Factor VII Coagulant Activity and Antigen Levels in Healthy Men Are Determined by Interaction Between Factor VII Genotype and Plasma Triglyceride Concentration

Steve E. Humphries, Anne Lane, Fiona R. Green, Jackie Cooper, George J. Miller

Abstract Ischemic heart disease is caused by a combination of and interaction between a number of genetic and environmental factors. In a study of a group of healthy men from the United Kingdom, such an interaction was identified between the levels of plasma triglycerides and genetic variation determining plasma levels of factor VII, a clotting factor that is associated with risk of ischemic heart disease. We previously reported a common genetic polymorphism of the factor VII gene that changes arginine at residue 353 to a glutamine (Arg353→Gln) and showed that healthy men who carry the allele for Gln353 had lower plasma levels of factor VII coagulant activity. This association is strongly confirmed in a new sample. Compared with 301 men with the allele for Arg353, 63 men with one or two alleles for Gln353 had levels of factor VII coagulant activity that were 20% lower (97.8% [95% confidence interval (CI), 95.2% to 100.4%] and 78.2% [CI, 73.8% to 82.9%], respectively; P<.0001), with similar genotype-associated differences observed for levels of factor VII antigen. The 6 men who were homozygous for the Gln353 allele had mean levels of factor VII coagulant activity and antigen that were lower by 40% and 50%, respectively. In an assay using bovine thromboplastin, which is specific for the cleaved (activated) form of factor VII, they had levels lower by 60%, suggesting that the major effect of the Gln353 substitution is to reduce the proportion of the circulating zymogen that is activated. Factor VII coagulant activity in this sample was strongly and positively correlated with plasma triglyceride levels (r=.225, P<.0001), but the relation was confined to only those with the allele for Arg353 (r=.325, P<.001). These differences associated with the Gln353 genotype are likely to be of clinical importance because in middle-aged men, an increase in factor VII coagulant activity of 25% is associated with an 82% increase in the risk of experiencing a fatal ischemic event over 5 years. Carriers of the allele for Gln353, who represent approximately 20% of the general population, have lower plasma levels of factor VII coagulant activity and thus a lower likelihood of experiencing thrombotic ischemic events. Because of interaction between genotype and environmental factors, this genetic protection against thrombotic ischemic events is likely to be maintained even in the face of an increase in risk due to environmental changes over time, including those that lead to the development of hypertriglyceridemia.

Key Words • factor VII • triglycerides • ischemic heart disease • gene-environment interaction

The Northwick Park Heart Study reports that a high plasma factor VII coagulant activity (factor VIIc) is associated with an increased risk of coronary heart disease (CHD), especially risk of a fatal CHD event,1,2 in middle-aged men.3 This association is independent of plasma cholesterol and fibrinogen concentrations.4 Factor VII is one of the vitamin K–dependent clotting factors synthesized principally in the liver and secreted as a single-chain glycoprotein zymogen of apparent Mr 48 000.5 In healthy adults it circulates at an average concentration of about 450 ng/mL6 with a half-life of about 5 hours.7 The active enzyme, factor VIIa, is generated by limited proteolysis of factor VII to produce a two-chain form that normally circulates at a concentration of about 4 ng/mL8 with a half-life of approximately 150 minutes.9 This cleavage can be effected by several activated coagulation factors, including factors Xlla, IXa, Xa, and thrombin.8 Factor VIIa possesses very little activity until bound to tissue factor, an integral cell surface–membrane protein that is expressed in the subendothelium and on activated macrophages and acts as an essential cofactor. The factor VIIa–tissue factor complex initiates coagulation by limited proteolysis of factors IX and X.10 Factor VIIc in a plasma sample is determined by an in vitro bioassay10 and is primarily a measure of the coagulant activity of factor VII in the sample after addition of tissue factor...
and a source of Ca\(^{2+}\) as well as a measure of factor VIIa generated from factor VII during the coagulant reaction.

We previously reported\(^{11}\) a strong association between a common polymorphism of the factor VII gene and plasma factor VIIc levels. In exon 8 of the gene a single base change (a glutamine [Gln] to arginine [Arg] substitution) in the coding for amino acid 353 leads to the replacement of Arg by Gln (designated Gln353).\(^{11}\) In a sample of healthy men from the United Kingdom, the frequency of the allele coding for the Gln353 allele was 0.1, and carriers of this allele had levels of factor VIIc 22% lower than the sample mean.\(^{11}\) Individuals homozygous for the Gln353 allele had both low factor VIIc and low factor VII antigen (factor VIIa) levels,\(^{11}\) suggesting that the amino acid substitution may alter the conformation of the protein, leading to its reduced secretion from the liver or increased catabolism. Similar findings have been reported in healthy women\(^{12}\) and in adults of Afro-Caribbean and Asian-Indian descent.\(^{13}\) Because of this reduction in levels of factor VIIc, it is likely that carriers of the allele for Gln353 would have a reduced risk of thrombotic CHD.

A striking feature of factor VIIc is its positive association with plasma triglyceride concentration. This relation extends to all levels of triglyceride, including those within the range found in normal, healthy individuals within the general population, and is associated mainly with the triglyceride components of the chylomicrons and very-low density lipoprotein fractions.\(^{14}\) In persistent hypertriglyceridemic states the increase in factor VIIc is also associated with a raised plasma concentration of factor VIIa.\(^{15}\) \(^{16}\) However, in a small study of Afro-Caribbean and Asian-Indian adults, this association between factor VIIc and plasma lipid levels appears to be much weaker in those individuals carrying the Gln353 allele than in those homozygous for the Arg353 allele.\(^{13}\) We therefore investigated this phenomenon in more detail in a larger sample of healthy men of Caucasian descent.

### Methods

#### Subjects and Biochemical Measures

The 364 men in this study were recruited from a medical practice in Surrey, England, with their informed consent. All were Europeans of Caucasian origin, aged 50 through 61 years, and without clinical evidence of CHD. A nonfasting blood sample was drawn by venipuncture using a Vacutainer system and minimal stasis. Cholesterol and triglyceride concentrations in serum were measured by enzymatic assays with reagents from Sigma (Poole, UK) and Boehringer (Mannheim, FRG), respectively. Plasma factor VIIc was measured by a one-stage semi-automated bioassy\(^{19}\) using either rabbit or bovine thromboplastin. Plasma factor VIIa was measured by an enzyme-amplified immunoassay (Novo Nordisk, Bagsvaerd, Denmark). Both factors VIIc and VIIa were expressed as a percentage of the activity and antigen levels given by standards; in the former, a pool of citrated normal human plasma was adjusted to the potency of an international primary standard (code labeled 84/665; National Institute of Biological Standards and Control [NIBSC], Potters Bar, UK) and in the latter case, a recombinant factor VII was supplied by Novo Nordisk. Plasma factor VIIa was determined by bioassay employing a soluble mutant tissue factor.\(^{2}\) Coagulation times were converted to factor VIIa concentration in nanograms per milliliter by comparison with a standard curve produced by serial dilution of a purified recombinant factor VIIa (code labeled 89668; potency, 0.115 mg/ampoule; NIBSC). Dilutions were performed with a commercial human factor VII-deficient plasma (George King Biomedical, Overland Park, Kan.). Prothrombin was measured by using a modification of the T לפרן venom method\(^{20}\) and factor X by an automated modification of the method of Denson,\(^{20}\) with results expressed as a percentage of the performance of a standard (Immuno, Vienna, Austria). Clotting times were determined on an H Amelung KC 10 coagulometer (American Hospital Supplies Ltd, Didcot, UK).

#### DNA Procedures

DNA was extracted from whole blood by a salting-out method.\(^{21}\) Enzymatic amplification of DNA was performed by polymerase chain reaction (PCR) using 10 µL DNA extract and thermostable Taq polymerase (Perkin Elmer-Cetus) according to the manufacturer’s instructions. Oligonucleotide primers for PCR were obtained from OSEWEL DNA Service. The PCR reactions were performed in a Cambio “intelligent heating block.” The oligonucleotide primers and the cycle times, temperatures, and conditions for Msp I genotype analysis have been described.\(^{11}\) Msp I digestion yielded a constant band of 40 bp irrespective of genotype. The common Mf allele (presence of cutting site, coding for the Arg353) gave bands of 205 bp and 67 bp, and the M2 allele (absence of cutting site, coding for the Gln353) gave a band of 272 bp as described.\(^{11}\)

#### Statistical Analysis

A \(\chi^2\) test was used to compare the observed numbers of each genotype with those expected for a population in Hardy-Weinberg equilibrium. Allele frequency was estimated by gene counting and \(\chi^2\) analysis. The distributions of factor VIIc and triglyceride levels were log transformed, where this reduced skewness and kurtosis, before statistical analysis. Mean factor VIIc levels of individuals of different Arg-Gln polymorphism genotypes were compared by one-way ANOVA. The relations of factors VIIc and VIIa with triglyceride concentration in individuals of different Gln353 polymorphism genotypes were examined by fitting separate least-square regression relations to the data for the whole sample and for men of different genotypes and testing for interaction. In all statistical tests, a probability value of less than .05 was taken as indicating significance.

#### Results

The distribution of factor VII genotypes in the 364 men examined was that expected for a sample in Hardy-Weinberg equilibrium, and the frequency of the allele for Gln353 was 0.095 (95% confidence interval, 0.074 to 0.116). The characteristics of men with different Arg353-Gln genotypes are summarized in Table 1. There were no statistically significant differences in age, body mass index, or serum cholesterol or serum triglyceride concentrations between those with and without the Gln353 allele. However, the 301 men possessing only the Arg353 allele had a factor VIIc and factor VIIa on average 25% and 27% higher, respectively, than those men with one or two alleles for Gln353. To explore further the possible mechanism of the effect of the Arg→Gln substitution, a number of additional measures of clotting factors were performed in the 6 men who were homozygous for the Gln353 allele; the results are summarized in Table 2. Compared with those men with only the Arg353 allele, plasma factor VIIa was lower on average by 30% of standard, and these men had lower mean factor VIIc (38% of standard) when the test was performed with the routine rabbit thromboplastin as a source of tissue factor. However, when the level of activated factor VII was measured by performing the
bioassay with a bovine thromboplastin, which does not support the activation of factor VII zymogen, factor VIIc was lower on average by 60% of standard. Low levels of factor VIIa were confirmed when factor VII activity was assessed with a soluble mutant tissue factor that also fails to support the activation of factor VII zymogen, factor VIIa.

The associations of factors VIIc and VIIag with serum cholesterol and triglyceride concentrations are shown in Table 3. Overall, there were positive and highly significant relations between both factor VIIc and cholesterol concentration. These results are illustrated in the Figure for the relation between genotype and levels of factors VIIc and VIIag in men in the lower, middle, and higher tertiles of plasma triglycerides. Men in the top tertile (≥2.0 mmol/L) with only the Arg353 allele had levels of factor VIIc 20% of standard higher than those in the lowest tertile (≤1.3 mmol/L), while for men with one or more alleles for Gln353 the corresponding values were 5% of standard lower. Similar effects were seen on levels of factor VIIag, with those with the Arg353 allele in the upper tertile of triglycerides having factor VIIag levels 20% of standard higher than those in the lowest tertile; for those with the Gln353 allele this increase was only 10% of standard.

In the majority of the men, similar measures were available at 1 and 2 years of follow-up; their results are presented in Table 4. In both groups there was a progressive, but similar, small and nonsignificant increase in mean body mass index (due to increasing weight) and a nonsignificant decrease in plasma triglyceride levels. However, in the group of men homozygous for the allele for Arg353 there was a significantly larger (22%) increase in factor VIIc over the 3-year period compared with a 14% increase seen in those with the allele for Gln353 (P=.03).

Discussion

The association between the allele for Gln353 and reduced levels of factor VIIc has now been observed in six different studies carried out in samples of patients with CHD and healthy men and women from England, Ireland, France, and the United States and of Afro-Caribbean and Asian-Indian origin (References 11 through 13 and A. Lane, PhD, et al, unpublished data) with the assays of factor VIIc having been performed in three different laboratories. The present study confirmed the very strong effect of this amino acid substitution on factor VIIc and showed that the factor VII genotype is also associated with a similar effect on levels of factor VIIag. In the European Caucasian samples we studied, healthy men with one or more alleles for Gln353 comprised around 20% of the population and had factor VIIc levels 20% to 25% lower than the sample mean. Results from the Northwick Park Heart Study show that in middle-aged men reduced levels of factor VIIc are associated with a reduction in risk of an acute CHD event within 5 years, with significantly fewer CHD events in the men in the low tertile of factor VIIc (below 98.5%) than in the middle or upper tertiles.\(^1\) This factor VII-Arg353-Gln polymorphism is unique in that it is common and exerts a large effect on levels of factors VIIc and VIIag even in heterozygous individuals. Possession of the factor VII-Gln353 variant is likely to confer a measure of protection against acute thrombotic events and thus reduce an individual's risk of thrombosis and myocardial infarction without producing bleeding problems, although prospective studies are required to confirm this prediction.

The molecular mechanism of the Gln353 effect on levels of factors VIIc and VIIag is not yet clear. A
Table 3. Regression Relations of Factor VII Coagulant Activity and Factor VII Antigen Concentration on Cholesterol and Triglyceride at Baseline in Men With and Without the Gln353 Allele

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Predictor Variable</th>
<th>All (n=364)</th>
<th>Arg-Arg (n=301)</th>
<th>Arg-Gln+Gln-Gln (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loge factor VII coagulant activity, % standard</td>
<td>Loge triglyceride*</td>
<td>.115 (.022)</td>
<td>.143 (.024)</td>
<td>-.015 (.055)</td>
</tr>
<tr>
<td></td>
<td>Coefficient (SE)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SRE (SE)</td>
<td>.062 (.012)</td>
<td>.077 (.012)</td>
<td>-.008 (.030)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>.8</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Coefficient (SE)</td>
<td>.068 (.012)</td>
<td>.068 (.013)</td>
<td>.068 (.028)</td>
</tr>
<tr>
<td></td>
<td>SRE (SE)</td>
<td>.069 (.012)</td>
<td>.069 (.013)</td>
<td>.069 (.028)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>.02</td>
</tr>
<tr>
<td>Loge triglyceride^</td>
<td>Coefficient (SE)</td>
<td>.163 (.022)</td>
<td>.181 (.024)</td>
<td>.082 (.048)</td>
</tr>
<tr>
<td></td>
<td>SRE (SE)</td>
<td>.088 (.012)</td>
<td>.098 (.013)</td>
<td>.044 (.026)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>.09</td>
</tr>
<tr>
<td>Cholesterol^</td>
<td>Coefficient (SE)</td>
<td>.078 (.012)</td>
<td>.090 (.013)</td>
<td>.029 (.026)</td>
</tr>
<tr>
<td></td>
<td>SRE (SE)</td>
<td>.080 (.012)</td>
<td>.091 (.013)</td>
<td>.029 (.026)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.0001</td>
<td>&lt;.001</td>
<td>.26</td>
</tr>
</tbody>
</table>

SRE indicates the change in factor VII coagulant activity or factor VII antigen to accompany a 1-SD change in the predictor (independent) variable.

*Interaction genotype × triglycerides, P = .007.
†Interaction genotype × triglycerides, P = .083.
‡Interaction genotype × cholesterol, P = .02.

Remote possibility is that this polymorphism is simply a marker of variation elsewhere in the gene, and a direct effect of the amino acid substitution is being investigated by in vitro expression studies and a comparison of the biochemical properties of the two factor VII types. One possibility for the lower plasma levels of factor VIIag might be that the conformational change produced by the Arg353→Gln substitution affects the processing of factor VII in the hepatocyte, leading to a reduced secretion of the protein. There are precedents for this type of effect, as for example the Z-variant of α-antitrypsin and some variants of the low-density lipoprotein receptor, and this effect could be examined by analysis in vitro of the kinetics of secretion of the factor VII–Gln353 molecule. Alternative explanations might be that in those with the factor VII–Gln353 molecule, feedback regulation of factor VII metabolism is altered, giving reduced hepatic synthesis, or that the factor VII–Gln353 molecule is cleared from the plasma more quickly than the Arg353 protein.

In agreement with earlier findings, lower levels of factor VII in the Gln353 homozygotes were not associated with any decrease in two other vitamin K–dependent clotting factors, factor X and prothrombin.

Bar graphs showing (left) mean factor VII coagulant activity (% standard) and (right) mean factor VII antigen concentration by tertiles of serum triglyceride concentration in men homozygous for the factor VII–Arg353 allele (shaded bars) and heterozygous or homozygous for the factor VII–Gln353 allele (black bars). Cutoff values for tertiles of plasma triglycerides were low tertile, ≤1.28 mmol/L; middle tertile, 1.29 to 2.02 mmol/L; and high tertile, ≥2.02 mmol/L.

Plasma triglyceride thirds
ever, in 5 of the 6 Gln353 homozygotes studied, plasma factor VIIa levels were well below the reported lower normal limit of 1.2 ng/mL, which raises the possibility that the factor VII-Gln353 variant may be relatively resistant to activation in vivo. However, this seems unlikely, since the plasma from Gln353 homozygotes cold activates normally (G. Miller and K. Mitropoulos, unpublished data, 1993), implying that the factor VII-Gln353 zymogen can be cleaved to its two-chain active species normally by factors XIa and IXa processes that do not require tissue factor. Using bovine tissue factor in the assay also gave very low estimates of factor VIIa in those homozygous for the Gln353 allele, and since the assay does not recognize the factor VII zymogen, this suggests a real reduction in factor VIIa levels in vivo in these individuals. However, a reduced affinity of the factor VII-Gln353 variant for bovine soluble mutant and normal human tissue factor, giving rise to low factor VIIc and VIIa activities in the in vitro bioassays, cannot be excluded. An alternative possibility is that the factor VII-Gln353 variant is cleared from the plasma more rapidly than the factor VII-Arg353 variant.

Although the Arg353→Gln substitution is close to the active site serine 344 in the primary sequence of factor VII, the preliminary three-dimensional structure of the factor VII molecule opposite to the active site (Dr Ted Tuddenham, personal communication, 1993). This peripheral location supports the possibility that the Arg353→Gln substitution influences interactions of factor VII with phospholipid surfaces or tissue factor, thereby having an indirect effect on factor VII activation and plasma levels. This sequence may, for example, be involved in the interaction between triglyceride-rich lipoproteins and the factor VII molecule, and the Gln353 substitution may therefore alter the strength of this interaction and affect the rate of cleavage to the two-chain form or alternatively the rate of removal from the circulation. It is thus possible that compared with carriers of the factor VII-Gln353 variant, the levels of activated factor VII in individuals homozygous for the Arg353 allele increase more rapidly in response to changes in plasma lipids after a fatty meal or to long-term changes in the intake of dietary fat. However, this remains to be tested. Such a mechanism might explain the greater rise in factor VIIc over time seen in men with the allele for Arg353. This may be related to small changes in environmental factors, such as adiposity or diet, or to changes in postprandial lipid metabolism, which are known to occur with advancing age and are reflected only partially in fasting triglyceride levels.

Factor VII genotype may be useful in determining those individuals who would most benefit from dietary or drug intervention to influence hypercoagulability and the risk of CHD by lowering triglyceride levels. The data in Table 3 imply that in men with only the allele for Arg353 a reduction of plasma triglycerides of 1.0 mmol/L, which could be achieved by lifestyle changes in diet, exercise, and weight loss, would be associated with an 11% of standard reduction in levels of factor VIIc. By contrast, in those with one or more alleles for Gln353 there would be little change or even a slight increase in levels of factor VIIc for such a reduction in triglyceride levels. Determination of the factor VII-Arg-Gln genotype can be easily performed by using methods suitable for automation (eg, with gene amplification methods using oligonucleotides labeled with fluorophores). The elucidation of the mechanisms underlying these effects will be important in understanding how environmental factors and an individual's genotype may interact to determine risk of CHD.

Acknowledgments

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