

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

M. Redondo, H.H. Watzke, B. Stucki, I. Sulzer, F. Demarmels Biasiutti, B.R. Binder, M. Furlan, B. Lämmle, W.A. Wuillemin

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

Received May 25, 1998; revision accepted September 25, 1998.

From the Central Hematology Laboratory, Inselspital, University Hospital Bern, Switzerland (M.R., B.S., I.S., F.D.B., M.F., B.L., W.A.W.); the Department of Hematology and Hemostaseology (H.H.W.), and the Department of Vascular Biology and Thrombosis Research (B.R.B.), University of Vienna, Austria.

Correspondence to Walter A. Wuillemin, MD, PhD, Central Hematology Laboratory, University Hospital, Inselspital, CH-3010 Bern, Switzerland. E-mail wwuillemin@insel.ch

© 1999 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>

found an increased prevalence of APC resistance in stroke patients,³¹ whereas other groups reported no association of APC resistance or factor V Leiden with coronary artery disease³¹⁻³³ or ischemic stroke,^{24,26,32} 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 twice for 10 minutes at 15°C to 18°C and was stored in polypropylene tubes at -70°C . A sample of 10 mL blood, collected into EDTA (Monovette[®], Sarstedt) and stored at -70°C , was available from 177 patients and 89 controls.

Coagulation Assays

Prothrombin time (PT) was performed using Thromborel[®]-S (Behringwerke). The clotting activity of FII (FII:C), FV (FV:C), FVII (FVII:C) or FX (FX:C) was measured by PT-based assays using the respective deficient substrate plasmas on a Fibrinometer and expressed as percentage of a normal human plasma pool (NHP). NHP was prepared from 42 healthy male volunteers and stored in small aliquots in liquid nitrogen. NHP was used as a standard for measurement of clotting factors, and was defined to contain 100% of clotting activity. Fibrinogen was determined according to the method of Clauss.³⁴

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 \rightarrow A transition is located was enzymatically amplified as described.^{12,21} PCR-products were subjected to a restriction digest with *Hind*III (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 *Mn*II. 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 (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 *Mn*II (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 assayed using a commercial activated partial thromboplastin time-based APC resistance assay (COATEST[®] APC[™] Resistance V, Chromogenix) on a Fibrinometer (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 in-house cutoffs (normal ≥ 2.2 , heterozygous > 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 χ^2 statistics. All probability values are 2-tailed and probability values below 0.05 were considered statistically significant. Statistical analysis was done using SigmaStat, version 1.0 (Jandell). Odds ratios (ORs) were calculated as a measure of relative risk in the standard unmatched fashion. Confidence intervals (CI) were calculated at the 95% level. ORs (and their 95% CI) were used to describe the association between coronary artery disease and prothrombin gene 20210G \rightarrow 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 FII:C quartile ($>102\%$) and 1 in the second lowest (90% to 94%), respectively. The patient with the prothrombin gene 20210G \rightarrow 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

	Patients (n=200) Males=174 Females=26	Controls (n=100) Males=87 Females=13	P-value
Age (years) median (range)	57 (32–72)	56 (32–74)	0.35*
Body mass index median (range)	26.1 (20.6–43.3)	24.7 (18.6–39.1)	0.002*
Arterial hypertension	48%	11%	0.001†
Diabetes mellitus	21%	0%	
Smoker	74%	36%	0.0001†
Cholesterol (mmol/L) median (range)	5.97 (3.09–9.75)	5.76 (3.72–8.65)	0.008*
Fibrinogen (g/L) median (range)	2.8 (1.8–4.8)	2.6 (1.7–4.5)	0.0001*

*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 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

TABLE 2. Clotting Activity of the Coagulation Factors II, V, VII, and X in Patients and Controls

	Patients	Controls	P-values*
FII:C (%)	96 (41–127)	95 (68–123)	0.977
FV:C (%)	111 (67–186)	103 (77–148)	<0.0001
FVII:C (%)	118 (66–204)	100 (74–144)	<0.0001
FX:C (%)	108 (28–153)	99 (59–142)	0.0054

The clotting activities were determined in 100 controls and 200 patients (FV:C), or in 129 non-anticoagulated patients (FII:C, FX:C), or 133 non-anticoagulated patients (FVII:C), respectively. Values are presented as median (range). *P-values were assessed using Mann-Whitney U-test.

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 20210 AG 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 detected in 11 patients (6.2%; 95% CI, 2.7% to 9.8%) and 6 controls (6.7%; 95% CI, 1.5% to 12%), respectively. All 11 patients and 6 controls with the factor V R506Q had APC sensitivity ratios <1.9, whereas 166 patients and 83 controls with normal factor V genotype had APC sensitivity ratios ≥2.2. No homozygous carrier for 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, either when assessed indirectly using the modified APC resistance assay (OR, 1.0; 95% CI, 0.4 to 2.8) or when assessed by factor V genotyping (OR, 0.9; 95% CI, 0.3 to 2.6).

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.

TABLE 3. Relative Risk of Myocardial Infarction in the Quartiles of the Coagulation Factors FII:C, FV:C, FVII:C, FX:C, and Fibrinogen

	Quartiles	Patients	Controls	OR	95% CI	P-value*
FII:C (%)	≤89	39	24	1.0		0.22
	90–94	16	26	0.4	0.17–0.85	
	95–102	40	25	1.0	0.48–2.01	
	>102	34	25	0.8	0.41–1.73	
FV:C (%)	≤96	32	25	1.0		0.006
	97–103	32	23	1.1	0.51–2.30	
	104–109	29	27	0.8	0.40–1.76	
	>109	107	25	3.3	1.76–6.60	
FVII:C (%)	≤93	18	25	1.0		0.0001
	94–100	13	26	0.7	0.28–1.71	
	101–110	20	27	1.0	0.45–2.38	
	>110	82	22	5.2	2.40–11.15	
FX:C (%)	≤90	25	24	1.0		0.51
	91–98	21	25	0.8	0.36–1.81	
	99–109	29	27	1.0	0.48–2.22	
	>109	54	24	2.2	1.03–4.52	
Fibrinogen (g/L)	≤2.2	16	24	1.0		0.0038
	2.3–2.5	33	22	2.3	0.98–5.17	
	2.6–2.8	61	29	3.2	1.46–6.83	
	>2.8	90	25	5.4	2.49–11.69	

*P-value of logistic regression after correction for possible confounders (age, sex, smoking habit, arterial hypertension, diabetes mellitus, cholesterol, and fibrinogen).

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,^{3,5} 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 4. Relative Risk for Myocardial Infarction of High FV:C Levels Depending on Smoking Status or Arterial Hypertension

FV:C	1. Quartile	1. Quartile	4. Quartile	4. Quartile
Smoking	–	+	–	+
Patients (n)	13	19	21	86
Controls (n)	19	6	13	12
OR	1.0	4.6	2.4	10.5
95% CI		1.5–14.7	0.8–6.3	4.1–26.5
OR		1.0		2.3
95% CI				0.8–6.8
FV:C	1. Quartile	1. Quartile	4. Quartile	4. Quartile
Hypertension	–	+	–	+
Patients (n)	15	17	53	54
Controls (n)	23	2	22	3
OR	1.0	13.0	3.7	27.6
95% CI		2.6–64.8	1.6–8.4	7.2–104.6
OR		1.0		2.1
95% CI				0.3–13.8

TABLE 5. Prevalence of the Prothrombin Gene 20210G→A Transition and Factor V Leiden Mutation Among Patients and Controls, and Relative Risk for Myocardial Infarction

	Patients (n=177)	Controls (n=89)	OR	95% CI
Prothrombin gene 20210 genotype				
Normal GG	176	85		
Heterozygous GA	1	4	0.1	0.01–1.1
Homozygous AA	0	0		
Factor V 506 genotype				
Normal RR	166	83		
Heterozygous RQ	11	6	0.9	0.3–2.6
Homozygous QQ	0	0		

FX:C levels were significantly elevated in patients compared with controls (Table 2). Patients with FX:C levels in the highest quartile (>109%) 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.¹² Carriers of the mutation tend to have higher prothrombin levels than noncarriers.^{12,16,17,19} Moreover, high prothrombin clotting activity, also in the absence of the 20210G→A transition, are associated with an increased risk for venous thrombosis.¹² 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^{16,20} 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%),¹⁴ England (0.7% to 2.6%),^{13,15,19} Netherlands (1.2% to 2.3%),^{12,23} Austria (2%),²¹ Spain (1.4%),²⁰ and Italy (4%).¹⁶ 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^{31,32,36} or ischemic cerebrovascular disease.³² 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.³⁰

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

This study was supported by a grant from the Swiss National Foundation for Scientific Research (3200-047016.96), and by a grant from the Department of Clinical Research, University Hospital, Inselspital, Bern, Switzerland.

References

- Falk E. Unstable angina with fatal outcome; dynamic coronary thrombosis leading to infarction and/or sudden death. *Circulation*. 1985; 71:699–708.
- Sherman CT, Litvack F, Grundfest W. Coronary angiography in patients with unstable angina pectoris. *N Engl J Med*. 1986;315:913–919.
- Davies MJ. The contribution of thrombosis to the clinical expression of coronary atherosclerosis. *Thromb Res*. 1996;82:1–32.
- Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WRS, Haines AP, Stirling Y, Imeson JD, Thompson SG. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet*. 1986;2:533–537.
- Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. *JAMA*. 1987;258:1183–1186.
- Miller GJ, Bauer KA, Barzegar S, Cooper JA, Rosenberg RD. Increased activation of the haemostatic system in men at high risk of fatal coronary artery disease. *Thromb Haemost*. 1996;75:767–771.
- Stormorken H, Sakariassen KS. Hemostatic risk factors in arterial thrombosis and atherosclerosis: The thrombin-fibrin and platelet-vWF axis. *Thromb Res*. 1997;88:1–25.
- Pankov JS, Folsom AR, Province MA, Rao DC, Eckfeldt J, Heiss G, Shahar E, Wu KK. Family history of coronary heart disease and hemostatic variables in middle-aged adults. *Thromb Haemost*. 1997;77:87–93.
- Woodward M, Lowe GDO, Rumley A, Tunstall-Pedoe H, Phillipou H, Lane DA, Morrison CE. Epidemiology of coagulation factors, inhibitors and activation markers: The Third Glasgow MONICA Survey II. Relationships to cardiovascular risk factors and prevalent cardiovascular disease. *Br J Haematol*. 1997;97:785–797.
- Cortellaro M, Boschetti C, Cofrancesco E, Zanussi C, Catalano M, de Gaetano G, Gabrielli L, Lombardi B, Specchia G, Tavazzi L, Tremoli E, della Volpe A, Polli E. The PLAT Study. Hemostatic function in relation to atherothrombotic ischemic events in vascular disease patients. Principal results. *Arterioscler Thromb*. 1992;12:1063–1070.
- Conlan MG, Folsom AR, Finch A, Davis CE, Sorlie P, Marucchi G, Wu KK. Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. *Thromb Haemost*. 1993;70:380–385.
- Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996;88:3698–3703.
- Cumming AM, Keeney S, Salden A, Bhavnani M, Shwe KH, Hay CRM. The prothrombin gene G20210A variant: prevalence in a U. K. anticoagulant clinic population. *Br J Haematol*. 1997;98:353–355.
- Hillarp A, Zöller B, Svensson PJ, Dahlbäck B. The 20210 A allele of the prothrombin gene is a common risk factor among Swedish outpatients with verified deep venous thrombosis. *Thromb Haemost*. 1997;78:990–992.
- Brown K, Luddington R, Williamson D, Baker P, Baglin T. Risk of venous thromboembolism associated with a G to A transition at position 20210 in the 3'-untranslated region of the prothrombin gene. *Br J Haematol*. 1997;98:907–909.
- Ferraresi P, Marchetti C, Legnani C, Cavallari E, Castoldi E, Mascoli F, Ardissino D, Palareti G, Bernardi F. The heterozygous 20210 G/A prothrombin genotype is associated with early venous thrombosis in inherited thrombophilias and is not increased in frequency in artery disease. *Arterioscler Thromb Vasc Biol*. 1997;17:2418–2422.
- Kapur RK, Mills LA, Spitzer SG, Hultin MB. A prothrombin gene mutation is significantly associated with venous thrombosis. *Arterioscler Thromb Vasc Biol*. 1997;17:2875–2879.
- Arruda VR, Annichino-Bizzacchi JM, Goncalves MS, Costa FF. Prevalence of the prothrombin gene variant (nt20210A) in venous thrombosis and arterial disease. *Thromb Haemost*. 1997;78:1430–1433.
- Makris M, Preston FE, Beauchamp NJ, Cooper PC, Daly ME, Hampton KK, Bayliss P, Peake IR, Miller GJ. Co-inheritance of the 20210A allele of the prothrombin gene increases the risk of thrombosis in subjects with familial thrombophilia. *Thromb Haemost*. 1997;78:1426–1429.
- Corral J, Gonzalez-Conjeiro R, Lozano ML, Rivera J, Heras I, Vicente V. The venous thrombosis risk factor 20210 A allele of the prothrombin gene is not a major risk factor for arterial thrombotic disease. *Br J Haematol*. 1997;99:304–307.
- Watzke HH, Schüttrumpf J, Graf S, Huber K, Panzer S. Increased prevalence of a polymorphism in the gene coding for human prothrombin in patients with coronary heart disease. *Thromb Res*. 1997;87:521–526.
- Arruda VR, Siquiera LH, Chiapparini LC, Coelho OR, Mansur AP, Ramires A, Annichino-Bizzacchi JM. Prevalence of the prothrombin gene variant 20210 G→A among patients with myocardial infarction. *Cardiovasc Res*. 1998;37:42–45.
- Doggen CMJ, Cats VM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors. Increased risk of

- myocardial infarction associated with factor V Leiden or prothrombin 20210A. *Circulation*. 1998;97:1037-1041.
24. De Stefano V, Chiusolo P, Paciaroni K, Casorelli I, Rossi E, Molinari M, Servidei S, Tonali PA, Leone G. Prothrombin G20210A mutant genotype is a risk factor for cerebrovascular ischemic disease in young patients. *Blood*. 1998;91:3562-3565.
 25. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. *Blood*. 1997;90:1747-1750.
 26. Longstreth WT, Rosendaal FR, Siscovick DS, Vos HL, Schwartz SM, Psaty BM, Raghunathan TE, Koepsell TD, Reitsma PH. Risk of stroke in young women and two prothrombotic mutations: Factor V Leiden and prothrombin gene variant (G20210A). *Stroke*. 1998;29:577-580.
 27. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature*. 1994;369:64-67.
 28. März W, Seysewitz H, Winkelmann B, Chen M, Nauck M, Witt I. Mutation in coagulation factor V associated with resistance to activated protein C in patients with coronary artery disease. *Lancet*. 1995;344:526-527.
 29. Holm J, Zöller B, Berntorp E, Erhardt L, Dahlbäck B. Prevalence of factor V gene mutation amongst myocardial infarction patients and healthy controls is higher in Sweden than in other countries. *J Intern Med*. 1996;239:221-226.
 30. Rosendaal FR, Siscovick DS, Schwartz SM, Beverly RK, Psaty BM, Longstreth Jr WT, Raghunathan TE, Koepsell TD, Reitsma PH. Factor V Leiden (Resistance to activated protein C) increases the risk of myocardial infarction in young women. *Blood*. 1997;89:2817-2821.
 31. van der Bom JG, Bots ML, Haverkate F, Slagboom E, Grobbee DE, Kluit C. Reduced response to activated protein C is associated with increased risk for cerebrovascular disease. *Ann Intern Med*. 1996;125:265-269.
 32. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke and venous thrombosis in apparently healthy men. *N Engl J Med*. 1995;332:912-919.
 33. Demarmels Biasiutti F, Merlo C, Furlan M, Sulzer I, Binder BR, Lämmle B. No association of APC resistance with myocardial infarction. *Blood Coagul Fibrinolysis*. 1995;6:456-459.
 34. Clauss A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. *Acta Haematol*. 1957;17:237-246.
 35. Doggen CJM, Manger Cats V, Bertina RM, Reitsma PH, Vandenbroucke JP, Rosendaal FR. A genetic propensity to high factor VII is not associated with the risk of myocardial infarction in men. *Thromb Haemost*. 1998;80:281-285.
 36. Emmerich J, Poirier O, Evans A, Marques-Vidal P, Arveiler D, Luc G, Aiach M, Cambien F. Myocardial infarction, Arg506 to Gln factor V mutation, and activated protein C resistance. *Lancet*. 1995;345:321-322.