

POLYMORPHISMS IN THE COAGULATION FACTOR VII GENE AND THE RISK OF MYOCARDIAL INFARCTION

LICIA IACOVIELLO, M.D., AUGUSTO DI CASTELNUOVO, M.S., PETER DE KNIJFF, PH.D., ANDRIA D'ORAZIO, B.S., CONCETTA AMORE, B.S., ROSA ARBORETTI, M.S., CORNELIS KLUFT, PH.D., AND MARIA BENEDETTA DONATI, M.D., PH.D.

ABSTRACT

Background High blood levels of coagulation factor VII are associated with a risk of ischemic vascular disease. Although factor VII levels may be genetically determined, the relation between genetic polymorphisms of factor VII, factor VII blood levels, and the risk of myocardial infarction has not been established.

Methods We performed a case-control study of 165 patients with familial myocardial infarction (mean [\pm SD] age, 55 \pm 9 years) and 225 controls without a personal or family history of cardiovascular disease (mean age, 56 \pm 8 years). The polymorphisms involving R353Q and hypervariable region 4 of the factor VII gene were studied. Factor VII clotting activity and antigen levels were also measured.

Results Patients with the *QQ* or *H7H7* genotype had a decreased risk of myocardial infarction (odds ratios, 0.08 [95 percent confidence interval, 0.01 to 0.9] and 0.22 [95 percent confidence interval, 0.08 to 0.63], respectively). For the R353Q polymorphism, the *RR* genotype was associated with the highest risk, followed by the *RQ* genotype and then by the *QQ* genotype ($P < 0.001$). For the polymorphism involving hypervariable region 4, the combined *H7H5* and *H6H5* genotypes were associated with the highest risk, followed in descending order by the *H6H6*, *H6H7*, and *H7H7* genotypes ($P < 0.001$). Patients with the *QQ* or *H7H7* genotype had lower levels of both factor VII antigen and factor VII clotting activity than those with the *RR* or *H6H6* genotype. Patients with the lowest level of factor VII clotting activity had a lower risk of myocardial infarction than those with the highest level (odds ratio, 0.13; 95 percent confidence interval, 0.05 to 0.34).

Conclusions Our findings suggest that certain polymorphisms of the factor VII gene may influence the risk of myocardial infarction. It is possible that this effect may be mediated by alterations in factor VII levels. (N Engl J Med 1998;338:79-85.)

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DURING the past decade evidence has accumulated that increased factor VII activity represents a risk factor for ischemic cardiovascular disease.¹⁻³ The Northwick Park Heart Study group reported that high levels of factor VII were independently associated with an increase in the risk of coronary events in middle-aged men.¹ More recently, this group showed that the level of factor VII was predictive of the risk of fatal but not nonfatal myocardial infarction.² The same trend

was observed in the Prospective Cardiovascular Münster Study.³ Other investigators, however, failed to find such associations.^{4,5}

Factor VII blood levels are influenced by both environmental and genetic factors. Triglyceride levels are a major determinant of factor VII levels in blood.⁶ Age, body-mass index, oral contraceptive use, and menopausal status have also been related to the levels and activity of factor VII.⁷ Recent studies have demonstrated that the levels of factor VII and their response to environmental stimuli are genetically determined. Green et al.⁸ reported a strong association between a common polymorphism (R353Q) of the factor VII gene and plasma factor VII levels; however, there was no association between the polymorphism and the risk of ischemic vascular disease.⁹ Another common polymorphism has been described in hypervariable region 4 of intron 7 of the factor VII gene and reported to be associated with levels of factor VII.^{10,11}

We investigated whether the risk of myocardial infarction is associated with polymorphisms in the factor VII gene, and whether these polymorphisms are associated with factor VII levels. To emphasize the contribution of genetic factors, we studied patients with a family history of thrombosis.

METHODS

Study Population

Case patients were patients over 45 years of age who had had a myocardial infarction and who were reported to have at least one first-degree relative who had had a myocardial infarction or a stroke (or both) before the age of 65 years.¹² The patients were selected from the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico trial population¹³ on the basis of an interview regarding their family history of thrombosis.¹⁴ Of the 239 case patients identified, 180 patients could be contacted and were willing to participate.

Controls were consecutive patients over the age of 45 years who were hospitalized for any clinical reason except myocardial

From the Unit of the Genetics of Vascular Risk Factors, Angela Valenti Laboratory of Thrombosis Pharmacology, Department of Vascular Medicine and Pharmacology (L.I., A.D.C., A.D'O., C.A., M.B.D.), and the Laboratory of Drug Epidemiology, Department of Clinical Pharmacology and Epidemiology (R.A.), Istituto di Ricerche Farmacologiche Mario Negri, Santa Maria Imbaro, Italy; and Gaubius Laboratory (L.I., P. de K., C.K.) and Medical Center of Genetics-Department of Human Genetics, Leiden University (P. de K.), Leiden, the Netherlands. Address reprint requests to Dr. Iacoviello at Angela Valenti Laboratory of Thrombosis Pharmacology, Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro, Italy.

infarction, stable or unstable angina, stroke, or transient ischemic attacks. Among the 309 control patients interviewed, 37 who reported a personal or family history of thrombosis or who had definite defects of the hemostatic system were excluded. Patients with chronic diseases were not considered unless they were hospitalized for reasons unrelated to these conditions.

We used a structured questionnaire to characterize both case patients and controls.¹⁴ The subjects were all Italian: 40 percent of both case patients and controls were from northern Italy, 15 percent from central Italy, 40 percent from southern Italy, and 5 percent from Sardinia. All patients provided written informed consent. The study was performed according to the Declaration of Helsinki of 1975 and was approved by the Mario Negri Sud ethics committee.

Laboratory Measurements and Techniques

The case patients were assessed five to seven months after their most recent ischemic event. Blood was collected from the patients between 8 and 10 a.m. after an overnight fast and after a 20-minute rest in the supine position. Blood was not collected from nine patients who were receiving oral anticoagulant drugs. Blood samples for DNA and biochemical analyses were available for 165 of 171 case patients (96 percent) and 225 of 272 controls (83 percent).

Hypervariable region 4 of intron 7 of the factor VII gene was amplified according to a modification of the method of Marchetti et al.¹⁵ Three alleles were identified: a common allele (H6) of 443 bp with six monomers, a less frequent allele (H7) of 480 bp with seven monomers of 37 bp, and a very rare allele (H5) of 406 bp with five monomers (Fig. 1).

The R353Q polymorphism was detected as described.¹⁰ The amplified fragments were digested with 5 U of *MspI* (GIBCO BRL, Gaithersburg, Md.) and then subjected to electrophoresis on 2.5 percent agarose gel. Fragments of 205 bp (the R allele) and 272 bp (the Q allele) were detected.

Factor VII clotting activity was determined by a one-stage clotting assay as follows: 100 μ l of plasma was diluted 1:10 in TRIS-hydrochloride buffer (50 mmol of TRIS per liter and 100 mmol

of sodium chloride per liter, pH 7.4), mixed with 100 μ l of human factor VII-deficient plasma (Sigma, St. Louis), and incubated for five minutes at 37°C in a coagulometer (model KC 4A, Amelung, Lemgo, Germany). Then, 200 μ l of a prewarmed (37°C) 1:1 mixture of recombinant human thromboplastin (Hemoliance, Cologno Monzese, Italy) and calcium dichloride (25 mmol per liter) was added, and the clotting time was automatically recorded on the coagulometer. Values were expressed as a percentage of the value in pooled normal plasma.

Plasma factor VII antigen was measured as total factor VII by a double-antibody enzyme-linked immunosorbent assay (Boehringer Mannheim Italia, Milan, Italy) that reacts with all species of factor VII.

Statistical Analysis

The variables factor VII clotting activity and antigen and triglyceride levels were analyzed on a logarithmic scale to remove positive skewness. The means were compared by analysis of variance or the Kruskal-Wallis test. Chi-square analysis or Fisher's exact test was used to compare discrete variables. The frequencies of the alleles and genotypes among case patients and controls were determined and compared by the chi-square test with the values predicted on the basis of the assumption of Hardy-Weinberg equilibrium. The coefficient of gametic linkage disequilibrium was calculated by likelihood methods in the control sample.¹⁶ The coefficient (*D'*) is reported as the ratio of the unstandardized coefficient to its maximal value. Odds ratios were calculated, together with their 95 percent confidence intervals. Dummy variables were created to assess the association of the various genotypes with familial myocardial infarction. Multiple logistic-regression analysis was performed with the Logistic procedure; confounding variables included in the model were age, sex, smoking status, and history of hyperlipidemia, hypertension, and diabetes. In a separate analysis, the effect of the genotype on the risk of myocardial infarction was also adjusted for factor VII activity. The effects of the H7 and H5 alleles, with H6 chosen as the reference allele, and the effect of the Q allele, with R chosen as the reference allele, were analyzed by the introduction of three dummy variables (0, 1, and 2) coding respectively for the number of H7, H5, and Q alleles. This model specifies a codominant multiplicative effect of the H7, H5, and Q alleles in determining the risk of myocardial infarction.

The association of factor VII levels with the genotypes was tested by one-way analysis of variance and covariance (with age, sex, and triglyceride levels as covariates) with use of the general linear model procedure. The Tukey-Kramer approach was used for multiple comparisons. Data for continuous variables were expressed as means \pm SD; a two-tailed P value of less than 0.05 was considered to indicate statistical significance. All computations were carried out with the SAS statistical package (SAS Institute, Cary, N.C.).¹⁷

RESULTS

Characteristics of the Study Population

The characteristics of the case patients and controls are shown in Table 1. There were no significant differences in age between the groups. The patients with familial myocardial infarction had a higher prevalence of common risk factors for atherosclerotic disease, as well as significantly higher levels of both factor VII clotting activity and factor VII antigen, than the control group.

Prevalence of Factor VII Polymorphisms

The genotypes and alleles at the factor VII loci in case patients and controls are shown in Table 2. The

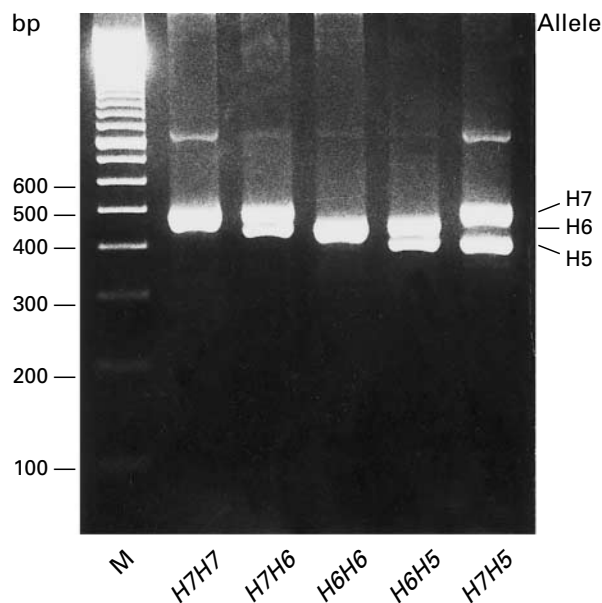


Figure 1. Five Genotypes of Hypervariable Region 4 of the Factor VII Gene.

In the first lane, M denotes the molecular-size marker.

distribution of the factor VII R353Q and hypervariable region 4 genotypes was virtually identical to that predicted on the basis of Hardy-Weinberg equilibrium, both in case patients and in controls (chi-square with 1 df=3.1, P=0.08, and chi-square with 1 df=0.01, P=0.9, respectively, for R353Q; chi-square with 2 df=1.5, P=0.5, and chi-square with 2 df=0.6, P=0.7, for hypervariable region 4). The two polymorphisms were in linkage disequilibrium, with a D' value of 0.65 (P<0.001).

The distribution of the genotypes differed significantly between case patients and controls (Table 2). Indeed, significantly fewer case patients had the QQ or the H7H7 genotype than controls. The distribution of genotypes for hypervariable region 4 was also significantly different between case patients and controls when those heterozygous for the H5 allele (H7/H5 or H6/H5) were considered separately (P=0.02 by Fisher's exact test) or together (P=0.01 by Fisher's exact test), or excluded from the analysis (chi-square with 2 df=6.3, P=0.04).

The allele frequencies of both polymorphisms also differed between case patients and controls (Table 2). Allele Q of the R353Q polymorphism was less frequent among case patients than controls (chi-square with 1 df=4.3, P=0.04). Allele H7 of the hypervariable region 4 polymorphism was significantly less frequent among case patients (chi-square with 1 df=6.9, P=0.008), whereas the relative frequencies of H6 (chi-square with 1 df=4.0, P=0.05) and H5 (P=0.04 by Fisher's exact test) were higher among case patients.

Odds Ratios for Familial Myocardial Infarction

The specific odds ratios for myocardial infarction with each R353Q and hypervariable region 4 genotype, with RR and H6H6 as the reference groups, are shown in Table 3. Homozygosity for the Q or H7 allele conferred significant protection from myocardial infarction. Indeed, patients with the QQ or H7H7 genotype had a decreased risk of myocardial infarction of 92 percent and 78 percent, respectively, after adjustment for age, sex, smoking status, and history of hyperlipidemia, diabetes, and hypertension. However, when these analyses were also adjusted for factor VII activity levels, the cumulative effect of factor VII genotypes in decreasing the risk of myocardial infarction was no longer significant (odds ratio for the QQ genotype, 0.18; 95 percent confidence interval, 0.01 to 2.17; odds ratio for the H7H7 genotype, 0.58; 95 percent confidence interval, 0.21 to 1.93), suggesting a role for factor VII in mediating the effect. For the R353Q polymorphism, the QQ genotype was associated with the lowest risk, followed by the RQ genotype, with the RR genotype as the reference group, in univariate (P=0.02) and multivariate (P<0.001) analyses. For the polymorphism involving hy-

TABLE 1. CHARACTERISTICS OF THE PATIENTS WITH FAMILIAL MYOCARDIAL INFARCTION AND THE CONTROLS.*

CHARACTERISTIC	CASE PATIENTS (N=165)	CONTROLS (N=225)	P VALUE
Age — yr	55±9	56±8	0.16
Factor VII clotting activity — %†	115±32	94±25	<0.001
Factor VII antigen — %†	100±21	95±21	0.02
Male sex — no. (%)	129 (78)	154 (68)	0.03
Smoking status — no. (%)‡			
Current smoker	94 (57)	52 (28)	
Former smoker	26 (16)	32 (17)	<0.001
Never smoked	45 (27)	104 (55)	
History of hyperlipidemia — no. (%)§	104 (63)	33 (18)	<0.001
History of hypertension — no. (%)¶	67 (44)	38 (20)	<0.001
History of diabetes — no. (%)	30 (19)	17 (9)	0.004

*Plus-minus values are means ±SD.

†Values are expressed as a percentage of the value in pooled normal plasma. The statistical analysis was performed on log-transformed values, but the untransformed mean values are given in the table.

‡Data on smoking status were missing for 37 controls.

§Data on history of hyperlipidemia were missing for 45 controls.

¶Data on history of hypertension were missing for 13 case patients and 32 controls.

||Data on history of diabetes were missing for 11 case patients and 32 controls.

TABLE 2. GENOTYPES AND ALLELE FREQUENCIES FOR R353Q AND HYPERVARIABLE REGION 4 POLYMORPHISMS AT THE FACTOR VII LOCI IN PATIENTS WITH FAMILIAL MYOCARDIAL INFARCTION AND IN CONTROLS.*

VARIABLE	CASE PATIENTS (N=165)	CONTROLS (N=225)	P VALUE†
Alleles — no. of alleles/total no. (%)			
R	277/328 (84.5)	352/448 (78.6)	
Q	51/328 (15.5)	96/448 (21.4)	0.04
H7	88/330 (26.7)	160/450 (35.6)	
H6	233/330 (70.6)	287/450 (63.8)	0.003
H5	9/330 (2.7)	3/450 (0.7)	
Genotype — no. of subjects/total no. (%)			
RR	114/164 (69.5)	138/224 (61.6)	
RQ	49/164 (29.9)	76/224 (33.9)	0.03
QQ	1/164 (0.6)	10/224 (4.5)	
H7H7	12/165 (7.3)	31/225 (13.8)	
H6H7	60/165 (36.4)	97/225 (43.1)	
H6H6	84/165 (50.9)	94/225 (41.8)	0.02
H7H5	4/165 (2.4)	1/225 (0.4)	
H6H5	5/165 (3.0)	2/225 (0.9)	

*Data on R353Q were missing for one case patient and one control. Because of rounding, not all percentages total 100.

†In each case the P value is for the overall difference between groups.

TABLE 3. RISK OF FAMILIAL MYOCARDIAL INFARCTION FOR DIFFERENT R353Q AND HYPERVARIABLE REGION 4 GENOTYPES.

GENOTYPE	UNIVARIATE ANALYSIS	MULTIVARIATE ANALYSIS*
	odds ratio (95% confidence interval)	
<i>RR†</i>	1.0	1.0
<i>RQ</i>	0.78 (0.50–1.21)	0.71 (0.38–1.33)
<i>QQ</i>	0.12 (0.02–0.96)	0.08 (0.01–0.91)
<i>H6H6†</i>	1.0	1.0
<i>H7H5</i> and <i>H6H5</i>	3.36 (0.88–12.8)	1.69 (0.16–17.3)
<i>H6H7</i>	0.69 (0.45–1.07)	0.62 (0.33–1.17)
<i>H7H7</i>	0.43 (0.21–0.90)	0.22 (0.08–0.63)

*The multivariate logistic-regression analysis was adjusted for age, sex, smoking status, and history of hyperlipidemia, hypertension, and diabetes.

†This is the reference group.

pervariable region 4, the highest risk was associated with the combined *H7H5* and *H6H5* genotypes, followed in descending order by the *H6H6* (odds ratio, 1.0; the reference group), *H6H7*, and *H7H7* genotypes in univariate ($P=0.008$) and multivariate ($P<0.001$) analyses.

The allele-specific risks of myocardial infarction associated with the Q allele, computed by a codominant model for the odds ratio, were 0.66 (95 percent confidence interval, 0.45 to 0.97) and 0.58 (95 percent confidence interval, 0.33 to 1.00), respectively, in univariate and multivariate analyses. The respective odds ratios for the H7 and H5 alleles were

0.67 (95 percent confidence interval, 0.49 to 0.92) and 3.33 (95 percent confidence interval, 0.87 to 12.7) in univariate analysis and 0.52 (95 percent confidence interval, 0.33 to 0.81) and 1.62 (95 percent confidence interval, 0.16 to 16.5) in multivariate analysis.

Sixty-eight percent of the patients reported a family history of myocardial infarction alone, 25 percent had a family history of both myocardial infarction and stroke, and the remaining 7 percent had a family history of stroke alone. When the patients with a family history of stroke were excluded, the *QQ* genotype (odds ratio, 0.13; 95 percent confidence interval, 0.02 to 1.05) and the *H7H7* genotype (odds ratio, 0.42; 95 percent confidence interval, 0.20 to 0.88) were both associated with a lower risk of myocardial infarction. This relation was also found when only patients with a family history of myocardial infarction were analyzed (*QQ* genotype: odds ratio, 0.21; 95 percent confidence interval, 0.02 to 1.13; *H7H7* genotype: odds ratio, 0.40; 95 percent confidence interval, 0.16 to 0.99).

Table 4 shows the effect of various combinations of genotypes for hypervariable region 4 and R353Q as compared with the *RRH6H6* combination. The *H7H7* genotype decreased the risk of myocardial infarction in combination with any R353Q genotype; homozygosity for both the Q and H7 alleles afforded the greatest protection. However, the small number of *QQ* carriers did not allow a formal statistical analysis to verify the effect of such combinations of genotypes.

TABLE 4. RISK OF MYOCARDIAL INFARCTION AND FACTOR VII LEVELS FOR DIFFERENT COMBINATIONS OF HYPERVARIABLE REGION 4 AND R353Q GENOTYPES.*

GENOTYPE	NO. OF CASE PATIENTS/CONTROLS	FACTOR VII CLOTTING ACTIVITY†		FACTOR VII ANTIGEN†		UNIVARIATE ANALYSIS	MULTIVARIATE ANALYSIS‡
		percent		percent			
		CASE PATIENTS	CONTROLS	CASE PATIENTS	CONTROLS		
<i>RRH6H6§</i>	77/78	120±32	99±25	104±21	101±18	1.0	1.0
<i>RQH6H6</i>	6/15	132±36	90±15	94±15	85±19	0.40 (0.15–1.10)	0.30 (0.06–1.40)
<i>RRH6H7</i>	28/50	117±31	106±25	101±21	107±18	0.57 (0.32–1.00)	0.40 (0.18–0.91)
<i>RQH6H7</i>	32/45	108±31	88±18	97±23	89±16	0.72 (0.42–1.25)	0.78 (0.35–1.73)
<i>RRH7H7</i>	3/7	80±20	95±24	82±16	85±18	0.43 (0.11–1.74)	0.30 (0.04–2.30)
<i>RQH7H7</i>	8/16	91±24	79±21	90±22	77±19	0.51 (0.21–1.25)	0.23 (0.06–0.81)
<i>QQH7H7</i>	1/7	60	49±12	60	65±20	0.14 (0.02–1.20)	0.06 (0.01–0.80)

*Plus–minus values are means ±SE. Nine case patients and three controls who were heterozygous for the H5 allele were not included in the analysis. Data on R353Q were missing for one case patient and one control. One control had the *QQH6H6* genotype, and two controls had the *QQH6H7* genotype.

†Values are expressed as a percentage of the value in pooled normal plasma. The statistical analysis was performed on log-transformed values, but the untransformed mean values are given in the table.

‡The multivariate logistic-regression analysis was adjusted for age, sex, smoking status, and history of hyperlipidemia, hypertension, and diabetes.

§This is the reference group.

TABLE 5. FACTOR VII CLOTTING ACTIVITY AND ANTIGEN LEVELS IN CASE PATIENTS AND CONTROLS, ACCORDING TO THE GENOTYPES FOR HYPERVARIABLE REGION 4 AND R353Q.*

GENOTYPE	FACTOR VII CLOTTING ACTIVITY		FACTOR VII ANTIGEN	
	CASE PATIENTS	CONTROLS	CASE PATIENTS	CONTROLS
	percent			
<i>RR</i>	134±4†	104±3	109±3	102±2
<i>RQ</i>	121±6†‡	86±3§	100±5†	84±3§
<i>QQ</i>	60	57±9§¶	60	67±7§
<i>H6H5</i> and <i>H7H5</i>	131±15	137±14**	104±12	121±12**
<i>H6H6</i>	134±4††	98±3**	109±4	95±3**
<i>H6H7</i>	132±6†‡‡	97±3**	106±5	97±2**
<i>H7H7</i>	95±13	72±6	94±10	71±5

*Plus-minus values are means ±SE. The Tukey-Kramer approach was used for statistical analysis, which adjusts for multiple comparisons in multivariate analysis of variance (with age, sex, and log triglyceride values as covariates). Factor VII levels are expressed as a percentage of the value in pooled normal plasma. The statistical analysis of factor VII clotting activity and antigen levels was performed on log-transformed values, but the untransformed mean values are given in the table.

†P<0.001 for the comparison with controls.

‡P=0.04 for the comparison with the *RR* genotype.

§P<0.001 for the comparison with the *RR* genotype.

¶P<0.001 for the comparison with the *RQ* genotype.

||P=0.02 for the comparison with the *RQ* genotype.

**P<0.001 for the comparison with the *H7H7* genotype.

††P=0.005 for the comparison with the *H7H7* genotype.

‡‡P=0.02 for the comparison with the *H7H7* genotype.

Association between Factor VII Polymorphisms and Factor VII Levels

In a multivariate analysis of variance, with age, sex, and triglyceride levels as covariates, the factor VII polymorphisms involving R353Q and hypervariable region 4 were significantly associated with the levels of factor VII clotting activity and antigen (Table 5). The effect was more evident for factor VII clotting activity in controls, owing to the small number of case patients with the *QQ* and *H7H7* genotypes. Patients with the *QQ* or *H7H7* genotype had the lowest levels of factor VII clotting activity and antigen, whereas those with the *RR* or *H6H6* genotype had the highest levels of factor VII clotting activity. Analysis for trend and multiple comparisons showed a codominant effect of the R and Q alleles, whereas the effect of the H7 allele was recessive. The association between factor VII levels and the combination of polymorphisms showed the same trend (Table 4): factor VII levels were the lowest in patients with the *QQH7H7* combination and highest in those with the *RRH6H6* combination.

Dividing values for factor VII clotting activity into thirds allowed us to compare the odds ratios for familial myocardial infarction among patients with

the lowest and intermediate levels with those among patients with the highest level. As shown in Figure 2, the risk of myocardial infarction was reduced by 85 percent and 70 percent, respectively, after adjustment for confounding variables. The results of the analysis of factor VII antigen levels were similar with respect to the lowest level, although the differences were not statistically significant.

DISCUSSION

We found that polymorphisms of the factor VII gene are associated with the risk of familial myocardial infarction. The frequencies of the Q allele of R353Q and of the H7 allele of polymorphisms involving hypervariable region 4 were significantly lower in patients with a family history of thrombosis than in controls, suggesting that these alleles protect against familial myocardial infarction. The presence of the H5 allele seems to be associated with an increase in the risk of familial myocardial infarction, but because of the very low frequency of this allele, a larger sample is necessary to confirm this association.

Patients with a family history of myocardial infarction or stroke (or both) were selected for this study because genetic variants related to the development of thrombosis should be found more frequently in these patients than in patients without such a family history. To increase the contrast further, subjects with a personal or family history of vascular disease were excluded from the control group. These selection criteria, together with ethnic differences, can explain the discrepancy between our results and the inconclusive results of other studies.^{9,18,19}

The distribution of the genotypes in the control group was similar to that recently reported by Bernardi et al.²⁰ in the general population in a region of central Italy and to that which we found in a larger sample of the Italian nonhospitalized population.²¹ However, it differed significantly from that found in other populations, such as in the Netherlands, Denmark, and the United Kingdom, where the frequencies of the rare alleles of both polymorphisms were lower.^{9,18,22} Since these rare alleles are associated with low levels of factor VII, the finding of lower frequencies could be in agreement with the higher incidence of myocardial infarction in such countries as compared with Italy,²³ further supporting the role of these polymorphisms in the development of or protection against myocardial infarction.

Both polymorphisms were significantly related to factor VII clotting activity and antigen levels, suggesting that the association between factor VII genotypes and myocardial infarction may occur as a result of the modulation of factor VII blood levels. Indeed, patients with the *QQ* or *H7H7* genotype, who had the lowest risk of myocardial infarction, also had the lowest levels of factor VII. Moreover,

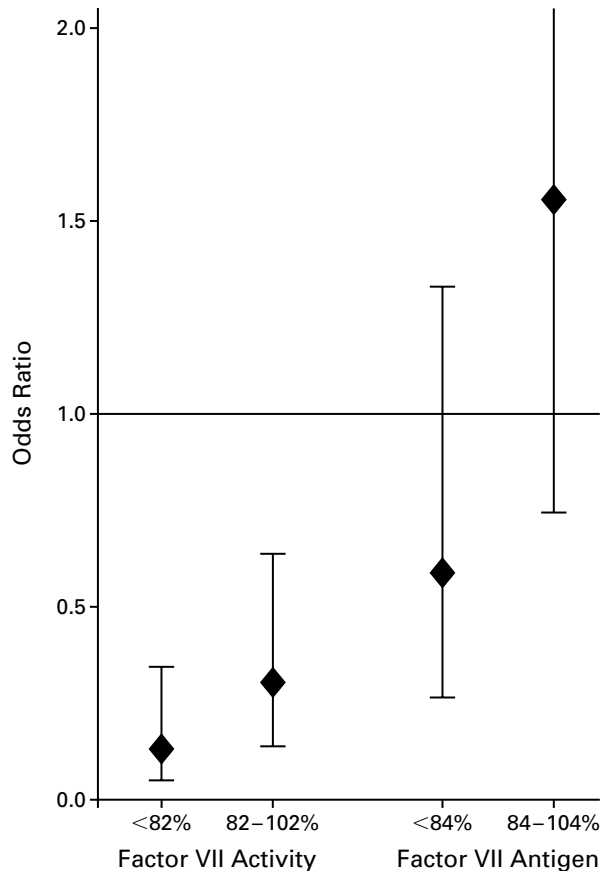


Figure 2. Odds Ratios (and 95 Percent Confidence Intervals) for Familial Myocardial Infarction among Patients with Low and Intermediate Levels of Factor VII Clotting Activity and Antigen as Compared with Patients with the Highest Level.

Factor VII values are expressed as a percentage of the value in pooled normal plasma.

the association of factor VII genotypes with a decreased risk of myocardial infarction was no longer significant once the multivariate analyses were adjusted for factor VII clotting activity, further supporting a role for the activation of factor VII in mediating the effect.

The way in which the factor VII polymorphisms involving R353Q and hypervariable region 4 modulate the levels of factor VII is only a matter of speculation. The amino acid substitution caused by the R353Q polymorphism has been shown to influence the correlation between plasma triglyceride levels and factor VII levels.²⁴ Moreover, the R353Q polymorphism is in strong linkage disequilibrium with a polymorphism in the factor VII promoter, whose functional relevance has been demonstrated by transfection experiments in hepatoma cells.²⁵

It is conceivable that the polymorphism involving

hypervariable region 4 is in linkage disequilibrium with the R353Q polymorphism. However, this DNA locus contains a consensus splice sequence at the 5' repeat, which, even if it is not translated, could be important in regulating the splicing of messenger RNA as it is formed.^{26,27}

In conclusion, our study supports the possibility of genetic control of myocardial infarction. Certain polymorphisms of the factor VII gene may protect against familial myocardial infarction, possibly by modifying factor VII blood levels. Prospective clinical trials are needed to investigate the clinical and therapeutic implications of our results.

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APPENDIX

The following clinical centers and investigators participated in the study: Asti General Hospital, Asti (M. Alciati); Avellino General Hospital, Avellino (G. Amoroso); Barletta General Hospital, Barletta (A. Messina); Sant'Orsola Hospital, Bologna (G. Palareti); Bozzolo General Hospital, Bozzolo (E. Franzì); Cagliari General Hospital (M. Sias) and Cagliari University Medical School (F. Marongiu), Cagliari; Casale Monferrato General Hospital, Casale Monferrato (M. Pezzana); Casarano General Hospital, Casarano (S. Ciricugno); Caserta General Hospital, Caserta (R. Di Sarò); Cento General Hospital, Cento (L. Orselli); Colleferro General Hospital, Colleferro (E. Venturini); Copertino General Hospital, Copertino (A. Calcagnile); Desio General Hospital, Desio (G. Iacuitti); Fidenza General Hospital, Fidenza (S. Callegari); Grosseto General Hospital, Grosseto (A. Cresti); Guastalla General Hospital, Guastalla (V. Manicardi); Lanciano General Hospital, Lanciano (A. Valerio); Legnago General Hospital, Legnago (P. Todesco); Leno General Hospital, Leno (A. Lanzini); Lodi General Hospital, Lodi (C. Pezzi); Lugo General Hospital, Lugo (T. Tognoli); Magenta General Hospital, Magenta (R. Turato); Mantova General Hospital, Mantova (A. Izzo); Mestre General Hospital, Mestre (G. Gasparini); Sacco Hospital (E. Rossi) and Niguarda Hospital (C. Corsini), Milan; Mirano General Hospital, Mirano (A. Zanocco); Monza General Hospital, Monza (F. Achilli); Cardarelli Hospital (F. Piantadosi) and Second University Medical School (D. De Lucia), Naples; Novi Ligure General Hospital, Novi Ligure (L. Fascioli); Nuoro General Hospital, Nuoro (G. Tupponi); Cervello Hospital (A. Ledda, I. Greco) and Benfratelli Hospital (R. La Malfa), Palermo; Perugia General Hospital, Perugia (S. Brando); Pescara General Hospital, Pescara (T. Bonfini); Pescia General Hospital, Pescia (L. Iacopetti); Piombino General Hospital, Piombino (S. Bechi); Pisa General Hospital, Pisa (U. Conti); Rieti General Hospital, Rieti (S. Orazi); Rimini General Hospital, Rimini (E. Bologna); First University Medical School, Rome (P. De Paolis); San Donà di Piave General Hospital, San Donà di Piave (P. Della Valentina); Savona General Hospital, Savona (A. Gandolfo); Casa Sollievo della Sofferenza, San Giovanni Rotondo (A. Vilella); Termoli General Hospital, Termoli (M. Esposito); Maria Vittoria Hospital, Turin (L. Mussano); Treviso General Hospital, Treviso (S. Perissinotto); Udine General Hospital, Udine (G. Feruglio, C. Fresco); Vasto General Hospital, Vasto (E. Bottari); and Fondazione S. Maugeri, Veruno (F. Soffiantino, M. Gattone).

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