Partial Gene Deletion in a Family With Factor X Deficiency

By F. Bernardi, G. Marchetti, P. Patracchini, S. Volinia, D. Gemmati, P. Simioni, and A. Girolami

The presence of gene lesions in coagulation factor X (FX, Stuart factor) was investigated in patients with FX deficiency or an FX abnormality (FX Friuli). The proposita had a heterozygous partial deletion of the FX gene with severe deficiency of FX activity and antigen. The lesion, which was inherited from her mother, removes the 3' portion of the gene coding for the catalytic domain of the factor. In this family, two differently affected FX genes are present, leading to double heterozygosity of the proposita and thus excluding consanguinity of parents. An apparently normal gene structure was observed in the other patient with FX abnormality, suggesting the presence of a small gene lesion.

©1989 by Grune & Stratton, Inc.

Factor X (FX, Stuart factor), a glycoprotein of the intrinsic and extrinsic pathways of blood coagulation, is a serine protease composed of a light [molecular weight (mol wt) 16,900] and a heavy (mol wt 42,100) chain.¹ The FX zymogen is activated by factor IXa or factor VIIa and converts prothrombin to thrombin. The amino-acid and cDNA sequences² and the structural organization of the gene³ have demonstrated that FX is similar to other vitamin K-dependent proteases, particularly to factor IX and protein C⁴,⁵ suggesting that they have evolved from a common ancestral gene.

FX deficiency (Stuart-Prower) is a coagulation disorder with a variable bleeding pattern¹ and is inherited as an autosomal recessive trait.⁶ The pedigree analysis has often suggested the presence of consanguinity.⁶,⁷ Both “true” deficiency and an abnormal FX molecule (FX Friuli)⁸ have been described.

To date, gene lesions responsible for FX inactivation have not been reported, apart from large chromosomal abnormalities involving chromosome 13, the localization site of the FX gene.¹¹-¹⁵ These chromosomal alterations cause additional defects, including FVII deficiency. Several restriction fragment-length polymorphisms (RFLPs) have been identified in FX gene.¹⁶-¹⁸ We used FX cDNA¹⁸ to investigate the presence of gene lesions in Italian patients with true deficiency or an abnormal molecule.

MATERIALS AND METHODS

Patients. A patient with true FX deficiency and her parents were studied. In addition, a patient with homozygous FX Friuli abnormality and her heterozygous daughter were also studied. The patients and family members consented to be studied, and the studies were performed with IRB approval. All these patients have been reported previously.¹⁹,²¹

Factor X clotting activity and antigen determination. FX activity was determined by several methods as reported previously.¹⁰,²¹ FX antigen was estimated by electroimmunoassay (EIA) with an antiserum supplied by Stago Laboratories (Asnieres, France).²²

Southern blot hybridizations. Blood samples were collected by standard procedures. DNA from the nuclei was isolated by the perchlorate method.²³ Six micrograms DNA was digested with TaqI, PstI, EcoRI, and HindIII restriction enzymes under conditions recommended by the manufacturers (New England Biolabs, Boston). All experiments involving recombinant DNA were performed according to the accepted safety guidelines. Both standard electrophoresis and pulsed-field gel electrophoresis²³ (pulse 7 seconds, 200 V, 16 hours, LKB 2015 Pulsaphor System [LKB, Bromma, Sweden]) were performed. Southern blotting technique was performed as previously described.²⁴

The hybridization (50% formamide, 10% dextran sulfate, at 42°C) and washing of filters were in accordance with the Gene Screen Plus transfer membrane method (New England Nuclear). A cDNA²⁸ spanning from codon 79 through 390 of the complete protein sequence and four subfragments obtained by TaqI/PstI or by DdeI digestions were used as probes. These probes were labeled with ³²P by the multiprime system (Amersham International, UK) or by nick-translation. Quantitative densitometry was performed on Southern blot autoradiographs with an LKB 2222 UltraScan XL (LKB, Bromma, Sweden) laser densitometer according to the manufacturer’s instructions.

RESULTS

The main coagulation features of the patients, whose FX gene structure was investigated, are summarized in Table 1. DNAs from patients and their relatives were digested with several restriction enzymes and hybridized to the cDNA probe covering from codon 79 through 390 of the complete sequence including the “pre-pro” leader sequence. If the numbering of amino acids in the plasma protein is considered, the cDNA contains codons 39 through 139 (the last) of the light chain and codons 1 through 210 of the heavy chain.

Figure 1 shows the TaqI and PstI patterns of the patient homozygous for FX Friuli (lane F) and of the family with a daughter with true FX deficiency. DNA fragments indistinguishable from normal were present in FX Friuli genes, confirming the results previously obtained.¹⁹

The 1.2-kilobase (kb) TaqI band, present only in subject N2, represents the less frequent allele of a normal TaqI RFLP,²⁸ the 1.6-kb band representing the more frequent allele. In contrast, an abnormal (2 kb) TaqI fragment is present in two members of the family with true deficiency. In addition, the DNA from these subjects shows bands with...
Fig 1. Abnormal FX gene restriction pattern. DNA samples from two normal subjects (N1, N2), a patient homozygous for FX Friuli (F), and members of a family with FX deficiency (Table 1) digested with TaqI and PstI restriction enzymes and hybridized to the FX cDNA. Heterozygotes for FX deficiency (C 81), double heterozygote for FX deficiency (s). Abnormal band. The faint 0.8-kb band is overexposed. A very faint 0.45-kb TaqI band is not apparent and is shown in Fig 3A.

Table 1. FX Activity and Antigen Assays in Patients With FX Deficiency or Abnormality

<table>
<thead>
<tr>
<th>Subject</th>
<th>FX Activity Extrinsic System</th>
<th>FX Activity RVV-Cephalin Mixture</th>
<th>FX Antigen Laurell</th>
<th>FX Antigen ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor X-deficient patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Proposita</td>
<td>4</td>
<td>6</td>
<td>&lt;10</td>
<td>7</td>
</tr>
<tr>
<td>(b) Heterozygous (mother)</td>
<td>38</td>
<td>40</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>(c) Heterozygous (father)</td>
<td>60</td>
<td>65</td>
<td>68</td>
<td>60</td>
</tr>
<tr>
<td>Factor X Friuli patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Homozygous</td>
<td>7</td>
<td>90</td>
<td>110</td>
<td>100</td>
</tr>
<tr>
<td>(b) Heterozygous (daughter)</td>
<td>45</td>
<td>95</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>Normal values</td>
<td>80-120</td>
<td>80-120</td>
<td>80-120</td>
<td>80-120</td>
</tr>
</tbody>
</table>

RVV, Russell's viper venom; ELISA, enzyme-linked immunosorbent assay.
Values are the mean of several observations on different occasions.

Table 2. Absorbance Areas and Ratios Obtained by Quantitative Laser Scanning of Southern Blots

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>TaqI</th>
<th>PstI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bands (kb)</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>F</td>
<td>1.1</td>
<td>1.9</td>
</tr>
<tr>
<td>P</td>
<td>1.5</td>
<td>2.4</td>
</tr>
<tr>
<td>M</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>FXF</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>N</td>
<td>1.1</td>
<td>1.9</td>
</tr>
</tbody>
</table>

F, P, M, FXF (Friuli), and N indicate subjects reported in Fig 1. Values are given in absorbance units. Since the 2.2-kb and 2.3-kb TaqI bands and the 2.7-kb and 2.9-kb PstI bands were not completely distinguished by the densitometer; areas including both peaks are shown.

*Abnormal band.
Fig 2. Pulsed-field gel electrophoresis (PFGE) of HindIII and EcoRI digests from a normal subject (N) and from the affected daughter (P) hybridized to the cDNA probe. *Abnormal bands.

Fig 3. TaqI (A) and EcoRI (B) digests were hybridized to cDNA fragments a, b, c, and d shown in Fig 4A. *Abnormal bands. N, normal subject; P, affected daughter; M, heterozygous mother.

Factor X Gene Alteration

The 3' portion of the gene was contained in the 5-kb fragment, recognized by probes b and d. The patient and her mother do not show EcoRI bands with normal intensity.

Figure 4B is a schematic diagram of the 3' part of FX gene and the localization of the deletion, inferred by the hybridization patterns. The 3' portion of the 8.2-kb EcoRI band (intron F) and the genomic region corresponding to the 3' portion of probe b through probes c and d (exon VII and the 5' part of exon VIII) are deleted in the pathologic FX gene.

The 5' portion of probe b, which does not overlap probe c and is contained completely within exon VI (codons 215 through 247), detects the normal 2.2-kb fragment and the additional 2-kb TaqI band. This exon VI sequence is probably the most 3' coding region present in the deleted gene. The abnormal gene fragments, detected in several digestions, are derived from the fusion of the DNA region 5' to the truncated intron F with sequences 3' to FX gene.

The 3' boundary of the lesion cannot be defined precisely with the probes used in this study; however, as inferred by the size of the abnormal EcoRI fragment, the deletion most probably includes the 3' EcoRI site.

Discussion

A partial and heterozygous gene deletion, causing a defect of FX antigen and activity was detected. This FX gene alteration removes a DNA fragment which is at least 5 kb and contains the exon VII and the 5' portion of exon VIII coding for the catalytic domain, a large part of the heavy chain of the protein. This protein domain is homologous in several serine proteases and is supposed to have been added through "exon shuffling" to several unrelated genes. A reverse event could have produced the gene deletion in this Italian patient. The presence in the genome of several homologous sequences could favor erroneous pairing. The deletion also offers the opportunity to investigate sequences 3' to FX gene, which could be useful in genetic studies of chromosome 13.

The stability of the sequences possibly originating from expression of the normal part of the altered FX gene appears to be greatly decreased, as demonstrated by the greatly reduced amount of FX antigen in the patient’s plasma. However, the presence of a partial and abnormal protein circulating at low levels, as an analogy with a partial deletion of the factor IX gene, cannot be excluded.

The patient’s mother, who is heterozygous for the FX deletion, shows (Table I) FX activity and antigen slightly lower than those of the father, who is heterozygous for a differently affected FX gene. When the parents were first investigated, this finding was interpreted as variation in the heterozygous state of the same gene. However, these results may reflect only normal variants since a bimodal distribution of FX clotting activities has been demonstrated. An alternative interpretation is that the residual expression of the pathologic FX gene present in the proposita’s father gives rise to levels of FX activity and antigen higher than those of the mother.

The present study shows that the affected daughter is
doubly heterozygous for two FX gene lesions: (a) the maternal partial gene deletion, and (b) a paternal small mutation which was not found in this analysis. These data exclude consanguinity of parents, often suggested in FX deficiency and, moreover, will further complicate classification of FX defects.

The presence of double heterozygotes has been described in several coagulation defects, such as prothrombin abnormalities (prothrombin Molise28), Hageman trait,24 and severe von Willebrand disease,3 and is compatible with the presence of several gene mutations even in small geographic areas.

DNA from the subjects carrying homozygous or heterozygous FX Friuli has an apparently normal gene pattern. This finding is in accordance with the possible presence of an amino-acid substitution.31

ACKNOWLEDGMENT

We thank Dr Michael Jaye for the FX cDNA probe and Dr Barbara Anderson for revising the manuscript.

REFERENCES


6. Stoll C, Roth MP: Partial 4q duplication due to inherited der(13)(t(4;13)(q26;q34) mat in a girl with a deficiency of factor X. Hum Genet 53:303, 1980


13. Scambler PJ, Williamson R: The structural gene for human...
FACTOR X GENE ALTERATION

coagulation factor X is located on chromosome 13q34. Cytogenet Cell Genet 39:231, 1985
Partial gene deletion in a family with factor X deficiency

F Bernardi, G Marchetti, P Patracchini, S Volinia, D Gemmati, P Simioni and A Girolami

Updated information and services can be found at:
http://www.bloodjournal.org/content/73/8/2123.full.html
Articles on similar topics can be found in the following Blood collections