Abstract Plasma factor VII activity (factor VIIc) is one of the independent risk factors for coronary artery disease and is controlled by both genetic and environmental factors. Several studies in healthy Caucasian subjects have revealed an association of a common genetic polymorphism at residue 353 (Arg→Gln) of the factor VII gene with plasma factor VIIc. We have investigated the influence of this polymorphism (factor VII Arg/Gln) on fasting plasma factor VIIc and antigen (factor VIIag) levels and its interaction with triglyceride levels in 185 healthy Dravidian Indians of both sexes (128 men, 57 women). The frequency of Gln353 has been found to be significantly higher in Dravidian Indians (0.29; confidence interval, 0.27 to 0.30) than in Caucasians (0.10). The distribution of factor VII Arg/Gln genotypes was at Hardy-Weinberg equilibrium. The carriers of the Gln353 allele had significantly lower plasma factor VIIc and factor VIIag in men (P<.05). The factor VII Arg/Gln polymorphism explained 13% and 11% of the total variance of plasma factor VIIc and factor VIIag, respectively, in men (P<.001) and 6% and 9% in women (P>.1). The genotype-specific correlation of factor VIIc and factor VIIag with triglyceride levels was stronger in carriers of the Gln353 allele (r=.38 and .41; P<.001) than in Arg353 homozygotes (r=.39 and .27; P=.19 and .005, respectively). (Arterioscler Thromb. 1994;14:1923-1927.)

Key Words • factor VII • triglycerides • coronary risk • gene-environment interaction • Asians

Factor VII is one of the vitamin K-dependent coagulation factors synthesized principally in the liver and secreted as a single-chain glycoprotein of M, 48,000. It is present in the plasma of healthy adults in a concentration of approximately 450 ng/mL with a half-life of approximately 5 hours.2,3 The active form (factor VIIa) is formed by limited proteolysis in the form of two polypeptide chains held together by a disulfide bond. Factor VIIa is present in plasma in low concentrations (approximately 1% of factor VII antigen [factor VIIag]) with a half-life of approximately 150 minutes.4,5 The cleavage of factor VII to factor VIIa is effected by several activated coagulation factors (XIIa, IXa, Xa) and thrombin. Factor VIIa binds to tissue factor, and this complex, in the presence of Ca2+, converts factor X to factor Xa.6

Factor VII activity (factor VIIc) has been shown to be an independent risk factor for the development and/or progression of coronary artery disease.7-12 However, the Progetto Lambardo Anti-Thrombosis (PLAT) Study and some other studies could not confirm the significance of factor VIIc in cardiovascular disease.13-15 Older and young healthy subjects with a family history of coronary artery disease have been found to have higher factor VIIc compared with those without such a family history.16-18 Another factor is that factor VIIc always shows a positive correlation with triglycerides and/or cholesterol although the mechanism is not clear.11,16-23 It has been suggested that factor VII binds to very-low-density lipoprotein (VLDL) and prolongs its half-life24,25 and that small amounts of factor VII are converted to factor VIIa through contact surface activation on VLDL.26-30 It has also been shown that factor VIIc depends on lipolytic activity via factor XII activity and that it is the free fatty acids released from the triglyceride core of chylomicrons that provide a contact surface for activation31,32; this reduces the fractional catalytic rate of factor VII, thereby enhancing the activation process.23 Furthermore, it has been suggested that factor VIIc decreases after weight reduction in obese subjects.33 It has also been shown that the postprandial rise of factor VIIc is not associated with an increase in factor VII protein in plasma.34 On the contrary, in persistent hypertriglyceridemia, the increase in factor VIIa is associated with a raised plasma concentration of factor VIIa.25,35

A strong association of factor VIIc has been observed with a common polymorphism of the factor VII gene in several Caucasian populations.36-38 The guanine to adenine substitution in codon 353 in exon 8 of the factor VII gene leads to the replacement of arginine by glutamine (Gln353). The frequency of Gln353 has been found to be around 0.1 in Caucasians, and the carriers of this allele had approximately 20% lower factor VIIc and factor VIIag. Individuals homozygous for the Gln353 allele had much lower factor VIIc and factor VIIag levels. A similar association has been observed in adults of Afro-Caribbean origin and Gujarati Indians in the United Kingdom.37 A genotype-specific association of
plasma factor VIIc and factor VIIag with triglyceride levels has also been observed, with a stronger correlation in Arg353 homozygotes compared with that in carriers of Gln353 in Caucasians and Gujaratis. However, all the above studies have been carried out in nonfasting subjects, which might have a confounding effect on genotype-specific correlation of plasma factor VIIc and factor VIIag levels with serum triglyceride levels because of variable postprandial status. This suggests that amino acid substitution may alter the conformation of the protein, leading to its reduced secretion or increased catabolism. An alternative mechanism could be that the amino acid substitution caused by the mutation in codon 353 of the factor VII gene may be involved in the interaction between triglyceride-rich lipoproteins (VLDL and chylomicrons) and the factor VII molecule.

We present here the results of a study of the association of this polymorphism of the factor VII gene with plasma factor VIIc and factor VIIag levels in a healthy population of Dravidian Indians in Singapore and examine the interaction with serum triglyceride levels.

Methods

Sample

One hundred and eighty-five healthy adults of both sexes (128 men, 57 women) of Dravidian Indian descent (20 to 78 years) formed the sample of the study. The Indians in Singapore are mostly Tamil-speaking and are second- or third-generation immigrants from southern India and Sri Lanka. They were recruited from factories and the community as voluntary participants of a healthy lifestyle promotion exercise. The average response rate was approximately 70%. Detailed medical and family histories were obtained from each individual on questionnaire forms. They were requested to report at the factory clinic or community center between 8 and 9 AM after an overnight fast. Their height and weight were recorded. Recumbent blood pressure and a 12-lead electrocardiogram were recorded after subjects had rested 30 minutes on a couch. A chest radiograph was also taken.

Blood samples were collected in Sarstedt vacuotainer tubes (5 mL in plain, 4 mL in 3.8% trisodium citrate, and 2 mL in EDTA) using a multisampling device with minimal stasis. Citrated plasma was separated within 1 hour and aliquoted in three Nunc cryotubes, which were immediately snap-frozen in liquid nitrogen and stored at -70°C until use. Cells were stored at -20°C for extraction of genomic DNA.

Drugstore plasma was separated within 1 hour and aliquoted in three Nunc cryotubes, which were immediately snap-frozen in liquid nitrogen and stored at -70°C until use. Cells were stored at -20°C for extraction of genomic DNA.

Plasma factor VIIc was measured by a one-stage semiautomatic bioassay on an H Amelung KC 10 coagulometer (Brownies Ltd). Plasma was thawed in a water bath at 37°C, brought to room temperature, and diluted 1:40 with glyoxaline buffer. To 100 μL of the diluted test plasma was added 100 μL of the factor VII-decient plasma. Coagulation was started by addition of a mixture of thromboplastin and 100 μL of 25 mmol/L CaCl2. The same batch of reagent kits and standard reference plasma obtained from Diagnostica Stago were used for the entire project, and factor VIIc was estimated following the manufacturer’s protocol. Two control plasma samples (Biopool International and Behring) were used as internal controls for factor VIIc. Plasma factor VIIc was estimated in batches of 10 test samples within 3 days of sample collection. The assays were repeated at a 1:80 dilution of the test plasma when clotting time was shorter than the 1:20 dilution of the standard. Plasma factor VIIag was measured by an enzyme-linked immunosorbent assay (Diagnostic Stago) and reference standard using an automated ELISA Reader (Bio-Rad 3550). Both factor VIIc and factor VIIag were expressed as a percentage of activity and antigen levels in the standards supplied by the manufacturers. Control plasma supplied by the manufacturer was used as internal control for factor VIIag.

Serum cholesterol and triglyceride levels were estimated on the Cobas Mira using commercially available kits (Roche). Body mass index (BMI) was estimated as weight (kilograms) divided by height (meters) squared.

DNA Procedures

Genomic DNA was extracted from citrated packed cells by the sarcosine method. Enzymatic amplification of DNA was carried out by polymerase chain reaction (PCR) in a final volume of 25 μL containing 0.25 to 0.5 μg DNA, PCR buffer with 2 mmol/L MgCl2, 25 μmol/L dNTPs, 1:10 dimethyl sulfoxide, 1.0 U thermostable Taq polymerase (BRL), and 100 nmol/L each of primers. PCR was carried out in an automated thermal cycler (model 480, Perkin-Elmer Cetus) in the following cycles: an initial cycle of 95°C for 5 minutes, 55°C for 1 minute, and 72°C for 2 minutes; followed by 30 subsequent cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes. The nucleotide sequences (5'-3') of the primers were GGAGAGCTCCCAATATCAC and ACGCACGCTTG-GCTTTCTC. Ten micromolars of each PCR product (312 bp) was digested with 20 U Msp I at 37°C. DNA fragments were separated by electrophoresis on a 4% agarose gel with ethidium bromide (NuSeive, Seakem; GTG=3:1) in Tris-ethylenediamine acetic acid/boric acid buffer (pH 7.6) at 200 V for half an hour. The gels were photographed over a UV transilluminator. Msp I digestion yielded a constant band of 40 bp. The common allele (presence of cutting site, coding for Arg353) produced two bands of 205 and 67 bp, whereas the Gln353 allele (absence of cutting site) gave one band of 272 bp.

Statistical Analysis

Allelic frequencies were calculated by the gene-counting method. Deviation in genotype distribution from that expected by the Hardy-Weinberg equilibrium was estimated by the χ² test. Plasma factor VIIc and factor VIIag levels were regressed on age, sex, BMI, and plasma triglyceride levels for genotype comparisons of factor VIIc and factor VIIag levels. One-way ANOVA with age, sex, BMI, and serum triglyceride levels as covariates was performed to estimate the extent of sample variance (R²×100) of factor VIIc and factor VIIag explainable in terms of factor VII polymorphism, and the level of significance was estimated by F and P values. All computations were carried out on IBM 3081 computer using the spssx statistical package.

Results

Table 1 shows the distribution of the Arg/Gln353 polymorphism of the factor VII gene in Dravidian Indians. The frequency of the allele coding for Gln353 (absence of Msp I cutting site) in the present series was found to be 0.29 (confidence interval [CI], 0.27 to 0.30), which is significantly higher than that reported in Caucasians (0.10; CI, 0.08 to 0.13) and Afro-Caribbeans in the United Kingdom (0.08; CI, 0.04 to 0.12). The distribution of the factor VII genotypes was at Hardy-Weinberg equilibrium. A similar high frequency of Gln353 (0.25) has been reported in the Gujaratis of the United Kingdom.37 The Gujaratis are immigrants from the northwestern state of Gujarat and speak Gujarati.

On the other hand, the Dravidian Indians are immi-
Table 1. Distribution of Factor VII Arg/Gln<sup>353</sup> Genotypes in Dravidian Indians

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Observations</th>
<th>No. Expected*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>92</td>
<td>94.2</td>
</tr>
<tr>
<td>Arg/Gln‡</td>
<td>80</td>
<td>75.6</td>
</tr>
<tr>
<td>Gln/Gln†</td>
<td>13</td>
<td>15.2</td>
</tr>
<tr>
<td>All</td>
<td>185</td>
<td>185.0</td>
</tr>
</tbody>
</table>

Arg indicates the presence of Msp I site; Gln, absence of Msp I site.
*Number of expected observations at Hardy-Weinberg equilibrium.
†Frequency of Gln<sup>353</sup> = 0.2865 (confidence interval, 0.27 to 0.30), consistent with Hardy-Weinberg equilibrium.

Table 2 shows the mean (SEM) plasma levels of adjusted factor VIIc and factor VIIag in relation to three genotypes of the Arg/Gln<sup>353</sup> polymorphism of the factor VII gene along with adjusted serum triglyceride levels in Dravidian Indians. The relatively low levels of factor VIIag in this series could not be caused by reference standard (Stago) as the control plasma used as internal control had expected values. Furthermore, the corresponding values of factor VIIc and factor VIIag in Chinese were 122% and 90%, respectively (N.S., et al, unpublished observations, 1994). Furthermore, the same batch of reagent kits and reference standards was used for the entire study. The Gln<sup>353</sup> homozygotes had lower serum triglyceride levels (111.2 mg/dL) than individuals with other genotypes. However, the carriers of Gln<sup>353</sup> had serum triglyceride levels (150.7±9.9 mg/dL) similar to those of Arg<sup>353</sup> homozygotes (149.0±10.6 mg/dL). Subjects carrying one or two Gln<sup>353</sup> alleles had significantly lower plasma factor VIIc (14% and 32%) and factor VIIag (9% and 20%) compared with Arg<sup>353</sup> homozygotes (P<.05). This influence was independent of the interaction of serum triglycerides, as plasma factor VIIc and VIIag values were adjusted for this in addition to age, gender, and BMI.

Table 3 shows the ANOVA statistics explaining the percentage contribution of factor VII Arg/Gln<sup>353</sup> polymorphisms in the sample variance of factor VIIc and factor VIIag in men and women. This factor VII polymorphism explained 13.4% and 11.2% of the total variance of plasma factor VIIc in men (P<.001). However, in women the influence was not statistically significant although the trend was apparent. The lack of influence of the factor VII gene on factor VIIc in women could be due to either the small sample size (n=57) or a gender-specific genotype association mediated by sex hormones. It will be interesting to examine the influence of the factor VII gene on plasma factor VIIc and factor VIIag in a larger sample of healthy premenopausal and postmenopausal women. Gender-specific association of apolipoprotein E polymorphism with plasma lipids has been well documented. Plasma factor VIIc and factor VIIag levels did not differ significantly between men and women after adjustment for age, BMI, and serum triglyceride levels in the present series (141% and 83% in men, 137% and 80% in women); serum triglyceride levels were significantly higher in men (161 versus 106 mg/dL), and this could have a confounding effect on the association. An increase in factor VIIc and factor VIIag has been reported after menopause. Earlier studies on the association of the factor VII Arg/Gln<sup>353</sup> polymorphism with plasma factor VIIc were carried out only in men.

Table 4 shows a genotype-specific correlation of factor VIIc and factor VIIag with serum triglyceride levels in the entire sample. A stronger correlation is observed between triglycerides and factor VIIc (r=.38, P<.001) and factor VIIag (r=.41, P<.001) in subjects carrying the Gln<sup>353</sup> allele than in those carrying only the Arg<sup>353</sup> allele (r=.09 and .27, respectively.)

Table 2. Triglyceride, Factor VII Activity, and Factor VII Antigen Levels According to Arg/Gln<sup>353</sup> Genotype in Dravidian Indians

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Triglycerides*</th>
<th>Factor VIIc†</th>
<th>Factor VIIag†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>92</td>
<td>149.0 (10.6)</td>
<td>158.0 (4.5)</td>
<td>88.7 (1.5)</td>
</tr>
<tr>
<td>Arg/Gln‡</td>
<td>80</td>
<td>150.7 (9.9)</td>
<td>135.8 (4.6)</td>
<td>81.1 (1.8)</td>
</tr>
<tr>
<td>Gln/Gln†</td>
<td>13</td>
<td>111.2 (14.6)</td>
<td>107.3 (8.3)</td>
<td>70.8 (3.1)</td>
</tr>
</tbody>
</table>

*Adjusted for age, gender, and body mass index.
†Adjusted for age, gender, body mass index, and triglycerides.

Table 3. Percentage of Sample Variance (R<sup>2</sup> x 100) In Plasma Factor VIIc and Factor VIIag Explained by Factor VII Arg/Gln<sup>353</sup> Genotype (ANOVA) In Dravidian Indians

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;x100</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIIc</td>
<td>128</td>
<td>13.4</td>
<td>10.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Factor VIIag</td>
<td>128</td>
<td>11.2</td>
<td>7.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIIc</td>
<td>57</td>
<td>6.1</td>
<td>2.2</td>
<td>.121</td>
</tr>
<tr>
<td>Factor VIIag</td>
<td>57</td>
<td>9.3</td>
<td>4.4</td>
<td>.170</td>
</tr>
<tr>
<td>Both sexes*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIIc</td>
<td>185</td>
<td>11.1</td>
<td>12.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Factor VIIag</td>
<td>185</td>
<td>10.9</td>
<td>12.4</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Sex as additional covariate.
TABLE 4. Spearman's Correlation of Plasma Factor VIIc and Factor VilaG With Triglycerides in Different Arg/Gln353 Genotypes in Dravidian Indians

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Factor VIIc r (P)</th>
<th>Factor VilaG r (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>92</td>
<td>.09 (.19)</td>
<td>.27 (.005)</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>93</td>
<td>.36 (&lt;.001)</td>
<td>.41 (&lt;.001)</td>
</tr>
</tbody>
</table>

Definitions as in Table 3.

of the genotype-specific correlation between plasma factor VIIc and factor VilaG with triglycerides was independent of serum triglyceride levels. However, in earlier studies in Caucasians and Gujaratis in the United Kingdom, an opposite genotype specificity with serum triglycerides was reported. Both of these studies were conducted in nonfasting subjects. The variable postprandial modification of factor VII activity through the formation of a contact-activation site on VLDL could have confounded the association. Furthermore, it has been suggested that the binding of factor VII protein to VLDL prolongs its half-life and that a small amount of factor VilaG is converted to activated factor VIIa through contact-surface activation. It has been shown that a postprandial increase in factor VIIc is not associated with a rise in factor VilaG, whereas in persistent hypertriglyceridemia the latter also rises. Correlation of factor VIIc with factor VilaG (Table 5) was also found to be stronger in those individuals carrying the Gln353 allele than in those with the Arg353 allele (r=.48 versus .26). There was no significant difference in the association of the Arg/Gln353 gene locus with plasma factor VIIc and factor VilaG levels in relation to smoking.

Discussion

The present study in fasting healthy individuals includes the confounding effect of a postprandial influence of serum triglyceride levels on factor VIIc and factor VilaG. The findings in the small sample of women indicate that the genotype association of plasma factor VIIc and factor VilaG could also be gender specific. All the parameters in the present study for association with factor VII genotypes were adjusted for age, BMI, and serum triglyceride levels. This study conducted in another independent sample of Asians confirms the association of factor VII Arg/Gln353 polymorphism with plasma factor VIIc and factor VilaG observed in Caucasian populations. Furthermore, it appears that the association is genotype specific, Gln353 having a stronger correlation of serum triglycerides with factor VIIc and factor VilaG. The frequency of the Gln353 allele in Dravidian Indians and Gujaratis is significantly higher (0.29) than that in Caucasians (0.10), and it would be expected that Indians would have lower factor VIIc levels. However, the interacting influence of other variables such as triglycerides and other vitamin K-dependent coagulation proteins may be involved in producing the higher factor VIIc levels observed in Indians. This is possibly supported by an observed stronger influence of serum triglycerides and factor VilaG with factor VIIc in the Gln353 genotype. Also, serum triglyceride levels in both the Gujaratis and Indians in Singapore are higher than levels in Caucasians. The average factor VIIc levels in the Gujaratis and present series of Indians have also been found to be significantly higher than levels in Caucasians.

We conclude that the influence of a factor VII Arg/Gln353 polymorphism on plasma factor VIIc and factor VilaG is present in all the populations studied so far and the interaction of serum triglyceride levels on the association is genotype specific, with possible variations depending on the prandial state of the subjects.

Acknowledgments

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