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Fibrinogen polymorphisms are not associated with the risk of myocardial infarction

C. J. M. DOGGEN,[1] R. M. BERTINA,[2] V. MANGER CATS[3] AND F. R. ROSENDAAL[1,2] [1]*Department of Clinical Epidemiology, Leiden University Medical Centre,* [2]*Haemostasis and Thrombosis Research Centre, Leiden University Medical Centre, and* [3]*Department of Cardiology, Leiden University Medical Centre, The Netherlands*

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Summary. In the *Study of Myocardial Infarctions Leiden*, we investigated the prevalence of three polymorphisms in the α- and β-fibrinogen genes among 560 patients with a myocardial infarction and 646 control subjects. Secondly, we studied the relationships between these polymorphisms and fibrinogen activity and antigen levels. The *Taq*I, *Hae*III and *Bcl*I polymorphisms in the fibrinogen gene were not associated with myocardial infarction. As we found an association of the rare B2 allele with fibrinogen levels and a similar, but weak, effect for the rare H2 allele, we conclude that a genetic propensity to high fibrinogen levels does not affect the risk of myocardial infarction. This is evidence against a causal role for fibrinogen levels in the aetiology of myocardial infarction.

Keywords: fibrinogen, myocardial infarction, polymorphisms, aetiology.

Fibrinogen has been shown to be an independent predictor of myocardial infarction. However, whether fibrinogen is a causal risk factor or is elevated as a result of either existing atherosclerosis or the presence of other cardiovascular risk factors remains unclear. Atherosclerosis is nearly always present before the development of myocardial infarction. It could well be that fibrinogen levels are elevated as an acute-phase reaction in the presence of atherosclerosis. A similar situation may exist with the presence of cardiovascular risk factors. For example, smoking is associated with increased fibrinogen levels and is simultaneously a risk factor for myocardial infarction. As a result, fibrinogen levels may appear to be associated with myocardial infarction but are, in fact, elevated as a consequence of smoking.

We studied the relationship between fibrinogen and myocardial infarction indirectly. Several polymorphisms in the genes encoding for the three separate chains of fibrinogen have been described. In some studies, these genotypes were associated with high levels of fibrinogen. When these genotypes are associated with higher levels, one would expect these genotypes to be present more often in patients who had a myocardial infarction, but only if fibrinogen plays a causal role in the development of myocardial infarction. In this paper, we present the results of the population-based case–control *Study of Myocardial Infarctions Leiden* (SMILE) with respect to the relationship between the *Taq*I polymorphism in the α-fibrinogen gene, the *Hae*III (−455 G/A) and *Bcl*I polymorphisms in the β-fibrinogen gene and myocardial infarction among 560 men with a first myocardial infarction and 646 control subjects. Secondly, fibrinogen levels were measured in the latter, and an association with the presence of certain alleles was looked for.

Correspondence: Prof. Dr F. R. Rosendaal, Department of Clinical Epidemiology, Bldg 1 C0-P, Leiden University Medical Centre, PO Box 9600, 2300 RC Leiden, The Netherlands. E-mail: F.R.Rosendaal@lumc.nl

METHODS

Patients were men below the age of 70 years with a first myocardial infarction. Controls were also men, frequency matched to the patients in 10-year age groups, who had undergone an orthopaedic intervention and had received prophylactic anticoagulants for a short period after this intervention. They did not have a history of myocardial infarction and had not used anticoagulants for at least 6 months before participation in this study. Both patients and controls were born in the Netherlands. Full details of the SMILE study have been published elsewhere (Doggen *et al*, 1998).

Morning fasting blood samples were drawn from the antecubital vein into two Sarstedt Monovette tubes containing 0·106 mol/l trisodium citrate. We separated the blood sample into cells and plasma by centrifugation for 10 min at 3000 *g* at room temperature. Genomic DNA was extracted

from the white blood cells by a salting-out method (Miller *et al*, 1988) The *Taq*I, *Hae*III and *Bcl*I polymorphisms were genotyped after amplification of relevant DNA regions by polymerase chain reaction (PCR) and digestion with the appropriate restriction enzymes as described previously (Thomas *et al*, 1991, 1995) Common alleles are coded as H1, T1 and B1, and the rare alleles are indicated as H2 T2 and B2 In the control subjects, fibrinogen activity was measured in plasma using the Clauss thrombin time method on a fully automatic coagulometer STA (Diagnostica Stago, Boehringer Mannheim) Fibrinogen antigen levels were measured with an enzyme-linked immunosorbent assay (ELISA)

Allele frequencies in patients and control subjects were compared by chi-square analysis A chi-square test was used to compare the observed numbers of each genotype with those expected for a population in Hardy–Weinberg equilibrium An odds ratio (OR) with a 95% confidence interval (95% CI) was calculated as a measure of relative risk Allele frequencies and means of fibrinogen are presented with the 95% CI Analysis of variance was used to compare differences between means

RESULTS

The frequencies of the common alleles, the *Taq*I, *Hae*III and *Bcl*I polymorphisms, were 0 71, 0 79 and 0 83, respectively, with no difference between patients and control subjects (Table I) The distribution of genotypes was as expected for a population in Hardy–Weinberg equilibrium For persons carrying the T2 allele of the *Taq*I polymorphism, the risk of myocardial infarction was not increased, with odds ratios at or close to unity, neither was the risk of myocardial infarction increased for carriers of the H2 allele of the *Hae*III polymorphism, nor for carriers of the B2 allele of the *Bcl*I polymorphism

Among control subjects, carriers of the T2 allele of the *Taq*I polymorphism had similar fibrinogen activity and antigen levels compared with homozygous carriers of the common allele (Table II) Fibrinogen levels appeared to be higher for heterozygous and homozygous carriers of the H2 allele of the *Hae*III polymorphism, although not significantly Antigen levels were higher for carriers of the rare allele of the *Bcl*I polymorphism compared with non-carriers, and a trend towards higher activity levels appeared as well

DISCUSSION

Possession of rare alleles of the *Taq*I polymorphism in the α-fibrinogen gene and the *Hae*III and *Bcl*I polymorphisms in the β-fibrinogen gene was not a risk factor for myocardial infarction Neither homozygous nor heterozygous carriers of rare alleles showed an increased or decreased risk compared with homozygous carriers of common alleles As we observed higher fibrinogen levels for carriers of the B2 allele of the *Bcl*I polymorphism, these data do not support a causal role for fibrinogen in the aetiology of myocardial infarction

Our results are in agreement with those of the Etude Cas-Temoins sur l'Infarctus du Myocarde (ECTIM) study, in which genotype frequencies of the *Hae*III and *Bcl*I polymorphisms were also similar in patients with a myocardial infarction and in control subjects (Scarabin *et al* 1993, Behague *et al*, 1996) Homozygous carriers of the rare allele of the *Taq*I polymorphism were less frequent among patients in Ireland, but not in France (Behague *et al*, 1996) The frequency of the rare allele of the *Hae*III polymorphism was virtually identical in patients with a myocardial infarction and in control subjects from Sweden (Green *et al*, 1993), in an elderly population (van der Bom *et al*, 1998), in individuals who underwent coronary angiography (Gardemann *et al*, 1997) and in men with and without ischaemic

Table I. Frequencies of the common alleles of *Taq*I *Hae*III and *Bcl*I polymorphisms among 560 patients and 646 control subjects and the risk of myocardial infarction for heterozygous and homozygous carriers of the rare alleles

Genotype of	Patients Number (%)	Control subjects Number (%)	Odds ratio (95% CI)
*Taq*I polymorphism			
T1T1	286 (51 1)	327 (50 6)	1
T1T2	228 (40 7)	265 (41 0)	1 0 (0 8–1 3)
T2T2	46 (8 2)	54 (8 4)	1 0 (0 6–1 5)
Allele frequency T1	0 71 (CI 0 69–0 74)	0 71 (CI 0 69–0 74)	
*Hae*III polymorphism			
H1H1	343 (61 3)	404 (62 5)	1
H1H2	199 (35 5)	211 (32 7)	1 1 (0 9–1 4)
H2H2	18 (3 2)	31 (4 8)	0 7 (0 4–1 2)
Allele frequency H1	0 79 (CI 0 77–0 81)	0 79 (CI 0 77–0 81)	
*Bcl*I polymorphism			
B1B1	378 (67 5)	444 (68 7)	1
B1B2	168 (30 0)	179 (27 7)	1 1 (0 9–1 4)
B2B2	14 (2 5)	23 (3 6)	0 7 (0 4–1 4)
Allele frequency B1	0 83 (CI 0 80–0 85)	0 83 (CI 0 81–0 85)	

Table II Fibrinogen activity and antigen levels in 646 control subjects according to the genotypes of the different polymorphisms

Genotype of	Number	Fibrinogen activity (g/l)		Fibrinogen antigen (g/l)	
		Mean (CI)	P value*	Mean (CI)	P value*
*Taq*I polymorphism					
T1T1	327	3 26 (3 19–3 33)		2 76 (2 68–2 83)	
T1T2	265	3 27 (3 18–3 36)		2 73 (2 64–2 81)	
T2T2	54	3 16 (2 98–3 33)	0 5	2 70 (2 54–2 86)	0 8
*Hae*III polymorphism					
H1H1	404	3 22 (3 16–3 29)		2 70 (2 63–2 76)	
H1H2	211	3 31 (3 21–3 40)		2 82 (2 72–2 91)	
H2H2	31	3 32 (3 03–3 61)	0 3	2 82 (2 52–3 12)	0 1
*Bcl*I polymorphism					
B1B1	444	3 22 (3 16–3 28)		2 69 (2 63–2 75)	
B1B2	179	3 34 (3 23–3 44)		2 85 (2 75–2 96)	
B2B2	23	3 30 (2 93–3 67)	0 1	2 79 (2 40–3 18)	0 03
Overall	646	3 25 (3 20–3 31)		2 74 (2 69–2 79)	

*P-value of analysis of variance comparing levels between the three different genotypes

heart disease (Tybjærg-Hansen *et al*, 1997) To our knowledge, only one study, which included Italian patients with familial myocardial infarction, found an increased risk for carriers of the rare allele of the *Bcl*I polymorphism (Zito *et al*, 1997) Given the study size of 560 patients and 646 control subjects, we would have been able to detect a significant excess (*P*-value $< 0\ 05$) of allele carriers in patients vs control subjects of about 8% (80% power), i e relative risks as low as 1 5 Thus our results, which show no association between polymorphisms and myocardial infarction, are not caused by lack of power

The frequency of the common allele of the *Taq*I polymorphism of 0 71 in our control subjects was similar to frequencies in healthy individuals from the UK, Ireland, France and Finland, which range from 0 72 to 0 75 (Humphries *et al*, 1987 Thomas *et al*, 1995, Behague *et al*, 1996, Rauramaa *et al*, 1997) The same was true for the frequency of 0 79 of the common allele of the *Hae*III polymorphism Frequencies ranging from 0 75 to 0 81 have been described (Thomas *et al*, 1991, Green *et al*, 1993, Scarabin *et al*, 1993, Behague *et al*, 1996, Gardemann *et al*, 1997, Tybjærg-Hansen *et al*, 1997 Margaglione *et al*, 1998, van der Bom *et al*, 1998, van't Hooft *et al*, 1999) Again the frequency of the *Bcl*I polymorphism of 0 83 in our control subjects was similar to that of other healthy populations in which the frequency ranged from 0 83 to 0 85 (Thomas *et al*, 1995, Behague *et al*, 1996, Rauramaa *et al*, 1997, Zito *et al* 1997)

No association existed between the *Taq*I polymorphism and fibrinogen levels, either in our study or in other studies (Humphries *et al*, 1987, Connor *et al*, 1992, Rauramaa *et al*, 1997) A trend towards increasing fibrinogen levels with the rare allele of the *Hae*III polymorphism (−455 A) was found (Thomas *et al*, 1991, Green *et al*, 1993, Tybjærg-Hansen *et al*, 1997, Gardemann *et al*, 1997, van der Bom *et al*, 1998, van't Hooft *et al*, 1999), although the association was not always significant (Connor *et al*, 1992, Margaglione *et al*, 1998), as in our own study Again, the rare allele of the *Bcl*I polymorphism seemed to be associated with increasing fibrinogen levels in some studies (Humphries *et al*, 1987, Zito *et al*, 1997), but not all (Connor *et al*, 1992, Rauramaa *et al*, 1997) We found that heterozygous carriers had the highest fibrinogen activity level although the association was only significant for the fibrinogen antigen level, not the activity level Altogether, this suggests that there probably is a weak relationship between the rare variants in the β-fibrinogen gene and fibrinogen levels

We conclude that the *Taq*I, *Hae*III and *Bcl*I polymorphisms in the fibrinogen gene are not related to myocardial infarction As we found an association of the B2 allele of the *Bcl*I polymorphism with fibrinogen levels and a similar but weak, effect for the H2 allele, we conclude that a genetic propensity to high fibrinogen levels does not affect the risk of myocardial infarction This is evidence against a causal role for fibrinogen levels in the aetiology of myocardial infarction

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