity of coagulation factors II, V, VII, and X, the prothrombin 20210A allele, and the factor V Leiden with myocardial infarction.

Methods

Patients, Controls, and Blood Samples

We investigated 200 (174 males, 26 females) survivors of myocardial infarction and 100 (87 males, 13 females) healthy controls. One control of the same sex and the same age (±5 years) was selected for every 2 patients. Patients were selected from the files of the Division of Cardiology of the University Hospital of Bern. Myocardial infarction had occurred at least 2 months before investigation. All patients except 2 had undergone coronary angiography. One-, two-, or three-vessel disease was present in 33.8%, 36.4%, and 26.8%, respectively, whereas 3% had angiographically normal coronary arteries. Patients and controls were considered smokers if they had smoked cigarettes >5 pack years. Arterial hypertension and diabetes mellitus were diagnosed according to the patient’s history and medical treatment. Body mass index (BMI) was available from 200 patients and 89 controls. This study was approved by the Ethics Committee of the University of Bern.

Blood was drawn from an antecubital vein with a 19-gauge butterfly needle and was collected into 2 10-mL plastic syringes (Monovette®, Sarstedt) containing 1 mL of 0.106 mol/L trisodium citrate. Plasma was prepared by centrifugation at 1500g for 10 minutes at 15°C to 18°C and was stored in polypropylene tubes at 4°C. Purified DNA in 500 mmol/L KCl, 25 mmol/L MgCl₂, 10 mmol/L Tris-HCl (pH 8.4), and 0.2 mmol/L ethidium bromide. PCR-products were subjected to a restriction digest with the corresponding restriction enzyme.

DNA Preparation

Preparation of genomic DNA from EDTA blood was performed using a commercial kit according to the manufacturer’s instructions (QiAmp® Blood Kit, QIAGEN Inc.).

Prothrombin Gene Genotype Analysis

A 345-bp fragment including the 3' untranslated region where the 20210G→A transition is located was enzymatically amplified as described. PCR-products were subjected to a restriction digest with HindIII (New England Biolabs) and then analyzed by polyacrylamide gel electrophoresis.

Factor V Leiden Genotype Analysis

The G1691A mutation in exon 10 was detected by the loss of a cleavage site for MnlI. A 286-bp fragment was amplified from genomic DNA using a commercial primer-mix (COASET® FV-506, Chromogenix). The PCR conditions were as follows: 5 μL of purified DNA in 5 μL of Tris-KCl-MgCl₂ buffer (100 mmol/L Tris, 500 mmol/L KCl, 25 mmol/L MgCl₂), 10 μL primer-mix, 6.6 μL 1.5 mmol/L of each nucleotide (dNTP-mix (Boehringer Mannheim), 23 μL distilled H₂O, and 2 U of Taq polymerase.

Chromogenix). The PCR conditions were as follows: 500 mmol/L KCl, 25 mmol/L MgCl₂), 10 μL primer-mix, 6.6 μL 1.5 mmol/L of each nucleotide (dNTP-mix (Boehringer Mannheim), 23 μL distilled H₂O, and 2 U of Taq polymerase (Life Technologies) overlaid with 50 μL of paraffin oil was heated in a Perkin Elmer Cetus DNA thermal cycler to 94°C for 5 minutes followed by 30 cycles of 93°C for 60 seconds, 62°C for 30 seconds, and 72°C for 90 seconds followed by a final 10 minutes at 72°C. Aliquots containing 17.5 μL PCR-product and 2 μL distilled water were digested for 3 hours at 37°C with 0.5 μL containing 1 U of MnlI (Fermentas Ltd). The fragments measuring 37-bp, 93-bp, and 156-bp for the 1691G allele, and 130-bp and 156-bp for the 1691A allele, were separated on 4% agarose gels and visualized with ethidium bromide.

Modified APC Resistance

Resistance to APC was assessed using a commercial activated partial thromboplastin time-based APC resistance assay (COATEST® APC™ Resistance V, Chromogenix) on a Fibrinintimer (Behringwerke). The patient plasma was diluted with 4 volumes of factor V-deficient plasma according to the manufacturer’s recommendation. Response to APC was expressed as the APC sensitivity ratio (ie, the quotient of the clotting time in the presence of APC divided by the clotting time obtained in the absence of APC). Factor V R506Q was diagnosed according to our APC sensitivity ratio as house cutoffs (normal at least 1.3 and ≤1.9, and homozygous ≥1.15).

Statistics

Medians or proportions were calculated for patients and controls for cardiovascular risk factors. The significance of any difference in medians was tested using the Mann-Whitney U-test (MWU), and the significance of any difference in proportions was tested using χ² statistics. All probability values were 2-tailed and probability values below 0.05 were considered statistically significant. Statistical analysis was done using SigmaStat, version 1.0 (Jandel). Odds ratios (ORs) were calculated as a measure of relative risk in the standard unmatched fashion. Odds ratios (ORs) and their 95% CI were used to describe the association between coronary artery disease and prothrombin gene 20210G→A transition, factor V Leiden mutation, FII:C, FV:C, FVII:C, and FX:C, respectively. To adjust for the effects of other coronary risk factors, we used logistic regression. Adjustments were made for the dichotomized risk factors sex, smoking status (yes/no), arterial hypertension (yes/no), diabetes mellitus (yes/no), and for age, cholesterol, and fibrinogen. Inclusion of BMI did not affect the results. Since BMI was not available for all controls, we excluded this variable from the final analysis. Logistic regression analysis was carried out with the SAS statistical package, release 6.12 (SAS Institute).

Results

Patients and Controls

We investigated 200 survivors of myocardial infarction and 100 healthy controls. Table 1 shows cardiovascular risk factors for patients and controls. The prevalence of arterial hypertension, diabetes mellitus, and smoking status (including former smokers) was significantly higher in patients than in controls. Median values of total cholesterol and fibrinogen were significantly higher in the patient group compared with the control group.

Factor II

We found no significant difference (P=0.977) between FII:C of the nonanticoagulated patients (n=129) and the control group (Table 2). High FII:C showed no association with myocardial infarction (Table 3). Three of the controls with the prothrombin gene 20210 GA genotype were in the highest ratio (≥102%) and 1 in the second lowest (90% to 94%), respectively. The patient with the prothrombin gene 20210G→A transition was anticoagulated and his FII:C value was therefore not analyzed.
**TABLE 1. Age, Sex, and Major Cardiovascular Risk Factors in Patients and Controls**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=200)</th>
<th>Controls (n=100)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) median (range)</td>
<td>57 (32–72)</td>
<td>56 (32–74)</td>
<td>0.35*</td>
</tr>
<tr>
<td>Body mass index median (range)</td>
<td>26.1 (20.6–43.3)</td>
<td>24.7 (18.6–39.1)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>48%</td>
<td>11%</td>
<td>0.001†</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>21%</td>
<td>0%</td>
<td>—</td>
</tr>
<tr>
<td>Smoker</td>
<td>74%</td>
<td>36%</td>
<td>0.0001†</td>
</tr>
<tr>
<td>Cholesterol (mmol/L) median (range)</td>
<td>5.97 (3.09–9.75)</td>
<td>5.76 (3.72–8.65)</td>
<td>0.008*</td>
</tr>
<tr>
<td>Fibrinogen (g/L) median (range)</td>
<td>2.8 (1.8–4.8)</td>
<td>2.6 (1.7–4.5)</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

*Mann-Whitney U-test, †Chi-square test.

**Factor V**
We found significantly elevated FV:C levels among the 200 patients compared with the controls (Table 2). Analysis of the relative risk for myocardial infarction associated with FV:C levels revealed that subjects in the highest quartile (>109%) had a 3.3-fold (95% CI, 1.8 to 6.6) increased risk compared with those in the first quartile (≤96%) (Table 3). This association was present in both the nonsmoking and the smoking subgroups (Table 4) and remained significant after correction for lipid and nonlipid vascular risk factors (Table 3). Smokers with FV:C levels in the highest quartile had a 10.5-fold (95% CI, 4.1 to 26.5) increased risk for myocardial infarction compared with nonsmokers in the lowest quartile. Furthermore, FV:C levels in the highest quartile were associated with an OR of 3.7 (95% CI, 1.6 to 8.4) and 2.1 (95% CI, 0.3 to 13.8) among subjects without arterial hypertension and among those with hypertension, respectively. Arterial hypertension combined with FV:C levels in the highest quartile was associated with a 27.6-fold (95% CI, 7.2 to 104.6) increased risk for myocardial infarction compared with the absence of arterial hypertension and FV:C levels in the lowest quartile (Table 4).

**Factor VII**
FVII:C levels among the 133 nonanticoagulated patients were significantly elevated when compared with the 100 controls (Table 2). Subjects with FVII:C levels in the highest quartile (>110%) were found to have a 5.2-fold (95% CI, 2.4 to 11.2) increased risk for myocardial infarction compared with those in the first quartile (≤93%) (Table 3). This association was unchanged when analyzed separately among nonsmokers and smokers (data not shown) and remained significant after correction for lipid and nonlipid vascular risk factors (Table 3). Smokers with FVII:C levels in the highest quartile had a 29.4-fold (95% CI, 8.8 to 98.1) increased risk for myocardial infarction in comparison to nonsmokers with FVII:C in the lowest quartile. Moreover, FVII:C levels in the highest quartile in the absence or in the presence of arterial hypertension, respectively, were associated with a 5.6-fold (95% CI, 2.2 to 14.3) and a 2.9-fold (95% CI, 0.4 to 22.9) increased risk for myocardial infarction (data not shown). High FVII:C levels combined with hypertension were associated with a 48.6-fold (95% CI, 9.6 to 244.7) increased risk for myocardial infarction compared with low FVII:C in the absence of arterial hypertension (data not shown).

**Factor X**
Levels of FX:C were significantly higher (P=0.0054) in nonanticoagulated patients (n=129) compared with those of the control group (Table 2). Patients with FX:C levels in the highest quartile (>109%) had a 2.2-fold (95% CI, 1.03 to 4.52) increased risk for myocardial infarction compared with those who had FX:C levels in the lowest quartile (≤90%) (Table 3). However, this association was not significant after adjustment for possible confounders (Table 3).

**Prothrombin Gene 20210G→A Transition**
One of 177 patients (0.6%; 95% CI, 0% to 1.6%) and 4 of 89 controls (4.5%; 95% CI, 0.2% to 8.8%) had the prothrombin 20210G→A genotype; no subject was homozygous (AA genotype). The AG genotype was not associated with an increased risk for myocardial infarction as shown in Table 5 (OR, 0.1; 95% CI, 0.01 to 1.1).

**Factor V Leiden Mutation and Modified APC Resistance**
A low APC sensitivity ratio (>1.3 and ≤1.9) corresponding to the factor V R506Q mutation was found in 12 of 200 patients (6%; 95% CI, 2.7% to 9.3%) and in 6 of 100 controls (6%; 95% CI, 1.3% to 10.7%). Out of the 177 patients and the 89 controls from whom DNA was available, the factor V Leiden mutation was found. As shown in Table 5, the factor V Leiden mutation was not associated with an increased risk for myocardial infarction compared with low FVII:C (OR, 0.1; 95% CI, 0.01 to 1.1).

**Discussion**
In the present study, we investigated the association of the FII:C, FV:C, FVII:C, and FX:C levels, of the prothrombin gene 20210G→A transition, and of the factor V Leiden mutation with myocardial infarction.
Our main finding was a strong and independent association between high FV:C levels and myocardial infarction. FV:C levels were significantly elevated in patients compared with controls (Table 2). Subjects with FV:C in the highest quartile (>109%) had a 3-fold increased risk for myocardial infarction (Table 3). This association remained significant after adjustment for possible confounders (Table 3). High FV:C levels combined with smoking or arterial hypertension increased the risk for myocardial infarction up to 27-fold (Table 4). To the best of our knowledge, this is the first report showing FV:C to be an independent risk factor for myocardial infarction.

Furthermore, we confirmed that elevated FVII:C levels are an independent risk factor for myocardial infarction (Table 3), which is in agreement with data from the Northwick Park Heart Study4 and the third Glasgow MONICA Survey II study.9 Moreover, we found up to 50-fold increased relative risk for myocardial infarction for high FVII:C levels in combination with smoking or arterial hypertension (data not shown). However, as discussed by others,35 on the basis of this case-control study, we cannot rule out the possibility that elevated FVII:C or FV:C levels are consequence of, rather than the cause of, coronary artery disease.


<table>
<thead>
<tr>
<th></th>
<th>Quartiles</th>
<th>Patients</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value*</th>
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<tr>
<td>FII:C (%)</td>
<td>89–89</td>
<td>39</td>
<td>24</td>
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<td></td>
<td>90–94</td>
<td>16</td>
<td>26</td>
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<td>95–102</td>
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<td>25</td>
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<td></td>
<td>&gt;102</td>
<td>34</td>
<td>25</td>
<td>0.8</td>
<td>0.41–1.73</td>
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<tr>
<td>FV:C (%)</td>
<td>90–109</td>
<td>32</td>
<td>25</td>
<td>1.0</td>
<td>0.006</td>
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<tr>
<td></td>
<td>97–103</td>
<td>32</td>
<td>23</td>
<td>1.1</td>
<td>0.51–2.30</td>
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<td></td>
<td>104–109</td>
<td>29</td>
<td>27</td>
<td>0.8</td>
<td>0.40–1.76</td>
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<tr>
<td></td>
<td>&gt;109</td>
<td>107</td>
<td>25</td>
<td>3.3</td>
<td>1.76–6.60</td>
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<tr>
<td>FVII:C (%)</td>
<td>93–93</td>
<td>18</td>
<td>25</td>
<td>1.0</td>
<td>0.0001</td>
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<td>13</td>
<td>26</td>
<td>0.7</td>
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<td>101–110</td>
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<td>0.45–2.38</td>
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<tr>
<td></td>
<td>&gt;110</td>
<td>82</td>
<td>22</td>
<td>5.2</td>
<td>2.40–11.15</td>
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<tr>
<td>FX:C (%)</td>
<td>90–90</td>
<td>25</td>
<td>24</td>
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<td>91–98</td>
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<td>25</td>
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<tr>
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<td>&gt;109</td>
<td>54</td>
<td>24</td>
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<td>1.03–4.52</td>
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<td>Fibrinogen (g/L)</td>
<td>&lt;2.2</td>
<td>16</td>
<td>24</td>
<td>1.0</td>
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<td>2.3–2.5</td>
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<td>22</td>
<td>2.3</td>
<td>0.98–5.17</td>
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<tr>
<td></td>
<td>2.6–2.8</td>
<td>61</td>
<td>29</td>
<td>3.2</td>
<td>1.46–6.83</td>
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<tr>
<td></td>
<td>&gt;2.8</td>
<td>90</td>
<td>25</td>
<td>5.4</td>
<td>2.49–11.69</td>
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</tr>
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</table>

*P-value of logistic regression after correction for possible confounders (age, sex, smoking habit, arterial hypertension, diabetes mellitus, cholesterol, and fibrinogen).
FX:C levels were significantly elevated in patients compared with controls (Table 2). Patients with FX:C levels in the highest quartile (>20%) had a 2-fold increased risk for myocardial infarction (Table 3). This association was not significant, however, after adjustment for lipid and nonlipid vascular risk factors (Table 3).

The prothrombin gene 20210G→A transition has been described as an independent risk factor for venous thrombosis. Carriers of the mutation tend to have higher prothrombin levels than noncarriers. Moreover, high prothrombin clotting activity, also in the absence of the 20210G→A transition, are associated with an increased risk for venous thrombosis. Our data show that the prevalence of the prothrombin gene 20210G→A transition is not increased in patients with myocardial infarction (0.6%) compared with healthy controls (4.5%) (Table 5). This result is in agreement with other reports indicating that the prothrombin 20210G→A transition should not be considered a risk factor for myocardial infarction in the general population. The prevalence of the prothrombin 20210 GA genotype among our Swiss healthy controls (4.5%) is rather high compared with that in other European countries such as Sweden (1.8%), England (0.7% to 2.6%), Netherlands (1.2% to 2.3%), Austria (2%), Spain (1.4%), and Italy (4%). We found that the FII:C levels were similar among patients and controls and were not associated with myocardial infarction (Table 2).

Factor V Leiden is known to be a common risk factor for venous thrombosis but it is still debated whether this mutation is associated with arterial thromboembolism. Our data show no association between factor V Leiden and myocardial infarction (Table 5). This is in agreement with several other studies, indicating that factor V Leiden is not a risk factor for coronary artery disease or ischemic cerebrovascular disease. However, a recent report showed a relatively high prevalence of the factor V Leiden mutation in young female smokers who had suffered from myocardial infarction.

In conclusion, our findings indicate that neither the factor V R506Q mutation nor the prothrombin gene 20210G→A transition are associated with myocardial infarction. We show for the first time that high FV:C is an independent risk factor for myocardial infarction, and confirm that high levels of FVII:C are an independent risk factor. Neither FII:C nor FX:C were found to be independent risk factors for myocardial infarction. Our data suggest that combinations of high coagulation factors (FV:C or FVII:C) and clinical cardiovascular risk factors (smoking, arterial hypertension) may result in more than additive risk for myocardial infarction. Further studies are needed to define the role of FV:C levels in coronary artery disease.

Acknowledgments

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