A Molecular Genetic Study of Factor XI Deficiency

By John F. Hancock, Kerstin Wieland, Roy E. Pugh, Uri Martinowitz, Sam Schulman, Vijay V. Kakkar, Peter B.A. Kernoff, and David N. Cooper

Factor XI deficiency is a rare bleeding diathesis found predominantly in Ashkenazi Jewish kindreds. A recent study of six Jewish patients identified three distinct mutations (Types I, II, and III) in the factor XI gene that were sufficient to fully define the genotypes of the patients. We have investigated 63 patients with factor XI deficiency and find overall allele frequencies of 44% for the type II mutation, 31% for the type III mutation, and 0% for the type I mutation. Therefore, 25% of the mutant factor XI alleles in our sample remain undefined. However, the distribution of mutant alleles is significantly different between Jewish and non-Jewish populations with hitherto undefined mutations accounting for 84% of the disease alleles in non-Jewish patients. Plasma factor XI:C levels were found to differ significantly between different homozygous and compound heterozygous genotypes and the inheritance of the II/III genotype was found to carry an increased risk of the most severe bleeding tendency. © 1991 by The American Society of Hematology.

Factor XI DEFICIENCY is a rare coagulopathy found predominantly but not exclusively in Ashkenazi Jewish kindreds where the defective allele occurs at a frequency of at least 4.3% (1). It also occurs much less commonly in non-Jewish populations. The disorder, first described by Rosenthal et al., is inherited as an autosomal incompletely recessive trait. Homozygous and heterozygous patients are identified by a severe or partial deficiency of factor XI, respectively, but both groups are at risk from increased bleeding. Unlike hemophilia A and B, the bleeding diathesis associated with factor XI deficiency is mild, typical symptoms including epistaxis, menorrhagia, bleeding after dental extraction, and prolonged postoperative or posttraumatic hemorrhage. However, rarer problems do include hemarthroses and muscle hematomas. This disorder is of particular interest because of the considerable variability in bleeding severity between individuals and the absence of any strong correlation with the plasma level of factor XI. Some investigators have reported that the bleeding tendency is consistent within an individual kindred but other studies have found that the bleeding tendency within families is unpredictable. The lack of any obvious correlation between clinical phenotype and plasma factor XI level may be a reflection of different underlying defects of the factor XI protein. Alternatively, other genetic factors may play a role in determining the clinical phenotype of a factor XI-deficient patient. This disorder may, therefore, represent a paradigm for other coagulopathies, including the thrombotic states where coagulation assays do not fully predict clinical severity.

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kindreds, and if so, are the frequencies of the mutant alleles similar in both populations?

MATERIALS AND METHODS

Patients

Patients attending the Royal Free Hospital, London, UK, formed the major group for this study. Factor XI levels and detailed clinical histories were available from a previous study of inheritance and bleeding in factor XI deficiency. Additional patients from Israel, Sweden, and other UK centers were also included; factor XI:C levels and clinical histories of these patients were provided by their attending physician.

Analysis of Mutant Alleles

High molecular weight genomic DNA was extracted from peripheral blood leukocytes. DNA (200 ng) was used in a polymerase chain reaction (PCR) of 40 cycles with 100 pmol each of oligonucleotide primer following the method of Saiki et al. The primers for the type I analysis were 5'-AGTGACCAACGAA-GAGTGCCA (5' end of exon 14) and 5'-TTGCATATATTTCCAT-TGGCTAAGA (5' end of intron N), annealing at 55°C; the primers for the type II analysis were 5'-GAATCTGGAAGGTAC-TCTCACTGG (5' end of intron D) and 5'-ATCGACCACTCGAAT-GTCCTG (5' end of intron E), annealing at 60°C; the primers for the type III analysis were 5'-ACAGTCTTGATTGTGATGTAT-GTC (3' end of intron G) and 5'-ACAGTCTTGATTGTGATGTGAA (5' end of intron J), annealing at 60°C. Denaturation was performed at 94°C for 30 seconds, annealing at the specified temperature for 15 seconds and extension at 72°C for 5 minutes. After 40 cycles the reactions were held at 72°C for 10 minutes. PCR products were digested with Mae I, Bsm I, and Sau3A I, respectively, and the digested products electrophoresed in 2.5% agarose gels containing ethidium bromide and visualised under UV light. Where appropriate (see Results), the validity of this restriction analysis was confirmed by direct sequencing of the PCR products using one or both of the amplification oligonucleotides as sequencing primers in dideoxy chain termination reactions using modified T7 DNA polymerase (Sequenase; US Biochemical Corp, Cleveland, OH).

Statistical Analysis

Analysis of variance and F-tests were used to test the null hypothesis of equality of multiple (more than two) means, t-tests used to test for equality of two means, and X*-tests used to compare two proportions. The analyses were performed according to established methods. Abbreviations used in reporting these analyses are: SEM, standard error of the mean; DF, degrees of freedom.

RESULTS

Patient Pedigrees

Patients were designated homozygous or heterozygous deficient according to their plasma factor XI:C levels. Of the 28 homozygous patients (0% to 20% FXI:C), 16 were unrelated sporadic cases and 12 were drawn from seven unrelated kindreds. The 35 heterozygotes (25% to 70% FXI:C) comprised six unrelated sporadic cases and 29 cases drawn from 11 unrelated kindreds. Thus, a total of 22 unrelated patients and 41 patients from 13 unrelated kindreds were included in the study. The pedigrees of each of these 13 kindreds are presented in the Appendix.

The clinical histories of 61 patients were assessed from available case notes by two physicians and the bleeding symptoms classified into one of three groups: 

None/trivial. No bleeding despite hemostatic challenge as a result of surgery, dental extraction, or trauma; or, very occasional trivial bleeding episodes not requiring clinical intervention.


Moderate. Recurrent bleeding in response to hemostatic challenges requiring specific treatment and/or blood transfusion; or one or more episodes of spontaneous hemorrhage.

Table 1 shows that there is no significant difference in the factor XI:C level among homozygotes in each of these groups or between heterozygotes with mild or no bleeding. Therefore, these data are consistent with previous studies in showing, first, that there is a poor correlation between plasma factor XI:C level and bleeding tendency and, second, that a substantial proportion of heterozygotes bleed excessively. In addition, it is apparent from the pedigrees given in the Appendix that the bleeding tendency within these families is variable.

Molecular Genetic Analysis

All patients were tested for the presence of the three mutations described by Asakai et al. For detection of the type I mutation, exon 14 plus the 5' region of the following intron (intron N) was amplified by PCR and the product digested with Mae I, Sau3A I, and Bsm I, respectively, and the digested products electrophoresed in 2.5% agarose gels containing ethidium bromide and visualised under UV light. Where appropriate (see Results), the validity of this restriction analysis was confirmed by direct sequencing of the PCR products using one or both of the amplification oligonucleotides as sequencing primers in dideoxy chain termination reactions using modified T7 DNA polymerase (Sequenase; US Biochemical Corp, Cleveland, OH).

Table 1. Plasma Factor XI:C Levels in homozygous and heterozygous deficient patients classified according to clinical severity

<table>
<thead>
<tr>
<th>Factor XI:C Level (U/dL)</th>
<th>Clinical Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>0-20</td>
<td>0.45 ± 0.45</td>
</tr>
<tr>
<td>n = 2</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>25-70</td>
<td>0.45 ± 0.45</td>
</tr>
<tr>
<td>n = 17</td>
<td>0.45 ± 0.45</td>
</tr>
</tbody>
</table>

Summary of patient clinical data. The clinical classification used to group patients is described in the text. Homozygous patients have plasma factor XI:C levels of 0 to 20 U/dL, while heterozygous patients have levels of 25 to 70 U/dL. Mean factor XI:C levels (±SEM) are given for each clinical group together with the number of patients (n) in each group. The plasma factor XI:C levels do not differ significantly between the clinical groups of homozygous patients (F = 0.77; DF = 3,24; P > .05) or heterozygous patients (t = 0.75; DF = 33; P > .05).
A Type I Mutation

MaellI

EXON 14 GAATC

pcu pcd

pcr product

Normal

Mutant

B

1 2 3 4 5 6 7 8

Fig 1. Type I analysis. (A) The PCR product obtained using the 5’ (pcu) and 3’ (pcd) primers described in Materials and Methods. The type I, G → A point mutation is indicated and a MaellI restriction map given for a wild-type and mutant PCR product. (B) An agarose gel analysis of four patients. We found no examples of the type I mutation and so all the patients shown have a wild-type restriction pattern. Lanes have been loaded in pairs with an uncut PCR product (odd lanes) run alongside a digested PCR product (even lanes). The position of a 100-bp marker is indicated alongside the gel. The fastest migrating band in each lane comprises excess oligonucleotide primers (21 bp and 24 bp) that obscure the 33-bp fragment released from the digested PCR product.

PCR product of 706 bp and digested with Sau3AI. In the wild-type product, a single Sau3AI site in exon 10 gives two fragments of 578 bp and 128 bp, but the presence of the T → C mutation in exon 9 creates a new Sau3AI site resulting in three fragments of 328 bp, 251 bp, and 128 bp (Fig 3). Figures 1, 2, and 3 illustrate these analyses, which follow a similar strategy to that devised by Asakai et al.13

The restriction analysis was validated by direct sequencing of certain PCR products. For example, in every case where the restriction analysis failed to identify a known mutation, sequencing of all three PCR products from that patient was performed to confirm that one of the previously defined mutations had not been missed. In addition, a random 10% of PCR products that scored positive for type II or type III mutations on restriction analysis were also sequenced to confirm the presence of the predicted point mutations. In no case was any discrepancy found between the restriction and sequencing analyses.

Table 2 shows that of the 91 mutant alleles expected in this group of patients, 37 had a type II mutation and 31 had a type III mutation. No example of a type I mutation was found. Therefore, 23 mutant factor XI alleles (25%) in our sample did not contain one of the previously described mutations. Among the non-Jewish patients, only two of the 19 expected mutant alleles contained a defined mutation, indicating that the majority of mutant factor XI alleles in this group were other than the three previously described.

A Type II Mutation

BsmI

EXON 5 GAATGC

pcu pcd

pcr product

Normal

Mutant

B

1 2 3 4 5 6 7 8 9 10 11 12

Fig 2. Type II analysis. (A) The PCR product obtained using the 5’ (pcu) and 3’ (pcd) primers described in Materials and Methods. The type II, G → T point mutation is indicated and a BsmI restriction map given for a wild-type and mutant PCR product. (B) An agarose gel analysis of six patients. Examples are shown of two patients homozygous for the type II mutation (lanes 1 through 4), two patients heterozygous for the type II mutation (lanes 5 through 8), and two normal patients (lanes 9 through 12). Lanes have been loaded in pairs for each patient, with an uncut PCR product (odd lanes) run alongside a digested PCR product (even lanes). The positions of 100-bp and 250-bp markers are indicated alongside the gel.
To obtain an unbiased estimate of these allele frequencies, the genotypes of all unrelated sporadic cases and the index case of each family group were analyzed. In two kindreds (groups 6 and 10), a mutation not present in the index case was identified in another family member. These two alleles were also included in the analysis. Of the 62 evaluable alleles analyzed in this manner, 16 (25.9%) contained undefined mutations (Table 3). Among the remainder, type II mutations were slightly more common than type III mutations. The analysis presented in Table 3 also shows that the distribution of mutations is significantly different between Jewish and non-Jewish populations ($\chi^2 = 61.98; P < .001$). In the latter, 84% alleles contained undefined mutations compared with 12% among Jewish patients.

**Correlation of Genotype and Phenotype**

We then investigated whether the measured plasma factor XI:C level correlated with factor XI genotype (Table 4). We found that the mean factor XI:C levels associated with the homozygous and compound heterozygous genotypes differed significantly ($F = 4.69; P < .01$). The statistical analysis was therefore taken further with those genotypes containing defined mutations (ie, II/III, 11/11, and III/III). The differences between mean plasma factor XI levels in this subset of genotypes were highly significant ($F = 11.12; P < .005$). The plasma factor XI:C levels associated with a II/II genotype were very low (range 0 U/dL to 1.5 U/dL, mean 0.62 U/dL), whereas those associated with the III/III genotype were the highest of this subset (range 3

**Table 2. Factor XI Gene Mutations**

<table>
<thead>
<tr>
<th>Type of Mutation</th>
<th>Ethnic Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>UND</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients</td>
<td>0</td>
<td>37</td>
<td>31</td>
<td>23</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Jewish</td>
<td>0</td>
<td>36</td>
<td>30</td>
<td>6</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Non-Jewish</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td>19</td>
</tr>
</tbody>
</table>

Summary of the factor XI gene mutations found in 28 homozygous and 35 heterozygous patients. The total number of expected mutations is therefore 91. Type I, II, and III mutations are those described previously by Asakai et al and are detailed in the text. Mutant alleles that were found not to contain any one of these three mutations are designated undefined (UND).

**Table 3. Estimation of Mutant Factor XI Allele Frequencies**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Type of Mutation</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>UND</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sporadic cases</td>
<td>0</td>
<td>19</td>
<td>12</td>
<td>7</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>All index cases</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>9</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>All cases</td>
<td>0</td>
<td>27</td>
<td>19</td>
<td>16</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Allele frequency</td>
<td>0</td>
<td>43.5</td>
<td>30.6</td>
<td>25.9</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Jewish patients:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cases</td>
<td>0</td>
<td>26</td>
<td>18</td>
<td>6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Allele frequency</td>
<td>0</td>
<td>52</td>
<td>36</td>
<td>12</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Non-Jewish patients:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cases</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Allele frequency</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>84</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

To obtain an estimate of gene frequencies, the mutations occurring in all sporadic cases plus the index cases of each family were analyzed. The upper part of the table gives the numbers of evaluable alleles and overall gene frequencies. The lower part of the table divides patients into Jewish and non-Jewish groups and gives gene frequencies for each group. The observed non-Jewish allele frequencies are significantly different from those expected for the alleles analyzed ($\chi^2 = 61.98$, $DF = 1; P < .001$). The Jewish allele frequencies were used to calculate the expected frequencies.
types (F = 6.69, DF = 5.22; P < .01) but not between the different heterozygous genotypes (F = 0.33, DF = 2.32; P > .05). The mean plasma factor XI:C levels in patients with homozygous and compound heterozygous genotypes containing defined mutations (II/II, II/I, and II/III) were analyzed further. The differences between the mean factor XI:C levels in this subset of genotypes were highly significant (F = 11.12, DF = 2.18; P < .005).

A summary of the plasma factor XI:C levels found in patients with specific genotypes. Mean plasma factor XI:C levels differ significantly between the different homozygous and compound heterozygous genotypes (F = 4.69, DF = 5.22; P < .01) but not between the different heterozygous genotypes (F = 0.33, DF = 2.32; P > .05). The mean plasma factor XI:C levels in patients with homozygous and compound heterozygous genotypes containing defined mutations (II/II, II/I, and II/III) were analyzed further. The differences between the mean factor XI:C levels in this subset of genotypes were highly significant (F = 11.12, DF = 2.18; P < .005).

Abbreviation: WT, wild type.

Table 4. Comparison of Plasma Factor XI:C Levels With Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency (n)</th>
<th>Range</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>II/III</td>
<td>13</td>
<td>0-6</td>
<td>2.87 ± 0.49</td>
</tr>
<tr>
<td>II/III</td>
<td>3</td>
<td>3-20</td>
<td>11.0 ± 4.9</td>
</tr>
<tr>
<td>II/III</td>
<td>5</td>
<td>0-1.5</td>
<td>0.62 ± 0.29</td>
</tr>
<tr>
<td>UND/UND</td>
<td>4</td>
<td>1-3</td>
<td>1.75 ± 0.42</td>
</tr>
<tr>
<td>III/UND</td>
<td>2</td>
<td>1.6-10</td>
<td>5.8 ± 4.2</td>
</tr>
<tr>
<td>III/UND</td>
<td>1</td>
<td>3-3</td>
<td>3.0</td>
</tr>
<tr>
<td>III/WT</td>
<td>13</td>
<td>34-65</td>
<td>52.5 ± 3.2</td>
</tr>
<tr>
<td>II/WT</td>
<td>10</td>
<td>30-70</td>
<td>52.7 ± 4.3</td>
</tr>
<tr>
<td>UND/WT</td>
<td>12</td>
<td>31-68</td>
<td>48.4 ± 5.2</td>
</tr>
</tbody>
</table>

First, a significant proportion (25%) of the mutant alleles causing factor XI deficiency in our patient sample remain to be identified. It is interesting that none of our patients possessed the type I (splice donor) mutation described by Asakai et al.15 These investigators identified the mutation in only one of 12 alleles studied. One interpretation of these observations is that whereas the type II (premature termination) and type III (amino acid substitution) factor XI gene mutations clearly predominate in Jewish populations, a number of individually uncommon mutations (eg, type I) account for the remaining 25% of disease alleles found mainly in non-Jews. We are currently sequencing the factor XI genes of patients with undefined mutations to address this hypothesis. It is also possible that one or more of these undefined mutations may be non-allelic, ie, they occur at other loci whose gene products are required for normal expression of the factor XI gene.

Second, as mentioned above, the frequencies of the two common factor XI gene mutations differ significantly between Jewish and non-Jewish patients. Indeed, the majority (84%) of the disease alleles in non-Jews remain undefined. These differences are probably not surprising in view of the early finding that non-Jewish homozygotes and heterozygotes possessed lower plasma factor XI levels than their Jewish counterparts.19 The high incidence of factor XI deficiency among Ashkenazi Jews was initially attributed to a founder effect. The results of our study are in part consistent with this hypothesis in that two distinct mutations account for 88% of all factor XI gene defects in this ethnic group. Our observation that these same mutations are rare in non-Jewish patients is also consistent with this interpretation, especially if several undefined mutations exist, rather than just a single mutation. The high frequency of factor XI deficiency among Ashkenazi Jews due to a minimum of four distinct mutations is nevertheless intriguing. Some selective advantage may well have accrued to heterozygous carriers of the mutant alleles to account for their high frequency in this ethnic group.20

Third, the comparative analysis of phenotype and genotype presented here suggests that patients with a II/III genotype are significantly more likely to experience moderate bleeding than patients with other genotypes. Of the patients with a II/III genotype, approximately 50% had a moderate bleeding tendency and 50% had a mild clinical picture. Indeed, six of seven of the patients who had a moderate bleeding tendency possessed this genotype. This result indicates that a knowledge of a factor XI-deficient patient's genotype may be of some use in predicting the
Fig 4. Eleven patient pedigrees. The plasma factor XI:C level is recorded in the patients' box or circle. Underneath each patient, in parentheses, is given their genotype followed, after a comma, by their bleeding phenotype according to the key given below. For example, ([II]*11, 2) indicates a double heterozygote II/*II genotype with a moderate bleeding diathesis. If a single mutation is recorded, the patient is a heterozygote. All kindreds are Jewish except for groups 9 and 11, which are non-Jewish. U, undefined mutation; 0, none/trivial bleeding; 1, mild bleeding tendency; 2, moderate bleeding tendency; -, data not available.
likely outcome to an elective hemostatic challenge. Our data also show that there is an association between plasma factor XI:C levels and a patient’s genotype. In other words, the mean factor XI:C levels associated with individual homozygous and compound heterozygous genotypes differ significantly. We speculate that, for the three genotypes containing only defined mutations, the associated factor XI:C levels may be rationalized as follows. Dimers of a completely truncated factor XI molecule lack the catalytic site and are probably also unstable. This inactivity and instability would be reflected in the very low factor XI levels associated with the II/II genotype. Conversely, the missense mutation associated with the III mutation would not be expected to alter the stability of the protein because a bulky hydrophobic residue is merely replaced by a smaller aliphatic amino acid. In this case, stable dimers of a III/III protein should be produced that have limited activity. Although we do not as yet have confirmatory factor XI:Ag data, the observed higher factor XI:C level of type I11 homozygotes (11 U/dL) compared with that of type II homozygotes (0.6 U/dL) is consistent with this interpretation. Coexpression of type II and type III polypeptides in the same cell will result in the formation of II/II and II/III homodimers plus heterodimers of II/III protein. The amount of III/III protein secreted from heterozygous II/III liver cells would, therefore, be approximately 25% of that secreted by a homozygous III/III liver cell. This hypothesis fits with the observed mean factor XI:C level associated with the II/III genotype (2.9 U/dL). The question then arises as to whether II/III dimers are formed and secreted in II/III heterozygotes. The formation of such dimers may serve to stabilize the type II truncated chain resulting in a molecule that, despite lacking significant coagulant activity, can still bind HMWK because both binding sites for this molecule (Phe 56-Ser 86) would be present. The greater phenotypic severity associated with the II/III genotype might, therefore, be explained by a combination of HMWK depletion, due to sequestration by inactive II/III molecules, and a reduced production (relative to the III/III genotype) of the weakly active III/III homodimer.

APPENDIX

Eleven patient pedigrees are listed (Fig 4).

ACKNOWLEDGMENT

We thank Angus McCraw for preparing DNA from the Royal Free patients, Dr Sabri Kemahli for help in compiling certain of the clinical data, and Dr Atul Mehta for helpful advice. We also acknowledge Drs B.T. Colvin, R. Vaughan Jones, G.L. Scott, A. Copplestone, B. Zoll, and P.H.B. Bolton-Maggs, who kindly contributed patient material for inclusion in this study.

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