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Carine J. M. Doggen, Volkert Manger Cats, Rogier M. Bertina and Frits R. Rosendaal  
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# Interaction of Coagulation Defects and Cardiovascular Risk Factors

## Increased Risk of Myocardial Infarction Associated With Factor V Leiden or Prothrombin 20210A

Carine J.M. Doggen, MSc; Volkert Manger Cats, MD; Rogier M. Bertina, PhD;  
Frits R. Rosendaal, MD

**Background**—A genetic variation located in the 3′-untranslated region of the prothrombin gene (prothrombin 20210 G→A) was recently described as a risk factor for venous thrombosis. We examined how the presence of this mutation affected the risk of myocardial infarction in a population-based case-control study. Furthermore, we studied the risk of myocardial infarction associated with the simultaneous presence of a coagulation defect (ie, the 20210 AG genotype of prothrombin or the factor V Leiden mutation) and major cardiovascular risk factors.

**Methods and Results**—Among 560 men with a first myocardial infarction before the age of 70 years, 1.8% were heterozygous carriers of the 20210 variant of the prothrombin gene. The control group consisted of 646 men who were frequency matched by age. In the latter group, the frequency of the 20210 AG genotype was 1.2%. The risk of myocardial infarction in the presence of the AG genotype was increased by 50% (odds ratio, 1.5; 95% confidence interval [95% CI], 0.6 to 3.8). The risk of myocardial infarction for carriership of factor V Leiden mutation was increased by 40% (odds ratio, 1.4; 95% CI, 0.8 to 2.2). When a coagulation defect was present (ie, the 20210 AG prothrombin genotype or the factor V Leiden mutation), the risk of myocardial infarction for carriers versus noncarriers was 1.4 (95% CI, 0.9 to 2.2). This risk was substantially increased when one of the major cardiovascular risk factors of smoking, hypertension, diabetes mellitus, or obesity also was present, with odds ratios varying between 3 and 6. These risks exceeded those of the single effects of the cardiovascular risk factors (ie, in the absence of the coagulation defect).

**Conclusions**—We conclude that in men the 20210 G→A variant of prothrombin is associated with an increased risk of myocardial infarction. The combined presence of major cardiovascular risk factors and carriership of a coagulation defect increases the risk considerably. (*Circulation*. 1998;97:1037-1041.)

**Key Words:** myocardial infarction ■ coagulation ■ thrombosis ■ genetics ■ risk factors

Recently, a genetic variation in the 3′-untranslated region of the prothrombin (clotting factor II) gene was described that was associated with an elevated prothrombin level.<sup>1</sup> Individuals with a G→A transition at nucleotide 20210 (AG genotype) had a mean prothrombin level of 1.32 U/mL, whereas individuals with the GG genotype had a significantly lower mean prothrombin level of 1.05 U/mL. Individuals with the AG genotype had a 2.8-fold increased risk of venous thrombosis compared with individuals with the 20210 GG genotype in the Leiden Thrombophilia Study (LETS). In this population-based case-control study, 6.2% of 474 unselected consecutive patients with a first, objectively confirmed episode of deep-vein thrombosis carried the prothrombin 20210A allele compared with 2.3% of 474 control subjects matched for age and sex. In a population of selected patients with a personal and family history of venous thrombosis, 18% were carriers of this allele.<sup>1</sup>

In only one study has the relation been studied of the prothrombin variant (20210 G→A) and myocardial infarction. In this case-control study in women 18 to 44 years old, the presence of the AG genotype increased the risk of myocardial infarction by 4.1-fold.<sup>2</sup> In the same study, the 1691 G→A mutation in the gene of clotting factor V (factor V Leiden), which causes resistance to activated protein C, was shown to be related to the occurrence of arterial thrombosis at a young age: the risk of myocardial infarction was 2.3-fold increased in heterozygotes for the factor V Leiden mutation.<sup>3</sup> The AG genotype of prothrombin gene and factor V Leiden mutation both increased the risk of myocardial infarction, particularly in the presence of other cardiovascular risk factors, such as smoking or metabolic risk factors (obesity, diabetes, hypertension, hypercholesterolemia).<sup>2</sup>

In men, the association of the prothrombin 20210 G→A genetic variant and myocardial infarction is unknown; we

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From the Department of Clinical Epidemiology, Department of Cardiology, Hemostasis and Thrombosis Research Center, Leiden University Hospital, Netherlands.

Correspondence to Prof Dr Frits R. Rosendaal, Department of Clinical Epidemiology, Leiden University Hospital, Bldg 1:CO-P, PO Box 9600, 2300 RC Leiden, Netherlands.

E-mail [rosendaal@rullf2.medfac.leidenuniv.nl](mailto:rosendaal@rullf2.medfac.leidenuniv.nl)

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therefore studied the association of the genetic variation in the 3'-untranslated region of the prothrombin gene and the factor V Leiden mutation with myocardial infarction in the population-based case-control Study of Myocardial Infarctions Leiden, which includes 560 men with a first myocardial infarction and 646 control subjects. Second, we examined the interaction between these genetic variations and other known cardiovascular risk factors with regard to the risk of myocardial infarction.

## Methods

### Subjects

We conducted the population-based case-control Study of Myocardial Infarctions Leiden between July 1994 and February 1997.

Cases consisted of men with a first myocardial infarction before the age of 70 years who were hospitalized in a university or general hospital in Leiden, the Netherlands, between January 1990 and January 1996. Two of the three characteristics of typical chest pain, a transient rise in cardiac enzymes to more than twice the upper limit, and ECG changes typical for myocardial infarction had to be present in the discharge report or hospital record of a patient with myocardial infarction. The study protocol was approved by the ethics committees of both hospitals.

Control subjects also were men, who were frequency matched to the patients (cases) by 10-year age groups, had undergone an orthopedic intervention between January 1990 and May 1996, and had received prophylactic anticoagulants for a short period after the intervention. The orthopedic intervention varied from a plaster cast for a ruptured hamstring to hip replacement. The control subjects were identified in the records of the Leiden Anticoagulant Clinic; they did not have a history of myocardial infarction and had not received anticoagulants for  $\geq 6$  months before participation in the study.

Both patients and control subjects were born in the Netherlands and were living in the Leiden region. Excluded were men with renal disease ( $n=10$ ), severe (neuro)psychiatric problems ( $n=28$ ), or a life expectancy of  $< 1$  year ( $n=16$ ). The response among the remaining patients and control subjects was 84.3% (560) and 77% (646), respectively.

All persons completed a questionnaire concerning the presence of cardiovascular risk factors such as smoking habits and alcohol consumption. For patients, all questions referred to the period before the myocardial infarction. The quetelet index was derived by dividing weight (kg) by squared height ( $m^2$ ). Persons were considered obese if their quetelet index exceeded  $30 \text{ kg}/m^2$ . Medication use and history of diabetes were determined through an interview with control subjects and retrieved from discharge letters for the patients. A person was classified as hypertensive or hypercholesterolemic when he was taking prescription drugs for these conditions. The variables of obesity, diabetes, hypertension, and hypercholesterolemia were grouped together as "metabolic risk factors."<sup>3</sup>

### Blood Collection and Laboratory Analysis

A morning fasting blood sample was drawn from the antecubital vein into two Sarstedt Monovette tubes containing  $0.106 \text{ mmol/L}$  trisodium citrate. We separated the blood sample into plasma and cells through centrifugation. High-molecular-weight DNA was extracted from the white blood cells according to a salting-out method.<sup>4</sup> The DNA was stored at  $4^\circ\text{C}$  until amplification. Analyzable DNA was available for 560 patients and 646 control subjects.

The status of the prothrombin variant (20210 G $\rightarrow$ A) was determined by the presence of a *Hind*III restriction site in the polymerase chain reaction fragment according to the method of Poort et al.<sup>1</sup> Genetic analysis of the factor V Leiden mutation (1691 G $\rightarrow$ A) was performed with polymerase chain reaction as described previously.<sup>5</sup>

Heterozygous or homozygous carriership of the prothrombin 20210A allele or heterozygous or homozygous carriership of factor V Leiden mutation was defined as a "coagulation defect."

### Statistical Analysis

An odds ratio (OR) was calculated as a measure of relative risk. This OR estimates the risk of a myocardial infarction in the presence of a risk factor relative to the absence of the particular risk factor, the reference category. A 95% confidence interval (CI) was calculated according to the method of Woolf.<sup>6</sup> When the CI did not include unity, the OR was different from unity at a significance level of  $\leq 5\%$ . Multiple logistic regression was performed to adjust for age, with CIs for the adjusted ORs calculated with the use of the standard errors of the coefficients estimated according to the maximum likelihood methods. Mean values are presented with standard deviation (SD). All computations were carried out with the SPSS for Windows Version 7.0 statistical package.

## Results

The characteristics of all patients and control subjects and separately for those below the age of 50 years are shown in Table 1. The mean age of the patients was 56.2 years (SD, 9.0 years), and that of control subjects was 57.3 years (SD, 10.8 years). A higher percentage of patients smoked and a lower percentage used alcohol compared with the control subjects (62.3% and 33.3% smoked and 80.4% and 86.8% consumed alcohol, respectively). The risk factors of obesity, diabetes, hypertension, and hypercholesterolemia were more often found in patients than in control subjects, with the most striking contrast in younger persons.

### Prothrombin 20210A and Factor V Leiden Mutation

In 10 of 560 patients (1.8%), the heterozygous (20210 AG) genotype of prothrombin variant was detected compared with 8 of 646 control subjects (1.2%). Homozygous (20210 AA) carriers were not found. The relative risk of myocardial infarction associated with prothrombin 20210A carriership was 1.5 (95% CI, 0.6 to 3.8) (Table 2). In the subgroup of 314 men aged  $\leq 50$  years, no increased risk was found (OR, 0.9; 95% CI, 0.1 to 6.7). Only 4 persons in this young group were carriers of the prothrombin 20210A allele: 2 patients and 2 control subjects.

The relative risk of myocardial infarction in the presence of factor V Leiden mutation was 1.4 (95% CI, 0.8 to 2.2) (Table 2). One patient was a homozygous carrier of factor V Leiden mutation (age 63 at the time of infarction). For the 314 men below the age of 50, the risk was 1.8 (95% CI, 0.8 to 3.9).

### Coagulation Defect

Overall, 48 patients (8.6%) and 39 control subjects (6.0%) had a coagulation defect (ie, was a carrier of either prothrombin 20210A or factor V Leiden mutation). One individual (age 64 years) who was member of the control group was heterozygous for both mutations. The relative risk of myocardial infarction in the presence of a coagulation defect was 1.4 (95% CI, 0.9 to 2.2) overall and 1.6 (95% CI, 0.8 to 3.4) for men below the age of 50.

### Coagulation Defect and Interaction

Among smokers with the coagulation defect, the risk of a myocardial infarction was increased sixfold compared with nonsmokers without the abnormality. In comparison, smokers without the abnormality had a relative risk of  $\approx 3$  (Table 3). A similar indication of a synergistic effect was found for other risk

**TABLE 1. Characteristics of Patients\* and Control Subjects in the Study of Myocardial Infarctions Leiden**

Characteristic	Overall		<50 y	
	Patients (n=560)	Control Subjects (n=646)	Patients (n=154)	Control Subjects (n=160)
Age, y (mean [SD])	56.2 (9.0)	57.3 (10.8)	44.4 (4.2)	42.5 (6.9)
Smoker, %	62.3	33.3†	77.3	41.3†
Alcohol user, %	80.4	86.8†	89	87.5
Obesity, %‡	17.2	16.4	22.7	13.1†
Diabetes, %	4.6	3.4	4.5	1.9
Hypertension, %§	18.9	16.6	7.1	4.4
Hypercholesterolemia, %§	2.1	1.7	1.9	0.6
Metabolic risk factor, %	36.6	30.5†	31.2	18.8†
Coagulation defect, %¶	8.6	6	12.3	8.1

\*Data refer to the period before myocardial infarction.

† $\chi^2$  test,  $P < .05$ .

‡For two persons, length and weight were not available.

§A person was classified as hypertensive or hypercholesterolemic if he was taking prescription drugs for these conditions.

||Presence of one of the four metabolic risk factors of obesity, diabetes, hypertension, and hypercholesterolemia.

¶Carriership of the prothrombin 20210A allele or factor V Leiden mutation was defined as a coagulation defect.

factors (Tables 3 and 4). The single effect of obesity, diabetes, hypertension, or hypercholesterolemia (ie, the presence of one or more metabolic risk factors) was small without the concomitant presence of coagulation defect, whereas a substantial

**TABLE 2. Frequency of the 20210 GA Genotypes in the Prothrombin Gene, 1691 GA Genotypes in the Factor V Gene, and Risk of Myocardial Infarction in 560 Patients and 646 Control Subjects**

Genotype	Patients, n (%)	Control Subjects, n (%)	Odds Ratio (95% CI)*
Prothrombin (FII)			
20210 GG	550 (98.2)	638 (98.8)	1.0†
20210 AG	10 (1.8)	8 (1.2)	1.5 (0.6–3.8)
20210 AA	...	...	...
Factor V (FV)			
1691 GG	522 (93.2)	614 (95.0)	1.0†
1691 AG‡	37 (6.6)	32 (5.0)	1.4 (0.8–2.2)§
1691 AA	1 (0.2)	...	...
Coagulation defect			
FII 20210 GG and FV 1691 GG	512 (91.4)	607 (94.0)	1.0†
FII 20210 AG or FV 1691 AG/AA	48 (8.6)	38 (5.9)	1.4 (0.9–2.2)
FII 20210 AG and FV 1691 AG	...	1 (0.2)	...

\*95% confidence interval.

†Reference category.

‡Thus, AG is heterozygous for factor V Leiden, and AA is homozygous for factor V Leiden.

§Odds ratio of AG and AA genotypes versus GG genotype.

||Odds ratio of factor 20210 AG and/or factor V Leiden mutation versus neither.

increase in the risk of myocardial infarction was found when the abnormality was present in combination with one of these risk factors, ranging from a threefold to sixfold increase relative to those with neither risk factor nor coagulation defect. No clear evidence for synergy was found for persons using alcohol. The individuals who account for the excess risks in the different strata are not the same in each stratum in that only 21 of 87 persons with a coagulation defect had two or more cardiovascular risk factors simultaneously.

## Discussion

The results of our population-based case-control Study of Myocardial Infarctions Leiden show that the mutation in the prothrombin gene (20210 G→A) increases the risk of myocardial infarction by 50% (OR, 1.5; 95% CI, 0.6 to 3.8). In patients, 1.8% were heterozygous for the prothrombin 20210A allele compared with 1.2% of control subjects. The risk of myocardial infarction was increased considerably when a coagulation defect (ie, the prothrombin 20210A allele or factor V Leiden mutation) was present simultaneously with a cardiovascular risk factor, such as smoking.

In a population-based case-control study among women aged 18 to 44 years, the risk of myocardial infarction associated with carriership of prothrombin 20210A allele was 4.1 (95% CI, 1.1 to 15.2), with 5.1% of the patients and 1.3% of the control subjects being carriers of the prothrombin 20210A allele.<sup>2</sup> This risk was more pronounced than among men aged  $\leq 50$  years in the present study, in whom we found no increased risk. It should be noted, however, that only 4 young men carried the prothrombin 20210A allele and that the CIs were wide. Factors such as hormonal status and the use of oral contraception might interact in the association of the prothrombin 20210 G→A genetic variant and myocardial infar-

**TABLE 3. Risk Effect of Smoking and a Metabolic Risk Factor, Without and With a Coagulation Defect**

Cardiovascular Risk Factor	Presence of Coagulation Defect	Patients, n (%) <sup>*</sup>	Control Subjects, n (%) <sup>*</sup>	Odds Ratio (95% CI) <sup>†</sup>
No smoking	No coagulation defect	193 (91.5)	403 (93.5)	1
	FVL/20210A <sup>‡</sup>	18 (8.5)	28 (6.5)	1.3 (0.7–2.5)
Smoking	No coagulation defect	319 (91.4)	204 (94.9)	3.3 (2.5–4.2)
	FVL/20210A	30 (8.6)	11 (5.1)	6.1 (3.0–12.5)
Non-metabolic	No coagulation defect	332 (93.5)	420 (93.5)	1
	FVL/20210A	23 (6.5)	29 (6.5)	1.0 (0.6–1.8)
Metabolic risk factor	No coagulation defect	180 (87.8)	187 (94.9)	1.3 (1.0–1.6)
	FVL/20210A	25 (12.2)	10 (5.1)	3.2 (1.5–6.7)

<sup>\*</sup>Percentages are calculated within each stratum of cardiovascular risk factor (ie, among the nonsmoker control subjects, 6.5% were carriers of the factor V Leiden or prothrombin 20210A).

<sup>†</sup>Odds ratios adjusted for age, with 95% confidence interval. Reference categories are noncarriers without the particular cardiovascular risk factor.

<sup>‡</sup>Factor V Leiden or prothrombin 20210A.

tion in women and account for the varying results between the sexes. In addition, the previous study included women living in western Washington, whereas our study was conducted in men born in the Netherlands.

The prothrombin 20210A allele was found to be a risk factor (OR, 2.8; 95% CI, 1.4 to 5.6) for deep-vein thrombosis in the LETS study<sup>1</sup> and two other recent reports.<sup>7,8</sup> This relative risk for venous thrombosis was more pronounced than that in our case-control study of myocardial infarction. A discrepancy in relative risks for venous and arterial thrombosis has also been demonstrated for genetic variations in other clotting factors. Factor V Leiden mutation (1691 G→A), for example, is the most common risk factor for venous thrombosis. Heterozygous carriers of the mutation have a sevenfold increased risk of

venous thrombosis<sup>9</sup>; homozygous individuals have a risk that is increased up to 80-fold.<sup>10</sup> However, results of studies in which the factor V Leiden mutation was examined as a potential risk factor for myocardial infarction are inconsistent,<sup>3,11</sup> most likely because the excess risk is less pronounced or present only in specific groups.

When we consider the risk of myocardial infarction in the presence of a coagulation defect (ie, carriership of the prothrombin 20210A allele or factor V Leiden mutation), synergy is found. Smokers, the obese, and persons with diabetes or hypertension (ie, persons with one or more metabolic risk factor) have, in combination with a coagulation defect, a higher risk of myocardial infarction compared with noncarriers with the particular cardiovascular risk factor. In each instance,

**TABLE 4. Risk Effect of Obesity, Diabetes Mellitus, Hypertension, and Hypercholesterolemia, Without and With a Coagulation Defect**

Cardiovascular Risk Factor	Presence of Coagulation Defect	Patients, n (%) <sup>*</sup>	Control Subjects, n (%) <sup>*</sup>	Odds Ratio (95% CI) <sup>†</sup>
Nonobese	No coagulation defect	427 (92.2)	505 (93.7)	1
	FVL/20210A <sup>‡</sup>	36 (7.8)	34 (6.3)	1.3 (0.8–2.0)
Obese	No coagulation defect	84 (87.5)	101 (95.3)	1.0 (0.7–1.3)
	FVL/20210A	12 (12.5)	5 (4.7)	2.8 (1.0–8.1)
Nondiabetic	No coagulation defect	491 (92.0)	586 (93.9)	1
	FVL/20210A	43 (8.0)	38 (6.1)	1.3 (0.9–2.1)
Diabetic	No coagulation defect	21 (80.8)	21 (95.5)	1.2 (0.7–2.3)
	FVL/20210A	5 (19.2)	1 (4.5)	5.9 (0.7–50.0)
Nonhypertensive	No coagulation defect	419 (92.3)	505 (93.7)	1
	FVL/20210A	35 (7.7)	34 (6.3)	1.2 (0.8–2.0)
Hypertensive	No coagulation defect	93 (87.7)	102 (95.3)	1.2 (0.9–1.6)
	FVL/20210A	13 (12.3)	5 (4.7)	3.3 (1.2–9.4)
Nonhypercholesterolemic	No coagulation defect	502 (91.6)	597 (94.0)	1
	FVL/20210A	46 (8.4)	38 (6.0)	1.4 (0.9–2.2)
Hypercholesterolemic	No coagulation defect	10 (83.3)	10 (90.9)	1.2 (0.5–2.9)
	FVL/20210A	2 (16.7)	1 (9.1)	2.4 (0.2–26.7)

<sup>\*</sup>Percentages are calculated within each stratum of cardiovascular risk factor (ie, among the nonobese control subjects, 6.3% were carriers of the factor V Leiden or prothrombin 20210A).

<sup>†</sup>Odds ratios adjusted for age, with 95% confidence interval. Reference categories are noncarriers without the particular cardiovascular risk factor.

<sup>‡</sup>Factor V Leiden or prothrombin 20210A.

the risk of the combination of the coagulation defect with a risk factor exceeded the risk of the single risk factor. This is in accordance with the results of the previous study in young women, although the synergy was far more striking in that study.<sup>2</sup> It should be noted that the overall risk of myocardial infarction is much lower in young women than in middle-aged and elderly men (based on Dutch estimates (SIG Health Care Information, National Medical Registration, tables on hospital admissions, 1992 to 1994; available from Maliebaan 50, PO Box 14066, 3508 SC Utrecht, Netherlands), with an incidence of 1.5/10 000 person-years for women aged 35 to 39 years and 60/10 000 person-years for men aged 55 to 59 years). This implies that in terms of public health or individual risk estimates, an 1.5-fold increased risk in elderly men may be more important than a fourfold increase in the risk among young women. For every group of 10 000 men aged 55 to 59 years, there is an increase in the number of individuals experiencing a myocardial infarction of 30, whereas among every 10 000 women aged 35 to 39 years, the additional number of patients with a myocardial infarction patients is 5. The high background risk in elderly men also explains why even with the large study population and powerful case-control design of the present study, effects are more difficult to detect than among young women and often do not reach statistical significance.

The frequency of the prothrombin 20210 AG genotype in control subjects in the Study of Myocardial Infarctions Leiden was 1.2%, which is similar to that of the control group of the case-control study among young women<sup>2</sup> and equal to the prevalence in 164 healthy plasma donors from the United Kingdom.<sup>7</sup> In the LETS study, however, the percentage among healthy persons was 2.3%.<sup>1</sup> We studied additional control groups in the Netherlands to investigate this apparent discrepancy: in patients with rheumatic arthritis, 5 of 291 (1.7%) were carriers of the prothrombin 20210A allele, whereas in 249 blood donors, 8 carriers (3.2%) were found (data not shown). These figures are in line with the actual frequency of the prothrombin variant of 2%.<sup>12</sup>

The present study has two limitations. First, the results are derived from men born in the Netherlands and do not necessarily apply to other populations. The second is that of necessity we studied patients who survived the myocardial infarction. It cannot be excluded that patients who died during the acute phase of the myocardial infarction more often carried the prothrombin 20210A allele or factor V Leiden mutation. We think this is unlikely, however, because survival of an individual after myocardial infarction is influenced by other factors, such as patient-induced delay and delay in receipt of effective assistance, which affect the time period from the onset of symptoms to the start of interventions such as thrombolytic therapy.<sup>12</sup> Several other factors influencing 30-day mortality rates are the level of systolic blood pressure, heart rate, Killip class, and localization of myocardial infarction.<sup>13</sup> It does not seem likely that the prothrombotic genotypes would play a major role.

In conclusion, the results of the present study show that genetic variations in thrombotic risk factors, such as prothrombin 20210A and factor V Leiden mutation, increase the risk of myocardial infarction in men. The risk is particularly increased

when other cardiovascular risk factors are present as well. Given the high frequency of these genetic abnormalities in the general population and the high prevalence of cardiovascular risk factors, the need for prevention or treatment of these latter risk factors is supported.

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