

A Genetic Propensity to High Factor VII Is not Associated with the Risk of Myocardial Infarction in Men

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Summary

Several studies have examined the relation between factor VII and coronary artery disease by measuring factor VII levels in plasma and some found an association between high levels and disease. This suffers problems of interpretation concerning the causality of high factor VII levels, because factor VII levels may be affected by atherogenic risk factors and may become elevated as a consequence of atherosclerosis. We investigated the association between a genetic variant (³⁵³Arg→Gln), shown to be related to factor VII levels, and myocardial infarction in a large case-control study, including 560 cases and 644 controls. Individuals carrying the ³⁵³Arg-Arg genotype seemed to have a lower risk of myocardial infarction (odds ratio 0.80 [95% confidence interval 0.60-1.06]). In this study, we confirmed higher factor VII antigen and activity level in 529 men homozygous for the ³⁵³Arg allele compared with 115 men carriers of the ³⁵³Gln allele (around 20% higher).

Our results indicate that a genetic propensity to high factor VII levels is not associated with the risk of myocardial infarction. Since we confirmed the association of the ³⁵³Arg-Arg genotype with higher factor VII levels, we conclude that high levels of factor VII are not a causal determinant of myocardial infarction.

Introduction

Factor VII is a vitamin K-dependent single-chain plasma glycoprotein which participates in the extrinsic pathway of blood coagulation. The relationship between plasma factor VII levels and the risk for coronary artery disease has been studied in several epidemiological studies. The Northwick Park Heart Study, a 16-year follow-up study in 1,382 middle-aged men, showed factor VII coagulant activity (factor VIIc) as an independent risk factor for fatal ischemic heart disease, but not for non-fatal events (1). In the Prospective Cardiovascular Münster Study of 2,780 healthy men in the age of 40-65 years, baseline factor VIIc did not differ significantly between 130 individuals who in 8 years of follow-up developed coronary events and those who did not. A trend toward higher factor VIIc values was reported for the 37 fatal coronary events (2). However, the Edinburgh Artery Study, a population-based cohort study among 1592 men and women found no difference in baseline factor VII levels between 166 persons who subsequently developed myocardial infarction compared with those without any

vascular event in 5 years follow-up (3). Several cross-sectional studies have reported increased factor VIIc in patients who had survived a myocardial infarction (4-6). One study also found elevated factor VII antigen (factor VIIag) levels in these patients (7). In contrast, several other reports did not reveal any increase in plasma factor VIIc or factor VIIag levels after myocardial infarction (8-10). One proposed explanation for the different findings between the NPHS and other studies, could be the use of the assay, which seems more sensitive to activated factor VII in the NPHS (11).

At best, the association between factor VIIc and factor VIIag levels and the risk for coronary artery disease is equivocal. And even if there was truly an association between factor VII and myocardial infarction, this still does not allow the conclusion of a causal relationship between high factor VII levels and the risk of myocardial infarction.

First, factor VII levels are related to age, lipid levels, obesity, blood pressure (12-14), which in themselves are independent risk factors for myocardial infarction. As a consequence, an apparent relation between factor VII and myocardial infarction may simply be the result of the association of factor VII with other risk factors. Studies that do not consider these and other possible confounders, can thereby lead to spurious results. Even if known confounders are taken into account, there may be unknown confounders affecting factor VII levels and the risk of disease.

Secondly, even if an unconfounded relation between factor VII level and myocardial infarction is established, high factor VII antigen or activity level could be a consequence of atherosclerosis rather than a cause of disease progression. This problem is not only limited to case-control studies. Since atherosclerosis develops early in life (15) and the severity of atherosclerosis may to a large extent determine the risk for myocardial infarction (16) this issue cannot be solved in prospective studies, because future cases will, on average, already at baseline have more advanced atherosclerosis.

A common polymorphism in codon 353 in exon 8 of the factor VII gene (³⁵³Arg-Gln) is related to plasma levels of factor VIIc and factor VIIag. Individuals homozygous for the ³⁵³Arg allele have higher factor VIIc and factor VIIag levels compared to carriers of the ³⁵³Gln allele (10, 17-28). Because of this, individuals homozygous for the ³⁵³Arg allele have been exposed to high factor VII levels throughout their lives. If elevated factor VII levels are a risk factor for myocardial infarction, these individuals will be at higher risk for myocardial infarction. Because the factor VII allele is already defined at birth and cannot be influenced by other factors, the causal relation between a genetic propensity to high factor VII levels and myocardial infarction can be studied, without interference by atherogenic risk factors or even atherosclerosis.

In this paper we present the results of a large population-based case-control study "Study of Myocardial Infarctions Leiden" (SMILE) including men who survived a first myocardial infarction and a control

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group of men frequency matched to cases on age. Firstly, the results of the association between the ³⁵³Arg-Gln polymorphism and myocardial infarction are presented. Secondly, the associations between the ³⁵³Arg-Gln polymorphism and factor VIIag and factor VIIc levels in healthy men are shown.

Materials and Methods

Patients and Controls

Men with a first myocardial infarction before the age of 70 were eligible. Potential cases were identified in computerized discharge records of one university and one general hospital, both in Leiden, the Netherlands. Two of the following three characteristics had to be identifiable in the discharge report or hospital care record to confirm acute myocardial infarction: typical chest pain, electrocardiographical changes indicative of evolving myocardial infarction or a transient rise in cardiac enzymes to more than twice the upper limit of normal. Patients who survived a first myocardial infarction between January 1990 and January 1996 were included. Nine individuals with renal disease were excluded. For practical and ethical reasons ten individuals with severe (neuro)psychiatric problems and eight individuals with a life expectancy less than one year were excluded. Of the remaining eligible cases 15.7% refused to participate and 560 cases took part in this study.

Controls were men identified from the records of the Leiden Anticoagulant Clinic and matched to the cases on 10 year age groups. These men had an orthopedic intervention between January 1990 and May 1996 and had routinely received prophylactic anticoagulation for a few weeks or months after this event. Controls were excluded if they had a history of myocardial infarction or had used oral anticoagulants in a 6-month period before participation in this study. One person with renal disease, 18 individuals with severe (neuro)psychiatric problems and eight individuals with a life expectancy less than one year were also excluded. Of the remaining controls 77.0% (644) was willing to take part in the study.

All persons completed a questionnaire concerning the presence of cardiovascular risk factors such as smoking habits. For cases all questions referred to the period before their myocardial infarction. The quetelet index was derived by dividing weight (kilograms) by squared height (meter²). Persons were considered obese if their quetelet index exceeded 30 kg/m². Medication use and history of diabetes were ascertained in an interview with controls and retrieved from discharge letters for the cases. A person was classified as hypertensive or hypercholesterolemic when he was taking prescription drugs for these conditions. The variables obesity, diabetes, hypertension and hypercholesterolemia were grouped together as "metabolic risk factors". All participants were living in the Leiden area and born in the Netherlands. The study protocol was approved by the Ethical Committees of both hospitals.

Blood Collection

A morning fasting blood sample was drawn from the antecubital vein in two Sarstedt Monovette® tubes containing 0.106 mM trisodium citrate. Blood of the citrated tubes was centrifuged for 10 min at 3000 g at room temperature. The citrated plasma was aliquoted in multiple tubes and immediately stored at -80° C. No blood samples could be obtained from one case and one control. The median time between myocardial infarction and blood collection was 2.6 years (range 0.2 to 6.0 years), and orthopedic intervention and blood collection 2.9 years (range 0.6 to 6.3 years).

DNA Analysis

Genomic DNA was extracted from the white blood cells by a salting-out method (29). The DNA was stored at 4° C. Amplification of a fragment of the factor VII gene was performed using the technique of polymerase chain reaction (PCR) with approximately 50 ng genomic DNA and thermostable *Taq* DNA polymerase (Perkin Elmer, New Jersey, USA) in a thermocycler (Biomed GmbH, West-Germany). The nucleotide sequence of the primers used were

respectively: 5'-GGG AGA CTC CCC AAA TAT CAC-3' and 5'-ACG CAG CCT TGG CTT TCT CTC-3' (17). The initial cycle consisted of a step at 91° C for 4 min; this was followed by 33 cycles of 94° C for 40 s, 55° C for 40 s and 71° C for 2 min. This results in a fragment of 312 bp, which is part of exon 8 of the factor VII gene. Fifteen µl of the PCR reactions were digested with 3 units of *Msp*I (20,000 U/ml) (Promega, USA) overnight at 37° C. DNA fragments were separated by electrophoresis on 2% agarose gels in 0.09 M tris, 0.09 M boric acid, 0.2 mM EDTA containing 0.5 µg/ml ethidium bromide, and visualized by means of ultraviolet light. The common allele coding for arginine (³⁵³Arg) gave fragments of 205 bp, 67 bp and 40 bp. The rare allele coding for glutamine (³⁵³Gln) gave fragments of 272 bp and 40 bp long. The technician was blinded to the status of the sample i.e. whether it was from a patient or a control subject. For one individual analyzable DNA was not available.

Factor VII Assays

Factor VIIag was determined using an enzyme-linked-immunosorbent-assay (30). Factor VIIc was assayed by a one-staged method using factor VII deficient plasma (31) and recombiplastin (Ortho Diagnostic Systems NV, Belgium). Assays were performed with a fully automatic STA (Diagnostica Stago, Boehringer Mannheim). All samples were measured in three different dilutions, with a coefficient of variation less than 10% in these three dilutions. Factor VIIag and factor VIIc were expressed as percentages of pooled normal plasma; 100% corresponds to factor VII antigen or activity present in pooled plasma. The intra-assay coefficient of variation (CV) of factor VIIag was 4.6% and for factor VIIc 1.7% (n = 12) based on samples with a level of about 95% and 123% respectively. The inter-assay CVs were respectively 3.5% and 4.1% (n = 12). Pooled normal plasma was prepared from the platelet free plasmas of 118 healthy men and women (not using oral contraceptives) in the age group 20 to 60 years.

Six individuals had extremely high values of factor VII antigen (≥ 300%) with factor VIIc values varying from 80 to 146%. To avoid an excessive influence of these outcomes on the overall results, we repeated all analyses after excluding these individuals. Since this did not affect the results more than in a trivial way, we will present the overall data.

Statistics

Means are presented with the standard deviation (sd). Allele frequencies in the cases and controls are compared by allele counting and chi-square analysis. The expected genotype distribution was assessed using the Hardy-Weinberg equation under equilibrium assumptions. A chi-square test was used to compare the observed numbers of each genotype with those expected for a population in Hardy-Weinberg equilibrium. 95% Confidence intervals (CI) of the allele frequencies were calculated from sample allele frequencies. Odds ratios (ORs) were calculated with the 95% confidence interval according to the method of Woolf (32). We calculated odds ratios for individuals homozygous for the ³⁵³Arg allele, which is an estimate of the relative risk of myocardial infarction for these individuals versus carriers of the ³⁵³Gln allele. Logistic regression was performed to adjust for age. To investigate selective survival after the myocardial event, we studied the distribution of the genotypes of interest according to the time elapsed between the myocardial infarction and the interview. A one-way analysis of variance was performed comparing mean factor VIIag and factor VIIc of ³⁵³Arg-Arg versus ³⁵³Arg-Gln and ³⁵³Gln-Gln genotype. Differences were considered significant at a p value of <.05. All computations were carried out by the SPSS for Windows Version 7.0 statistical package.

Results

The mean age of the 560 patients was 56.2 (9.0) years at the time of infarction compared to 57.3 (10.8) years in the 644 controls at the time of the interview. Other characteristics of both groups are shown in Table 1. The differences in presence of risk factors between patients and controls were more evident in the subgroup younger than 50 years of age.

The frequency of the ³⁵³Arg allele was 0.89 (CI 0.87 - 0.91) in patients and 0.91 (CI 0.89 - 0.92) among controls ($p = 0.10$). The frequency of the ³⁵³Arg allele among patients was not associated with time elapsed since myocardial infarction, i.e. there was no indication of selective survival by genotype or survival bias. The distribution of factor VII genotype in controls was as expected for a population in Hardy-Weinberg equilibrium. As shown in Table 2 the odds ratio for myocardial infarction for individuals homozygous for the ³⁵³Arg allele was 0.80 (CI 0.60 - 1.06) as compared to heterozygous and homozygous carriers of the ³⁵³Gln allele and did not change after adjustment for age. In men younger than 50 years the odds ratio for homozygous ³⁵³Arg carriers was 0.49 (CI 0.28 - 0.84); so the risk for myocardial infarction among homozygous carriers of the ³⁵³Arg allele was significantly and two-fold reduced in younger men. The distribution of factor VII genotype in this age group was also in Hardy-Weinberg equilibrium. The frequency of the ³⁵³Arg allele in men under the age of 50 years was 0.85 (CI 0.81 - 0.89) in patients and 0.92 (CI 0.88 - 0.95) among controls ($p < .01$).

The geometric means of factor VIIag and factor VIIc in the healthy controls were 109.9 (45.7) % and 116.8 (23.0) % respectively. The 529 controls homozygous for the ³⁵³Arg allele had a mean factor VIIag and factor VIIc level of 114.2 (48.4) % and 120.3 (22.1) %, compared to a mean level of 90.4 (21.8) % and 100.6 (20.1) % in 111 heterozygotes. Four homozygotes for the ³⁵³Gln allele had a mean factor VIIag of 83.3 (10.3) % and a mean factor VIIc of 100.0 (21.1) % (Table 3).

Discussion

A genetic propensity to high plasma factor VII levels is not a causal determinant for myocardial infarction. The ³⁵³Arg-Arg genotype of the ³⁵³Arg-Gln polymorphism in factor VII is less often present in cases who survived a myocardial infarction than in controls, while this genotype is clearly related to elevated levels of factor VII antigen and factor VII coagulant activity in healthy individuals. If high factor VII was a causal determinant for myocardial infarction one would expect this genotype to be more frequent among cases compared to controls. In other words, men homozygous for the ³⁵³Arg allele have been exposed to relatively high factor VII antigen and activity levels throughout their whole life, but nevertheless have a lower risk for myocardial infarction. This makes it very unlikely that factor VII levels play an etiological role in the development of myocardial infarction.

While studies are discordant with respect to the association between factor VII levels and the risk of myocardial infarction, and the ³⁵³Arg-Gln polymorphism and the risk of myocardial infarction, there is agreement that this polymorphism is associated with plasma factor VII levels. Individuals homozygous for the ³⁵³Arg allele had levels of factor VIIc 15 to 25% higher than carriers of the ³⁵³Gln allele (17-19, 21, 23, 25). Homozygous carriers for the ³⁵³Arg allele also had higher factor VIIag level (21, 23) and factor VIIa level (28, 33). Our results are consistent with these studies with respect to the relation between genotype and factor VIIc and VIIag levels. The frequency of the ³⁵³Arg allele of 0.91 in controls in our study is similar to frequencies of about 0.9 reported in healthy individuals from the United Kingdom, Ireland and France (17-19, 21, 23, 25, 33).

The molecular mechanism of the ³⁵³Gln effect on plasma levels of factors VIIc and VIIag has been investigated in several studies. It could be a neutral marker in linkage disequilibrium with a functional variation elsewhere in the gene, e.g., with an insertion of a decanucleotide in the gene promotor (34). This insert results in reduction in factor VII promoter activity compared with the more common allelic sequence

Table 1 Characteristics of patients* and controls in the "Study of Myocardial Infarctions Leiden"

	Overall		< 50 years	
	560 cases	644 controls	154 cases	160 controls
Age, years (mean(sd))	56.2 (9.0)	57.3 (10.8)	44.4 (4.2)	42.5 (6.9)
Current smokers (%)	62.3	33.1 [†]	77.3	41.3 [†]
Alcohol users (%)	80.4	86.8 [†]	89	87.5
Obesity (%) [‡]	17.2	16.3	22.7	13.1 [†]
Diabetes (%)	4.6	3.3	4.5	1.9
Hypertension (%) [§]	18.9	16.5	7.1	4.4
Hypercholesterolemia (%) [§]	2.1	1.7	1.9	0.6

* data refer to the period prior to myocardial infarction

[†] chi-square test, p -value < 0.05

[‡] obesity is present as the Quetelet index exceeds 30 kg/m². For two persons height and weight were not available.

[§] a person was classified as having hypertension or hypercholesterolemia if he was taking prescription drugs for these conditions

Table 2 Distribution of genotype in patients and control subjects

Genotype	Cases	Controls	
	N (%)	N (%)	
³⁵³ Arg-Arg	440 (78.6)	529 (82.1)	OR = 0.80 (0.60 - 1.06) [*]
³⁵³ Arg-Gln	115 (20.5)	111 (17.2)	OR = 1.0 [†]
³⁵³ Gln-Gln	5 (0.9)	4 (0.6)	
Total	560	644	
< 50 years			
³⁵³ Arg-Arg	110 (71.4)	134 (83.8)	OR = 0.49 (0.28 - 0.84) [*]
³⁵³ Arg-Gln	42 (27.3)	26 (16.3)	OR = 1.0 [†]
³⁵³ Gln-Gln	2 (1.3)	0 (0.0)	
Total	154	160	

* OR denotes odds ratio of ³⁵³Arg-Arg versus ³⁵³Arg-Gln + ³⁵³Gln-Gln genotype

[†] Reference category ³⁵³Arg-Gln + ³⁵³Gln-Gln genotype

Table 3 Factor VIIc and factor VIIag levels in control subjects according to genotype of ³⁵³Arg-Gln polymorphism

Genotype	N (%)	Factor VIIag [*]		Factor VIIc [*]	
		Mean (sd)	P value [†]	Mean (sd)	P value [†]
³⁵³ Arg-Arg	529 (82.1)	114.2 (48.4)	<.001	120.3 (22.1)	<.001
³⁵³ Arg-Gln	111 (17.2)	90.4 (21.8)		100.6 (20.1)	
³⁵³ Gln-Gln	4 (0.6)	83.3 (10.3)		100.0 (12.1)	
Total	644	109.9 (45.7)		116.8 (23.0)	

* factor VIIag and factor VIIc expressed as percent of pooled normal plasma

[†] P value of one-way anova comparing mean factor VIIag and factor VIIc level of ³⁵³Arg-Arg versus ³⁵³Arg-Gln + ³⁵³Gln-Gln genotype

(35). A study among 705 men in the United Kingdom showed that this insert frequently cosegregated with ³⁵³Arg-Gln genotype, thereby making it almost impossible to separate the effects of both polymorphisms (23). However, among 99 Polish blood donors, the ³⁵³Arg-Gln genotype occurred in the absence of the insert in approximately a third of them and mean factor VII levels were still lower in persons heterozygous for ³⁵³Gln allele compared with persons homozygous for the

³⁵³Arg allele in this particular subgroup, indicating an effect of the ³⁵³Arg-Gln polymorphism independent of the insert (36). A second explanation of the effect of the ³⁵³Arg-Gln polymorphism is a functionally different factor VII molecule. It is possible that the charge change, associated with the replacement of arginine by glutamine, influences the interaction of factor VII with phospholipid surfaces or co-factors, thus having an indirect effect on plasma levels and cleavage of the inactive single chain zymogen to factor VIIa. Since levels of both factor VIIag and factor VIIc are low in homozygotes for the ³⁵³Gln allele substitution of the arginine by a glutamine could also affect the processing of factor VII in the hepatocyte, thus leading to a reduced synthesis and secretion by the liver (36, 37). An increased catabolism of the factor VII protein is yet another possibility (19, 38).

The present study showed individuals with the ³⁵³Arg-Arg genotype having a lower risk for myocardial infarction with an odds ratio of 0.80 (CI 0.60 - 1.06), which is most pronounced in younger individuals; odds ratio of 0.49 (CI 0.28 - 0.84). It appears that genetic risk factors stand out most clearly in the young, who have not yet accumulated age-associated risk factors as much as the elderly. Thus, homozygosity for the ³⁵³Arg allele may be associated with a reduced risk of myocardial infarction. This genotype is associated with higher factor VII levels, which offers no simple explanation for our observations. An explanation may be that the ³⁵³Arg-Arg genotype is linked to another genotype that is the "actual" cause of the effect on myocardial infarction. Which-ever genotype this is, needs to be established.

Since effects might be restricted to specific subgroups, we examined the association of the ³⁵³Arg-Gln polymorphism with myocardial infarction in those with additional risk factors, i.e. smoking or a metabolic risk factor (hypertension, hypercholesterolemia, diabetes, obesity). In these subgroups, although small, the risk appeared even stronger associated with the ³⁵³Arg-Arg genotype (data not shown).

Obviously, our study did not include patients who died during the acute phase of myocardial infarction and therefore we cannot completely exclude an effect of survival bias. Thus, if the genotype that increases factor VII levels would be a very strong risk factor for fatal myocardial infarction without affecting the probability of non-fatal myocardial infarction, an underrepresentation of this genotype among survivors could be the result. This would be a possible explanation for the lower percentage of ³⁵³Arg-Arg genotype among surviving patients. However, it is difficult to imagine that high factor VII levels only had an effect on the occurrence of fatal myocardial infarction and no effect at all on non-fatal myocardial infarction. It is therefore unlikely that the absence of information on the genotype of patients who died affected our conclusion, because survival after myocardial infarction is influenced most by extraneous factors such as patient delay and delay in providing effective assistance, which affect the time-frame from onset of symptoms and start of interventions such as thrombolytic therapy. Other factors influencing 30-day mortality are the level of systolic blood pressure, heart rate, Killip class and localisation of myocardial infarction (39). A second point is, that a marked effect on mortality of a genotype with an allele frequency of 90 % in the general populations is genetically implausible to the extreme.

The willingness to participate in our study was high (overall response over 80 percent), so selective nonresponse is not a plausible explanation for any of our results; especially since this would assume nonresponse determined by factor VII genotype.

Several smaller studies examined the relation between the ³⁵³Arg-Gln polymorphism and myocardial infarction. The ECTIM study, a multicenter case-control study in men, reported an overall nonsignificant association of homozygosity for the factor VII ³⁵³Arg allele with an

increased risk of myocardial infarction. Only one of the four centers, Lille in France, found a lower risk, i.e. an odds ratio of 0.66 (0.29 - 1.47) based on 46 cases and 140 controls (25). In a case-control study in Sweden including 94 men with myocardial infarction before the age of 45 an odds ratio of 1.81 (0.79 - 4.13) for the ³⁵³Arg-Arg genotype could be calculated (40). A case-control study of 165 patients with familial myocardial infarction in Italy found that persons with the ³⁵³Arg-Arg genotype had an increased risk of myocardial infarction; an odds ratio of 1.42 (0.93 - 2.18) could be calculated (41). In the latter study the frequency of the Gln allele among controls was very high, even when compared to other Italian healthy persons (28). This could have explained part of the increased odds ratio. The nonsignificantly increased risks in the Swedish and Italian study contrast to our findings, which indicate a lower risk. Some of the discrepancies between studies may be the result of chance variation, which is most likely to occur in small studies. However, our study included a large number of men living in one specific area and has high statistical power.

We have conducted a large case-control study, and conclude that a genetic propensity to high factor VII levels is not related to the risk of myocardial infarction in men. Therefore, it appears unlikely that high factor VII is a causal determinant for myocardial infarction.

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