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 proposa presented 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 proposa 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.

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**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.

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bands (kb)</th>
<th>TaqI</th>
<th>Ratio 1.3/1.6</th>
<th>Ratio 1.6/2.2 + 2.3</th>
<th>PstI</th>
<th>Ratio 2.7 + 2.9/3.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>1.1</td>
<td>1.9</td>
<td>5.5</td>
<td>0.57</td>
<td>0.2</td>
<td>1.4</td>
</tr>
<tr>
<td>P</td>
<td>1.5</td>
<td>2.4</td>
<td>3.3</td>
<td>0.62</td>
<td>0.3</td>
<td>2.5</td>
</tr>
<tr>
<td>M</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>0.51</td>
<td>0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>FXF</td>
<td>1.4</td>
<td>2.4</td>
<td>7.0</td>
<td>0.58</td>
<td>0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>N</td>
<td>1.1</td>
<td>1.9</td>
<td>5.7</td>
<td>0.57</td>
<td>0.2</td>
<td>1.3</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.*

intensity lower than normal both in TaqI (2.3 kb, 2.2 kb, mother’s and daughter’s lanes) and PstI (3.8 kb, mother’s lane) digests. Normal intensity of the 3.8-kb PstI band is observed in digest from the daughter, when a double amount of the DNA is loaded (12 μg).

Quantitative analysis of TaqI and PstI digests was performed by laser densitometry and relevant absorbance values are shown in Table 2. The absorbance ratios clearly confirm the presence of bands with intensity reduced to one-half.

EcoRI fragments, separated by pulsed-field gel electrophoresis, are shown in Fig 2 together with the HindIII pattern. A large (17 Kb) abnormal EcoRI fragment and a short (3.6 kb) HindIII abnormal fragment are detectable in DNA from the daughter (lane P). The presence of additional fragments and the reduction of intensity of FX gene bands are compatible with a heterozygous partial deletion of this gene.

This lesion was further investigated in hybridizations with cDNA fragments as probes. Figure 3 shows TaqI and EcoRI patterns hybridized with the cDNA fragments shown in Fig 4A.

In the propositus, fragment a detects TaqI bands (1.3 and 1.6 kb) with normal size and intensity, suggesting that the corresponding genomic region is intact. DNA from the affected subject and her mother hybridized with fragment b shows three TaqI bands, including the additional abnormal fragment (2.0 kb).

In DNA from the proposita and her mother, the hybridization patterns of probe c, which partially overlaps probe b, show only bands with reduced intensity. This probe recognizes a 0.45-kb TaqI band which, being too faint, is not
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.28

The presence of double heterozygotes has been described in several coagulation defects, such as prothrombin abnormalities (prothrombin Molise29), Hageman trait,24 and severe von Willebrand disease,30 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

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