A Common Genetic Polymorphism Associated With Lower Coagulation Factor VII Levels in Healthy Individuals

Fiona Green, Cecily Kelleher, Helen Wilkes, Anne Temple, Tom Meade, and Steve Humphries

We have identified a genetic polymorphism of factor VII that is strongly associated with plasma factor VII coagulant activity (factor VIIc) in healthy individuals from the United Kingdom. This polymorphism was detected afterMsp I digestion of polymerase chain reaction–amplified genomic DNA. In a sample of 284 men, the frequency of the M2 allele (loss of cutting site) is 0.1, and individuals with the M1M2 genotype have factor VIIc levels 22% below the sample mean (p < 0.0001). Msp I genotype was found to be the strongest predictor of factor VIIc, accounting for 20.2% of the variance, with cholesterol accounting for an additional 3.5%. The base change that gives rise to the Msp I polymorphism is a G-to-A substitution in the codon for amino acid 353, leading to replacement of arginine (Arg) with glutamine (Gln) in the protein product of the M2 allele (designated Gin 353). Three individuals homozygous for the M2 allele have both low factor VIIc and low factor VII protein concentrations. The conformation of the Gin 353 molecule may be different from that of the Arg 353 protein, affecting its intracellular processing, secretion, turnover in plasma, or activity. In view of its association with lower factor VIIc levels, possession of the M2 allele may confer protection against thrombosis and myocardial infarction. (Arteriosclerosis and Thrombosis 1991;11:540–546)

Factor VII is a serine protease found in plasma and is one of the vitamin K–dependent coagulation factors, along with prothrombin (factor II), factors IX and X, and proteins C and S (for review, see Reference 1). Factor VII is synthesized principally in the liver and is secreted as a single-chain glycoprotein of apparent M, 48,000.2 Cleavage of human factor VII to factor VIIa, a two-chain form held together by disulfide bonds, results in a 20- to 25-fold increase in enzyme activity.2 This cleavage can be effected by a number of activated coagulation factors, including factors XIIa, Xa, and IXa and thrombin.2 In the presence of tissue factor and calcium ions, factor VIIa converts factor X to factor Xa in the initiating reaction of the extrinsic coagulation pathway.3

Several prospective and case–control studies have shown that increased coagulability and reduced fibrinolytic capacity are associated with an increased risk of ischemic heart disease (IHD) and other vascular disorders.4-11 The Northwick Park Heart Study (NPHS) showed that raised plasma factor VII coagulant activity (factor VIIc) is associated with an increased risk of IHD, an association that is particularly striking for events occurring within 5 years.4 Preliminary results from the Prospective Cardiovascular Münster Study confirm this observation.5 In the NPHS, an increase of one standard deviation in factor VIIc (25% of standard) was found to be associated with a 62% increased risk for IHD within the next 5 years, whereas a rise in cholesterol level by one standard deviation was associated with a 47% increase in risk.4 The effects were independent of each other and are probably due to dietary influences on both cholesterol and factor VIIc.4 The effect of dietary lipids on factor VIIc is thought to be mediated through the activation of the intrinsic coagulation pathway by large, negatively charged lipoprotein particles in the plasma, such as very low density lipoprotein (VLDL) and chylomicrons.12-17 Thus, dietary fat may predispose to IHD through short-term effects on coagulability as well as through longer-term effects on atherogenesis.
As well as the effect of dietary lipid intake, there are a number of other factors that influence factor VIIc levels. Age and body mass index (BMI) and, in women, use of oral contraceptives and onset of menopause are associated with higher levels. Cigarette smoking has been shown not to affect factor VIIc. Factor VII is not considered an acute-phase reactant; however, repeated sampling of several individuals has revealed that there is considerable within-individual variation in factor VIIc levels. In the general population, some 47% of the total variance in factor VIIc levels is attributable to variability between individuals.

It is of interest to examine the role of genetic variation in determining plasma factor VIIc levels, as this may provide insight as to genetic predisposition to increased thrombosis and IHD. As yet, no studies have estimated the heritability of factor VIIc. There are a number of reports of inherited factor VII deficiency, which appears to be associated with mild bleeding problems. The cDNA and the gene encoding human factor VII have been cloned and the nucleotide sequence reported. The gene lies on chromosome 13 and is closely linked to the gene encoding coagulation factor X. We have identified a DNA polymorphism in the human factor VII gene that is associated with between-individual differences in factor VIIc levels and propose a mechanism to explain this association.

Methods

Subjects

The sample used for preliminary investigations was from northwest London and has been described previously. Participants in this study were enrolled after a call for volunteers to nearby industrial groups; however, no information was available on the response rate. This sample comprises 91 men and women, but factor VIIc measurements and DNA for genotyping were both available for only 71 of these individuals, of whom 14 were female and 16 were smokers.

The second and larger sample used in this study consisted of healthy men recruited from general practices throughout the United Kingdom as part of the screening for the Thrombosis Prevention Trial (TPT). The trial involved an invitation to all men aged 45–69 years who had no previous history of a myocardial infarction or who were otherwise ineligible for antithrombotic therapy. Individuals of African and Afro-Caribbean origin were excluded from the study. Seven variables were measured; these were smoking history, family history of IHD, BMI (weight/height squared), blood pressure, serum total cholesterol, plasma fibrinogen, and factor VIIc. The present sample was drawn from four selected practices whose physicians agreed to recall a randomly selected group of screened men for genotype analysis. Of the 600 screenees who were invited to participate, only 115 (40.5%) were smokers. Since these men were all recruited from the screening stage before any selection for entry into the TPT, they are not at high risk for IHD and, therefore, the response rate of about 50% (284 of 600) should not introduce bias regarding factor VIIc levels or allele frequency. The mean factor VIIc level in this sample (97%) was comparable with that (95%) of the whole group of 600 men invited to participate.

Factor VII Assays

Factor VIIc was measured by a one-stage biological assay. Factor VII antigen concentration was determined using a commercially available enzyme-linked immunosorbent assay kit (Diagnostica Stago, Franconville, France). Values are expressed as a percentage of a standard. The coagulant activity of some other vitamin K–dependent coagulation factors, such as factors II and X, were measured by standard techniques; factor IX was measured as described in Reference 25 except for substitution of factor IX for factor VIII–deficient plasma.

DNA Procedures

DNA was extracted from whole blood by the Triton lysis method. All restriction enzymes were purchased from Anglian Biotec Ltd., Colchester, England, or Boehringer Mannheim U.K. Ltd., Lewes, England. Southern blots were performed as previously described using the human factor VII cDNA (kindly provided by Earl Davie, University of Washington, Seattle) as the probe. Either full-length factor VII cDNA (2.5 kb), excised from the plasmid using EcoRI, or a 366-bp Pst I fragment containing the end of the coding region of exon 8 but excluding the tandem-repeat region, was radiolabeled using the random-primer method for use as probes.

Enzymatic amplification was performed using the technique of polymerase chain reaction (PCR) with 50 ng–1 μg genomic DNA and thermostable Taq polymerase (Perkin-Elmer-Cetus, Cambridge, England) according to the manufacturer's instructions. Oligonucleotide primers for PCR and direct sequencing were obtained from Oswel DNA Service (Dept. of Chemistry, University of Edinburgh, Scotland), and the sequencing primer was supplied high-performance liquid chromatography (HPLC) purified. The PCR reactions were performed in a Cambio intelligent heating block (Beaconsfield, England). The oligonucleotide primers for Msp I genotype analysis were oligonucleotides 5 and 6 (Figure 1). The initial cycle consisted of steps at 93°C for 3 minutes, 55°C for 1 minute, and 72°C for 2 minutes. The 35 subsequent cycles were 93°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes. Twenty microliters of the PCR reactions were digested with 10 units of Msp I at 37°C. DNA fragments were separated by electrophoresis through 2% agarose gels in 40 mM Tris acetate, 1 mM EDTA, pH 7.7,
Statistical Analysis

Mean factor VIIc levels of individuals of different Msp I polymorphism genotype were compared by one-way analysis of variance. 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 frequencies in different groups were compared by gene counting and $\chi^2$ analysis. A stepwise regression analysis was performed to assess the extent to which Msp I genotype, age, BMI, cholesterol levels, and family history of early IHD (self-reported IHD event in first-degree relative in men <55 years and in women <65 years) influence factor VIIc levels in these population samples. Logarithmic transformation (base 10: log 10) of the factor VIIc levels was used in the one-way analysis of variance, multiple regression, and correlation analysis. Factor VIIc means in the different genotypes are presented untransformed for simplicity.

Results

Southern blot analysis was employed to screen a panel of nine unrelated individuals for common polymorphisms of the human factor VII gene using the enzymes Msp I, Ava II, Taq I, Nco I, Xba I, Kpn I, Pvu II, Sst I, Xmn I, Pst I, BamHI, HindIII, EcoRI, and Nae I with the full-length human factor VII cDNA as a probe. Only for the enzyme Msp I was a polymorphism detected, with fragments of either 1.11 kb or 1.17 kb (not shown). The variable site giving rise to this polymorphism was mapped to exon 8 of the gene (Figure 1) by comparison of restriction fragment size with Msp I sites revealed in the published nucleotide sequence of the gene. This was confirmed by Southern blot analysis using the 366-bp Pst I fragment comprising part of exon 8 of the cDNA as the probe. This small fragment detected the polymorphism as predicted from examination of the nucleotide sequence (not shown).

Oligonucleotides were designed to use the PCR to amplify the region containing the variable Msp I site, together with a nearby constant Msp I site to act as an internal positive control for Msp I digestion (Figure

By guest on October 15, 2017 http://atvb.ahajournals.org/ Downloaded from
The common allele, which cuts with \textit{Msp I}, was designated \textit{M1}, and the allele that does not cut was designated \textit{M2} (Figure 2).

Initially, the \textit{Msp I} genotype of each of the 71 individuals from northwest London was determined. The frequency of the \textit{M2} allele was 0.06, and the sample was found to be in Hardy–Weinberg equilibrium for this polymorphism. Factor VIIc levels were found to be strongly associated with \textit{Msp I} genotype ($p<0.0001$; Table 1). Individuals with genotype \textit{M1M1} had a mean factor VIIc level 103% of standard, the heterozygotes had a lower mean level of 64%, and the one individual with genotype \textit{M2M2} had the lowest level of 33%. Age did not account for these differences since the mean ages of individuals in the three genotype groups were not significantly different from each other or from the sample mean of 42 years (Table 1).

To confirm or refute these results, we determined \textit{Msp I} genotypes in a second sample consisting of 284 men from the TPT screening. The frequency of the \textit{M2} allele was 0.11, which was not significantly different from that in the northwest London sample (0.06), and the genotype distribution was also in Hardy–Weinberg equilibrium in this sample. As in the northwest London sample, \textit{Msp I} genotype was strongly associated with factor VIIc levels ($p<0.0001$), confirming our initial observation. Individuals with genotype \textit{M1M1} had the highest mean factor VIIc level, heterozygotes had an intermediate mean level, and the two \textit{M2M2} homozygotes had among the lowest levels (Table 2). Differences in means of variables among the three genotypes were significant only for factor VIIc levels (Table 2).

In both samples, individuals heterozygous for the \textit{Msp I} polymorphism account for as much as 20% of the population and have factor VIIc levels well below their respective sample means (Tables 1 and 2). In the TPT sample of men, possession of the \textit{M2} allele was associated with factor VIIc levels 22% below the sample mean (Table 2).

Stepwise regression of log10 factor VIIc on age, BMI, \textit{Msp I} genotype, cholesterol, and family history of early IHD showed \textit{Msp I} genotype to be the strongest predictor of log10 factor VIIc, accounting for 20.2% of the variance. Cholesterol was an independent positive significant predictor, accounting for an additional 3.5% of the variance in log10 factor VIIc. This is consistent with the observed correlation of log10 factor VIIc and cholesterol (Table 3).

Three individuals homozygous for the \textit{M2} allele were recalled and blood samples taken for repeat measurement of factor VIIc, for determination of factor VII antigen concentration, and for measurement of other coagulation factors. In these individuals, the factor VIIc levels were repeatably low, and...
Table 3. Pearson Product-Moment Correlations Between Continuous Variables in Thrombosis Prevention Trial Sample

<table>
<thead>
<tr>
<th>Variable</th>
<th>Logio factor VIIc</th>
<th>Cholesterol</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>0.183*</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Age</td>
<td>0.011</td>
<td>0.005</td>
<td>...</td>
</tr>
<tr>
<td>BMI</td>
<td>0.072</td>
<td>0.151†</td>
<td>-0.074</td>
</tr>
</tbody>
</table>

BMI, body mass index.
*p<0.002.
†p<0.02.
For all other values, p>0.05.

The association of the M2 allele of the Msp I polymorphism with low factor VIIc levels is highly significant, even when individuals homozygous for the M2 allele are excluded. This observation was initially made in a small sample of men and women and subsequently confirmed in a larger sample of men. Because of the method of selection of the latter sample from general practices, it is likely to be representative of healthy men in the United Kingdom. In this larger sample (TPT), possession of the M2 allele was associated with factor VIIc levels 22% below the sample mean. This is evident even in the homozygous state (not shown).

To ascertain whether the sequence change creating the Msp I polymorphism alters an amino acid in the factor VII protein molecule, sequence analysis was undertaken. Direct sequencing of PCR-amplified genomic DNA from individuals of different Msp I genotype revealed that the base change causing the polymorphism is a G (M1 allele)-to-A (M2 allele) substitution in the second position of the codon for amino acid 353 (not shown; numbering from the first amino acid of the mature form of the single-chain protein). This would lead to substitution of arginine (Arg) in the protein product of the M1 allele (designated factor VII Arg 353), with glutamine (Gln) in the product of the M2 allele (factor VII Gln 353).

Discussion

The association of the M2 allele of the Msp I polymorphism with low factor VIIc levels is highly significant, even when individuals homozygous for the M2 allele are excluded. This observation was initially made in a small sample of men and women and subsequently confirmed in a larger sample of men. Because of the method of selection of the latter sample from general practices, it is likely to be representative of healthy men in the United Kingdom. In this larger sample (TPT), possession of the M2 allele was associated with factor VIIc levels 22% below the sample mean. This is evident even in heterozygotes, who comprise about 20% of the population. Results from the NPHS showed that in middle-aged men, elevated levels of factor VIIc were associated with an increased risk of an acute IHD event within 5 years, with significantly fewer IHD events in men whose levels were in the lower tertile of factor VIIc (below 98.5%) than in the middle and upper tertiles. This shows that lower factor VIIc level, as observed in individuals heterozygous for the Msp I polymorphism, is associated with a reduced incidence of IHD and suggests that possession of the M2 allele may confer a measure of protection against acute thrombotic events. In agreement with this suggestion, in the TPT sample of healthy men the percentage of men reporting a first-degree relative with IHD was lower among individuals of genotype M1M2 (20%) than among individuals of genotype M1M1 (29%), although these differences were not statistically significant.

A factor VIIc level of 75%, the mean for the M1M2 heterozygotes in the TPT sample, would not be expected to lead to severe bleeding problems since individuals with 20–50% factor VIIc (heterozygotes for congenital deficiency) suffer only mild bleeding characterized by epistaxis and easy bruising. However, the clinical picture seems to be somewhat variable since even a factor VIIc level as low as 1% is, in one case, associated with a very mild bleeding tendency. The individual with genotype M2M2 from the northwest London sample whose factor VIIc level was only 33% of standard has no history of bleeding problems. Therefore, possession of the M2 allele of this polymorphism, even in the homozygous state, is unlikely to be associated with any clinical sequelae.

The effects of cholesterol and Msp I genotype on factor VIIc level are independent and in opposite directions. Stepwise regression analysis showed Msp I genotype to be by far the strongest predictor of factor VIIc levels, accounting for 20.2% of the observed variation in levels. Cholesterol was positively correlated with factor VIIc (Table 3), and the correlation coefficient of 0.18 is consistent with, although slightly lower than, the 0.24 observed in the Wembley study. Cholesterol was found to account for an additional 3.5% of the variance when included in the linear regression model, while neither age, BMI, nor family history were significant predictors of factor VIIc in this sample.

Factor VII is a member of the serine protease family, and the heavy chain of factor VIIa contains the three principal residues involved in the catalytic activity, including serine at position 344. The Arg-to-Gln substitution resulting from the Msp I polymorphism is at position 353, which lies within the serine loop, close to the active-site serine 344. Since this amino acid substitution results in a charge change within a region of the enzyme whose tertiary structure is necessarily constrained, it seems likely that the conformation of the factor VII Gln 353 molecule may be substantially altered. It is possible that this affects the catalytic properties of the enzyme and/or the intracellular and extracellular processing of the molecule. Although the association we have detected...
is between Msp I genotype and measured factor VIIc, it is highly likely that we are detecting a relation with factor VII protein concentration. Population studies have shown that there is a strong correlation between plasma factor VIIc and factor VII protein concentration (r = 0.8).14,15 The three individuals homozygous for the M2 allele have both low factor VIIc levels and reduced levels of factor VII protein (Table 4), which suggests that in these individuals, factor VII protein may be of near-normal specific activity but present at reduced protein concentration in plasma. One explanation for this observation is that a conformational change resulting from this amino acid substitution could affect intracellular processing and lead to reduced secretion of the factor VII molecule. There are a number of precedents for this type of effect, for example, the Z-variant of α-antitrypsin26 and some variants of the LDL receptor.27 Confirmation of such a mechanism awaits experiments to analyze the catalytic properties and the kinetics of secretion of the factor VII Gln 353 molecule.

The finding that the base change in the factor VII gene giving rise to this Msp I polymorphism leads to an amino acid substitution affecting the properties of the factor VII protein provides an explanation at the molecular level for the observed association of the M2 allele with lower factor VIIc. The evidence suggests that this is due largely to a concomitant reduction in factor VII concentration. Although our results strongly suggest that the Msp I polymorphism is a functional base change that directly affects the properties of the protein product, it remains a possibility that it is a neutral marker in linkage disequilibrium with a functional base change elsewhere in the factor VII gene. This possibility can be formally excluded only by generation of the M2 allele base substitution in an M1 allele by site-directed mutagenesis and subsequent analysis of the properties of the protein product.

This factor VII Arg 353→Gln polymorphism is unusual in that it is a common polymorphism that has a large effect on factor VII levels, even in individuals heterozygous for the polymorphism. Possession of the factor VII Gln 353 variant is likely to reduce an individual’s risk of thrombosis and myocardial infarction without producing bleeding problems, although case-control studies will be required to confirm this prediction.

Acknowledgments

The authors acknowledge the secretarial assistance of Elaine Osman in the preparation of this manuscript and the technical expertise of Gail Simpson, Yvonne Stirling, and David Howarth. We thank Patrick Brennan for selecting the TPT sample and George Miller for helpful discussions.

References


25. Ott R, Pfeiffer RA: Evidence that activities of coagulation factors VII and X are linked to chromosome 13(q34). *Hum Hered* 1984;34:123–126


**KEY WORDS** • factor VII • polymerase chain reaction • thrombosis • myocardial infarction • ischemic heart disease • genetic polymorphisms
A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals.

F Green, C Kelleher, H Wilkes, A Temple, T Meade and S Humphries

doi: 10.1161/01.ATV.11.3.540

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/11/3/540

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/