

No Evidence of Association Between Prothrombotic Gene Polymorphisms and the Development of Acute Myocardial Infarction at a Young Age

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Background—We investigated the association between 9 polymorphisms of genes encoding hemostasis factors and myocardial infarction in a large sample of young patients chosen because they have less coronary atherosclerosis than older patients, and thus their disease is more likely to be related to a genetic predisposition to a prothrombotic state.

Methods and Results—This nationwide case-control study involved 1210 patients who had survived a first myocardial infarction at an age of <45 years who underwent coronary arteriography in 125 coronary care units and 1210 healthy subjects matched for age, sex, and geographical origin. None of the 9 polymorphisms of genes encoding proteins involved in coagulation (G-455A β -fibrinogen: OR, 1.0; CI, 0.8 to 1.2; G1691A factor V: OR, 1.1; CI, 0.6 to 2.1; G20210A factor II: OR, 1.0; CI, 0.5 to 1.9; and G10976A factor VII: OR, 1.0; CI, 0.8 to 1.3), platelet function (C807T glycoprotein Ia: OR, 1.1; CI, 0.9 to 1.3; and C1565T glycoprotein IIIa: OR, 0.9; CI, 0.8 to 1.2), fibrinolysis (G185T factor XIII: OR, 1.2; CI, 0.9 to 1.6; and 4G/5G plasminogen activator inhibitor type 1: OR, 0.9; CI, 0.7 to 1.2), or homocysteine metabolism (C677T methylenetetrahydrofolate reductase: OR, 0.9; CI, 0.8 to 1.1) were associated with an increased or decreased risk of myocardial infarction.

Conclusions—This study provides no evidence supporting an association between 9 polymorphisms of genes encoding proteins involved in hemostasis and the occurrence of premature myocardial infarction or protection against it. (*Circulation*. 2003;107:1117-1122.)

Key Words: genes ■ myocardial infarction ■ coagulation ■ platelets ■ fibrinolysis

Although the role of environmental factors in the development of acute myocardial infarction has been clearly established, the role of genetic factors (particularly those related to thrombogenesis) is still undefined. A number of relatively small case-control studies, usually of middle-aged and elderly patients, have assessed the association between various polymorphisms of genes encoding factors involved in the hemostatic system, each of which was usually studied separately, and susceptibility to or protection against myocardial infarction.^{1,2} However, the results were conflicting or of borderline statistical significance, not only because of the inadequate sample sizes of the majority of these studies but also because of the confounding effect of atherosclerosis, which is highly prevalent in middle-aged and elderly patients. When acute myocardial infarction occurs in the young, there is usually less coronary atherosclerosis, and the prevalence of normal or near-normal coronary angiograms is high, particularly in women.³ It is therefore biologically plausible that changes in hemostasis factors leading to prothrombotic phenotypes of hypercoagulability, heightened platelet function, hypofibrinolysis, and hyperhomocystinemia (as well as their

genetic determinants) play a relevant role in younger patients with myocardial infarction. In this matched case-control study, we evaluated the association between 9 of the most frequently investigated polymorphisms of genes encoding hemostasis factors and the occurrence of myocardial infarction. The study is distinguished by 2 main features: its sample size and the choice of case patients who had developed myocardial infarction at a young age.

Methods

Case and Control Patients

This nationwide case-control study involved 125 Italian coronary care units. The 1210 case patients (cases) were patients hospitalized for a first myocardial infarction before the age of 45 years who underwent coronary arteriography at the time of hospitalization. The 1210 control patients (controls) were healthy subjects unrelated to the cases but individually matched with them in terms of age, sex, and geographical origin who were enrolled from among the staff of the same participating hospitals; they had no history of thromboembolic disease. The cases and controls were enrolled between January 1998 and January 2001.

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All of the participants were given a standardized questionnaire concerning cardiovascular risk factors, medical diagnosis, lifestyle, and medication. The collected data included age, sex, and traditional risk factors such as a family history of ischemic heart disease, smoking, high serum cholesterol levels, diabetes, hypertension, and cocaine use. Alcohol intake and the levels and pattern of physical exercise were also recorded. The data relating to the cases were collected at the time of their first myocardial infarction and those relating to the controls at the time of the hospital evaluation for study enrollment. All of the study participants agreed to give blood samples for DNA analysis and cholesterol measurements. The Institutional Review Boards of the participating hospitals approved the study, and the cases and controls gave their written informed consent.

Definitions

Acute myocardial infarction was defined as resting chest pain lasting >30 minutes accompanied by ST-segment elevation evolving into pathological Q waves and was confirmed by the presence of total creatinine kinase or MB fraction levels of more than twice the upper normal limit. The absence of any narrowing in coronary diameter was considered evidence of a normal coronary artery, a narrowing of <70% (50% in the case of the left main coronary artery) was considered nonsignificant coronary artery stenosis, and a narrowing of >70% (50% in the case of the left main coronary artery) was considered significant coronary artery stenosis.

A positive family history was defined as the presence of at least 1 first-degree relative (parent, offspring, or sibling) who had developed coronary artery disease before the age of 55 years for men and 65 years for women. The subjects were considered to have hypertension if they had been diagnosed as hypertensive or were taking antihypertensive medication. Their body mass index (BMI) values were categorized as normal weight (18.5 to 25 kg/m²), preobese (>25 to 30 kg/m²), or obese (>30 to 35 kg/m²), the last including WHO classes I, II, and III; underweight subjects were excluded from the analysis because of their small number. In terms of smoking, the subjects were classified as current, former, or never smokers on the basis of self-reports: current smokers were those who reported smoking regularly during the 3 years preceding the myocardial infarction; former smokers, those who had smoked regularly for at least 3 years but not during the year preceding the infarction; and never smokers, those who had never smoked regularly or had smoked regularly for <3 years. Never and former smokers were aggregated in the single category of nonsmokers. The subjects were considered to have diabetes if they had ever been diagnosed as having type I or II diabetes by a physician. Hypercholesterolemia was defined as a fasting total serum cholesterol level of >200 mg/dL (5.2 mmol/L) or the intake of antihypercholesterolemic medications. Cocaine use was classified as chronic, occasional, or absent at the time of the index infarction or enrollment. Physical activity was considered habitual if the subjects engaged in moderately intense exercise for >30 minutes every day or vigorous exercise for >45 minutes twice a week or >20 minutes 3 times a week. Any other level of physical activity was considered occasional, and no exercise was also considered as a separate category. Alcohol consumption was quantified on the basis of self-reports, with moderate consumption being defined as the intake of 10 to 30 g ethanol/d and high consumption as >30 g ethanol/d. In the statistical analysis, alcohol consumption was considered a dichotomous variable (yes/no), with moderate and high consumers being aggregated in the same category.

Blood Collection and DNA Analysis

Blood was drawn from the antecubital vein into 3 tubes containing 0.106 M trisodium citrate and separated into plasma and red cells by centrifugation. DNA was isolated from white blood cells by the salting-out method. The investigators who performed the determinations were blinded as to whether the sample was from a case patient or a control. The following polymorphisms of genes encoding proteins involved in blood coagulation, platelet function, and fibrinolysis were analyzed.

Genes Encoding Proteins Involved in Blood Coagulation

G455A Polymorphism of the -Fibrinogen Gene

The G-to-A substitution located in the -fibrinogen gene promoter was detected by means of polymerase chain reaction (PCR) and digestion with the *Hae*III restriction enzyme (New England BioLabs). The digestion products were visualized by electrophoretic separation on 2% agarose gel.⁴

G1691A Polymorphism of the Factor V Gene (Factor V Leiden)

The G-to-A substitution was detected by amplifying a region of exon 10 and the adjacent intron of the factor V gene by PCR.⁵ The 220-bp fragment was digested with the *Mnl*II restriction enzyme (New England BioLabs) and visualized on 2% agarose gel.

G20210A Polymorphism of the Prothrombin (Factor II) Gene

For the direct identification of the G-to-A substitution in the prothrombin gene, genomic DNA was amplified by use of the 5' primer in exon 14 and a mutagenic primer in the 3' untranslated region.⁶ The 345-bp fragment was digested with the *Hind*III restriction enzyme (New England BioLabs) and visualized on 2% agarose gel.

G10976A Polymorphism of the Factor VII Gene

The G-to-A substitution leading to the replacement of arginine 353 by a glutamine residue was detected by PCR followed by digestion with the *Msp*I restriction enzyme (New England BioLabs). The digestion products were visualized on 2% agarose gel.⁷

Genes Encoding Proteins Involved in Platelet Function

C807T Polymorphism of the Glycoprotein Ia Gene

The mutagenic primers described by Reiner et al⁸ were used to amplify genomic DNA, and the PCR products were digested by the *Taq*I restriction enzyme (New England BioLabs). The digestion products were visualized on 4% agarose gel (2% standard agarose + 2% NuSieve agarose).

C1565T Polymorphism of the Glycoprotein IIIa Gene

The C-to-T substitution was detected by PCR followed by digestion with the *Msp*I restriction enzyme. The fragments were visualized on 3% agarose gel.⁹

Genes Encoding Proteins Involved in Fibrinolysis

G185T Polymorphism of the A Subunit Factor XIII Gene

The G-to-T substitution leading to the replacement of a valine by a leucine residue was detected by PCR followed by digestion with the *Bsa*HI restriction enzyme (New England BioLabs). The digestion products were visualized on 3% agarose gel (2% standard agarose + 1% NuSieve agarose).¹⁰

4G/5G Polymorphism of the Plasminogen Activator Inhibitor Type 1 Gene

The single-allele insertion/deletion is situated in the gene promoter 675 bp upstream of the start of transcription and produces a sequence of either 4 or 5 guanine bases. The genotype was determined by PCR amplification of genomic DNA using the allele-specific primers insertion 5G allele, deletion 4G allele, and a common downstream primer.¹¹ The PCR products were visualized on 2% agarose gel.

Gene Encoding Proteins Involved in Homocysteine Metabolism

C677T Polymorphism of the Methylenetetrahydrofolate Reductase Gene

The C-to-T transition leading to the replacement of an alanine by a valine was detected by PCR. The PCR products were digested by

HinfI enzyme restriction (New England BioLabs)¹² and visualized on 3% agarose gel (2% standard agarose + 1% NuSieve agarose).

Sample Size

Before the study was started, the sample size was calculated on the basis of the expected relative risk of the mutant allele versus the wild-type allele, the allelic frequency of the mutant allele, the desired power, and significance. The allelic frequencies of the 9 polymorphisms were obtained from the published data compiled by Tang and Tracy¹³ and the results of a previous study carried out in a group of young Italian patients and controls.¹⁴ They ranged from 2% for the factor V and prothrombin polymorphisms to 46% for the methylenetetrahydrofolate reductase (MTHFR) polymorphism, 7 of the 9 polymorphisms having a frequency higher than 10%.^{13,14} A sample size of 1210 matched pairs has an $\approx 70\%$ power of detecting an OR of 1.5 with a significance of 5% if the frequency of the mutant allele is 2%. For frequencies of 10% or higher, the power of detecting an OR of 1.5 (which was considered to be as high as can be expected in the context of a multifactorial disease) increases to $>90\%$.

Statistical Analysis

The dependence of disease risk (the response variable) on traditional and genetic explanatory factors was analyzed with a conditional logistic regression model; significance was tested by use of the likelihood ratio test and Wald's test.^{15,16} In the regression, the response variable was defined to take the value of 1 in the cases and 0 in the controls, whereas the set of explanatory variables included traditional nongenetic and genetic risk factors. In the case of traditional risk factors having >2 ordered levels (eg, BMI and physical activity), a test for trend was performed to assess the departure from linearity. If there was significant evidence of departure from linearity, the factor was included in the model as a categorical variable; otherwise, it was included as a quantitative variable.

The effect of each explanatory factor was expressed as the OR and 95% CI computed from the corresponding estimated regression coefficient in the model. Adjusted ORs were obtained by use of a model that included the factor of interest and all of the remaining factors we wanted to adjust for; unadjusted ORs were obtained by use of a model that included only the factor of interest. The effect of each traditional nongenetic risk factor on the risk of myocardial infarction was estimated by adjustment for all the nongenetic risk factors with the exception of family history. The OR for family history was estimated by adjustment for all of the remaining factors.

The 9 genetic factors considered in the analysis represented the individual's genotype at the 9 loci mentioned in the preceding section. Each of these loci was modeled as diallelic, with the wild type denoted 0 and the mutant allele 1. For each locus, a genetic risk factor was defined as taking the value $x=0$ when the locus genotype was 0/0, $x=1$ when it was 0/1, and $x=2$ when it was 1/1. To check for genotyping errors and/or possible stratifications in the sample, the deviation from Hardy-Weinberg equilibrium was tested for each polymorphism in the control group.

The effect of each gene polymorphism was estimated by use of a multiplicative penetrance model (a genotype of 1/1 representing the greatest risk, 0/1 an intermediate risk, and 0/0 the lowest risk), which best agrees with the known association between genotypes at the various polymorphisms and the corresponding phenotypic variations. We fitted a model including 1 genetic risk factor at a time (as a quantitative variable) and all of the nongenetic risk factors found to be statistically significant in the first step of the analysis.

Results

The case sample consisted of 1061 men and 149 women, whose mean age at the time of myocardial infarction was 39 ± 5 years: 11% had normal coronary arteriograms, 10% nonsignificant stenosis, and 69% significant stenosis.

Table 1 shows the frequency distribution and the unadjusted and adjusted ORs with their 95% CIs for the

TABLE 1. Traditional Risk Factors and Risk of Myocardial Infarction: Frequencies in Cases and Controls, Unadjusted and Adjusted ORs

Characteristic	Cases, % (n=1210)	Controls, % (n=1210)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Smoking				
No	12.3	49.2	1.0	1.0
Yes	87.7	50.8	7.4 (5.8–9.6)	7.6 (5.5–10.4)
Diabetes				
No	92.2	99.1	1.0	1.0
Type I+type II	7.8	0.9	10.2 (5.1–20.2)	7.4 (2.8–19.1)
Hypertension				
No	75.2	94.4	1.0	1.0
Yes	24.8	5.6	6.1 (4.4–8.3)	4.5 (3.0–6.8)
Family history				
No	65.8	87.2	1.0	1.0
Yes	34.2	12.8	3.8 (3.0–4.8)	4.0 (2.9–5.6)
BMI				
Normal weight	36.1	56.6		
Preobese	44.0	36.8	2.1 (1.8–2.4)	1.6 (1.3–1.9)
Obese class I–III	19.9	6.6		
Hypercholesterolemia				
No	36.9	51.2	1.0	1.0
Yes	63.1	48.8	1.9 (1.6–2.2)	1.4 (1.1–1.8)
Alcohol				
No	40.0	45.0	1.0	1.0
Yes	60.0	55.0	1.2 (1.0–1.5)	1.1 (0.9–1.5)
Cocaine use				
No	98.0	99.3	1.0	1.0
Yes	2.0	0.7	3.1 (1.4–6.9)	2.3 (0.8–6.7)
Physical exercise				
No	51.2	37.1		
Occasional	23.7	28.5	0.7 (0.6–0.8)	0.7 (0.6–0.9)
Routine	25.1	34.4		

Values are percentages.

traditional nongenetic risk factors in the 1210 cases and controls. BMI and physical activity were analyzed as quantitative variables, because the test for trend did not reveal any evidence of a statistically significant departure from linearity. Smoking and, to a lesser extent, diabetes and hypertension were the traditional nongenetic risk factors with the strongest effect; the effects of BMI and hypercholesterolemia were significant, but their magnitudes were smaller. The significant effect of family history was less than that of smoking but more than those of BMI and hypercholesterolemia. Physical exercise had a statistically significant linear protective effect. The effect of alcohol consumption and cocaine use lost significance after adjustment for the remaining nongenetic risk factors, particularly smoking.

Table 2 shows the frequency distribution of the genotypes and the unadjusted and adjusted ORs with their 95% CIs for each of the 9 polymorphisms, none of which showed a

TABLE 2. Gene Polymorphisms and the Risk of Developing Myocardial Infarction: Genotype Frequencies in Cases and Controls, Unadjusted and Adjusted ORs

Polymorphism	Cases, % (n=1210)	Controls, % (n=1210)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
G-455A β -fibrinogen gene				
0/0	60.5	61.5		
0/1	35.1	32.9	1.0 (0.9–1.1)	1.0 (0.8–1.2)
1/1	4.4	5.6		
G1691A factor V gene				
0/0	96.9	96.4	0.9 (0.5–1.3)	1.1 (0.6–2.1)
0/1	3.1	3.6		
G20210A prothrombin gene				
0/0	96.7	96.8		
0/1	3.3	3.1	1.0 (0.6–1.5)	1.0 (0.5–1.9)
1/1		0.1		
G10976A factor VII gene				
0/0	71.8	71.3		
0/1	26.5	26.9	1.0 (0.8–1.1)	1.0 (0.8–1.3)
1/1	1.7	1.8		
C807T platelet glycoprotein Ia gene				
0/0	41.3	39.9		
0/1	44.3	46.0	1.0 (0.8–1.1)	1.1 (0.9–1.3)
1/1	14.4	14.1		
C1565T platelet glycoprotein IIIa gene				
0/0	73.0	71.5		
0/1	24.1	25.4	0.9 (0.8–1.1)	0.9 (0.8–1.2)
1/1	2.9	3.1		
G185T factor XIII				
0/0	64.4	65.2		
0/1	31.0	30.0	1.0 (0.9–1.2)	1.1 (0.9–1.4)
1/1	4.6	4.8		
4G/5G PAI-1 gene				
0/0	27.7	28.3		
0/1	48.7	48.6	1.0 (0.9–1.1)	1.0 (0.8–1.2)
1/1	23.6	23.1		
677T MTHFR gene				
0/0	30.7	30.0		
0/1	45.2	51.2	1.0 (0.9–1.1)	0.9 (0.8–1.1)
1/1	24.1	20.8		

0/0 indicates genotype associated with the presence of 2 wild-type alleles; 0/1, the presence of 1 wild-type and 1 mutant allele; 1/1, the presence of 2 mutant alleles.

significant association, whether it was adjusted or unadjusted for the nongenetic risk factors.

Discussion

The importance of hemostasis in the pathogenesis of acute myocardial infarction has been firmly established by pathological and angiographic findings of coronary thrombosis and by the knowledge that the plasma levels of the proteins involved in the hemostatic mechanism (such as fibrinogen, factor VII, tissue plasminogen activator antigen, and its

principal inhibitor) are associated with susceptibility to or protection against myocardial infarction, especially in younger patients.^{17,18} In addition, a family history of myocardial infarction is a well-established risk factor in the young,^{19–21} and this may mean that a genetic component is particularly important in these patients.

This matched case-control study was designed to assess whether or not 9 previously evaluated polymorphisms of genes encoding proteins involved in hemostasis had an effect on the risk of myocardial infarction in a series of patients who

developed the disease before the age of 45 years. The main feature of this study is the type of patients, who were selected on the basis of the likelihood of experiencing a myocardial infarction because of a prothrombotic state rather than atherosclerosis. Moreover, the sample size (1210 cases and 1210 matched controls) is much larger than that of most previously reported studies on hemostasis-related genes.^{1,2} The cases and controls were matched by place of origin, as well as by age and sex, to avoid any possible sample stratification that might lead to spurious associations. None of the 9 polymorphisms deviated from Hardy-Weinberg equilibrium, thus providing no evidence of population stratification and/or genotyping error. The allelic frequencies of all of the polymorphisms were very similar to those reported in the literature,¹³ except for the MTHFR gene polymorphism, the frequency of which is relatively higher in the Italian population.^{14,22}

The traditional nongenetic risk factors for cardiovascular disease, such as smoking and family history, were highly associated with premature myocardial infarction, in general agreement with previous reports of smaller series.³ Despite our appropriate study design and adequate sample size, none of the polymorphisms investigated were associated with the occurrence of myocardial infarction, unlike the findings of several other reports reviewed by Lane and Grant¹ and Reiner et al.² As a typical example of discrepant results, a pilot study of 200 Italian patients with premature myocardial infarction (different from those involved in this study) found that the C1565T polymorphism of the platelet glycoprotein IIIa gene was associated with an increased risk of myocardial infarction, particularly in smokers.¹⁴

One limitation of this study is that only patients who had survived a myocardial infarction were enrolled. It cannot be excluded that prothrombotic mutations may be associated with more severe myocardial infarctions and a larger number of early deaths; thus, their effect on the risk of myocardial infarction may be underestimated. Only prospective studies on large series of young healthy individuals followed up until they reach the end points of myocardial infarction and cardiac death could overcome the limitation of this study.

In conclusion, on the basis of this analysis of the most frequently investigated polymorphisms of genes encoding hemostasis factors, it seems that the role of an inherited predisposition to thrombosis in a complex, polygenic, and multifactorial disease such as myocardial infarction is weak in itself and weaker than that of traditional nongenetic risk factors. From a clinical perspective, there is apparently no evidence supporting the usefulness of screening individuals at risk using the studied gene polymorphisms. However, other recently evaluated gene polymorphisms not considered in this study may be important contributors in combination with traditional risk factors or acquired prothrombotic stimuli.^{23,24}

Appendix

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