Factor XI (plasma thromboplastin antecedent) deficiency in Ashkenazi Jews is a bleeding disorder that can result from three types of point mutations

(coagulation/genetic defect/polymerase chain reaction)

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ABSTRACT  Factor XI (plasma thromboplastin antecedent) deficiency is a blood coagulation abnormality occurring in high frequency in Ashkenazi Jews. Three independent point mutations that result in a blood coagulation abnormality have been identified in the factor XI gene of six unrelated Ashkenazi patients. These mutations either disrupt normal mRNA splicing (type I), cause premature polypeptide termination (type II), or result in a specific amino acid substitution (type III). The three different genotypes were present in the six patients as type I/II, type II/III, and type III/III. Thus far no correlation was found between the three genotypes and the bleeding tendency in these patients.

Factor XI deficiency was first reported as a hemophilia-like syndrome by Rosenthal and coworkers in 1953 (1). This deficiency differs from classic hemophilia, however, in that the bleeding tendency is usually mild or absent, and either sex can be affected (for reviews, see refs. 2 and 3). Generally, spontaneous bleeding, such as hemarthrosis and purpura, is rare, and hemorrhage most often occurs only after trauma or minor surgery, such as circumcision, tonsillectomy, or dental extraction. Factor XI deficiency is rather unusual, however, in that an extreme variation in bleeding manifestations exists, ranging from a complete lack of symptoms to hemorrhage requiring multiple blood transfusions. Factor XI deficiency is inherited as an autosomal recessive trait (4) in which bleeding may occur in homozygotes and, perhaps, in heterozygotes (5). Factor XI deficiency is typically characterized by the virtual absence of factor XI coagulant activity as well as a lack of cross-reacting material in the circulating plasma (6). Thus far, a major portion of the factor XI-deficient patients have been found in the Ashkenazi Jewish population. In Israel, homozygotes occur at a frequency of about 1 in 190, whereas the heterozygote frequency is about 1 in 8 (7). Accordingly, factor XI deficiency is one of the most frequent genetic disorders in this ethnic group. Factor XI deficiency has also been described in patients of other ethnic backgrounds, but these occurrences are less common (6, 8).

The recent characterization of the normal factor XI gene in our laboratory (9) has made it possible to study the genetic abnormalities in six patients from the Ashkenazi population. In this report, three different mutations present in these patients are identified.

MATERIALS AND METHODS

Construction of Genomic Libraries, Cloning, and DNA Sequencing. High molecular weight DNA was isolated from peripheral lymphocytes (50 ml of whole blood) of six patients designated as Cleveland 1–6 (CL1-CL6). DNA from CL3 was digested with EcoRI restriction enzyme followed by fractionation on a 5–25% (wt/vol) NaCl gradient (10). The 18-kb DNA fraction was then pooled, ligated into a λ vector (Stratagene), and packaged with Gigapack Gold (Stratagene) in vitro. Seven recombinant phage (CL3-1 to CL3-7) were isolated by plaque hybridization using a radiolabeled factor XI cDNA probe (9). Five genomic DNA fragments generated by EcoRI and HindIII digestion of three phage clones (CL3-1, CL3-2, and CL3-6) were subcloned into pTZ vectors. Single-stranded DNA from pTZ was prepared from the medium of pTZ-transformed Escherichia coli cells superinfected with M13K07 helper phage. All the exons and exon-intron boundaries were sequenced by the dideoxy chain-termination method (11) with synthetic oligonucleotides as primers.

Alternative Strategy for Rapid Identification of Mutations. Thirteen pairs of oligonucleotide primers were used to amplify all the exons and intron/exon boundaries of the factor XI gene by the polymerase chain reaction (PCR) for DNA sequence analyses. These primers were changed slightly from an exact complementary DNA sequence with the gene to produce convenient restriction sites for cloning and sequencing. Each of the 12 independent exons and their intron/exon boundaries were amplified except exons 8, 9, and 10, which were amplified as a single segment of 711 base pairs (bp). Five or more M13 phage clones derived from each amplified fragment were sequenced to improve the likelihood that both alleles in CL4 were being examined.

Analysis of the Mutant Allele. The PCR was done as recommended by Perkin–Elmer/Cetus. Cloned DNA (0.1 μg) or genomic DNA (0.5 μg) was added to 100 pmol of each oligonucleotide primer. The annealing temperature was 60°C. The cycle was repeated 35 times followed by digestion with Mae III for type-I analysis, Bsm I for type-II analysis, and Sau3AI for type-III analysis. The digested DNA was electrophoresed on a 2% NuSieve GTG agarose and 1% SeaKem agarose (FMC) containing ethidium bromide.

RESULTS AND DISCUSSION

Type-I Mutation (Splice Junction Mutation). Six unrelated patients from Cleveland and Cuyahoga County in northern Ohio of Ashkenazi ancestry were designated CL1–CL6. Each individual had 10% or less of the normal plasma factor XI coagulant activity and factor XI antigen (Table 1). Southern blot analysis of the DNA from each of the six patients revealed no apparent deletions or rearrangements in their factor XI gene (data not shown). Furthermore, the restriction maps for each patient generated by >25 restriction endonuclease(s) were different from the normal map. This finding implicates a point mutation in the splice junction.

Abbreviations: PCR, polymerase chain reaction; CL1–6, designation for six different patients deficient in factor XI.
Table 1. Genotypes and bleeding manifestations among the six Ashkenazi Jews from Cleveland with factor XI deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>PTA titer, U/ml</th>
<th>Genotype</th>
<th>Bleeding symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL1</td>
<td>36</td>
<td>Male</td>
<td>0.02</td>
<td>II/III</td>
<td>Trivial</td>
</tr>
<tr>
<td>CL2</td>
<td>50</td>
<td>Female</td>
<td>0.06</td>
<td>II/III</td>
<td>None</td>
</tr>
<tr>
<td>CL3</td>
<td>44</td>
<td>Male</td>
<td>&lt;0.01</td>
<td>I/II</td>
<td>Mild</td>
</tr>
<tr>
<td>CL4</td>
<td>55</td>
<td>Female</td>
<td>0.03</td>
<td>III/III</td>
<td>Mild</td>
</tr>
<tr>
<td>CL5</td>
<td>48</td>
<td>Female</td>
<td>0.04</td>
<td>II/III</td>
<td>Moderate</td>
</tr>
<tr>
<td>CL6</td>
<td>64</td>
<td>Female</td>
<td>0.10</td>
<td>II/III</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

U, units; PTA, plasma thromboplastin antecedent.

*Consanguineous parents.

clases were identical with those of the normal gene. These results suggested that the genetic defects in these individuals involved small nucleotide changes or point mutations.

A detailed analysis was made of the defective factor XI genes in one patient (CL3) by DNA sequence analysis. The abnormal factor XI genes present in two adjacent EcoRI fragments of 18 kilobases (kb) in length (Fig. 1A) were cloned into the vector λ DASH (Stratagene), and seven genomic clones were isolated (CL3-1 to CL3-7). One clone (CL3-1) covered the 5’ half of the gene, and the six remaining clones (CL3-2 to CL3-7) covered the 3’ half of the gene, as shown by restriction mapping.

The sequences of all 15 exons, their adjacent intron junctions, as well as the 5'- and 3'-flanking regions of the gene were then determined in clones CL3-1 and CL3-2 by the dideoxy chain-termination method (11) with specific synthetic oligonucleotide primers. These analyses showed that the defective factor XI gene was identical in DNA sequence to the normal gene in the regions examined, except for a single base substitution at the splice junction boundary of the last intron (intron N). This mutation interrupts the coding region of the mRNA between amino acids Lys-185 and Gly-186 just before the active-site, Ser-188, in the light chain. In this boundary, the G in the obligatory 5'-splice donor dinucleotide GT (12) was changed to an A (Fig. 1B). This splice junction mutation (designated type-I mutation) could give rise to the following abnormally processed mRNAs: (i) The entire intron N might be unspliced and retained in the mRNA. Translation of this mRNA would extend the polypeptide 5 residues beyond exon 14 to a stop codon located within intron N (9). This change would result in a truncated polypeptide of 559-amino acid residues that lacks an active-site serine. (ii) Cryptic splice sites could be used, as has been shown in β-thalassemia (13, 14), and would lead to premature termination of translation. (iii) Exon 14 may be omitted, resulting in the splicing of exon 13 directly to exon 15 analogous to a defective gene for phenylalanine hydroxylase (15, 16). Translation of this mRNA would result in a substantial deletion in the polypeptide before the active-site serine. Furthermore, exons 13 and 15 belong to different splice junction types (9), and this difference would cause a shift in the reading frame of the mRNA. In addition, problems with abnormal sequences derived from aberrantly spliced transcripts would probably be unstable and rapidly degraded.

(iv) The abnormally spliced mRNAs may not be transported into the cytoplasm and may never be translated (14). Splice junction abnormalities have also been seen for hemophilia B (17) and Tay–Sachs disease (18–20).

The type-I mutation changed the nucleotide sequence of GTAAC to ATAAC at the splice junction boundary and abolished a Mae III restriction site in the gene for factor XI of CL3 (Fig. 2A). Thus, this mutation could be identified by directly screening for the absence of this restriction site in a fragment generated by the PCR from total genomic DNA (21) as well as phage genomic DNA (Fig. 2A). The choice of the fragment size for the PCR analysis was influenced by the presence of a normal Mae III site located 99 bases upstream from the splice junction in exon 14. Synthetic oligonucleotide primers (PCL1 and PCL2) were prepared to amplify a 162-bp fragment of the factor XI gene. The fragment was then digested with Mae III and analyzed on a 1% agarose gel containing 2% NuSieve. Experiments with clones CL3-2 (Fig. 2B, lane 3) as well as CL3-4 and CL3-5 (data not shown) indicated the presence of the type-I mutation, whereas clones CL3-6 (Fig. 2B, lane 2) and CL3-7 (data not shown) contain the normal Mae III restriction site. Thus, the latter two clones (CL3-6 and CL3-7) were derived from a different defective factor XI allele. This fact suggested that CL3 is a genotypically compound heterozygote with only one of the factor XI alleles carrying the type-I mutation. Genotypic heterozygocity was confirmed by direct PCR and Mae III analysis with total genomic DNA in which characteristic bands corresponding to both cleaved and uncleaved products were seen (Fig. 2B, lane 4). Analysis of the genomic DNA from the remaining five patients by this technique showed that none of these individuals carried the type-I mutation (Fig. 2B, lanes 5–9). Hence, of the 12 defective factor XI genes examined in this investigation, only 1 had a type-I mutation.

Type-II Mutation (Stop Codon Formation). Because CL3 is homozygous in phenotype but heterozygous in genotype, clone CL3-6 was chosen to identify the second mutation because it did not carry the type-I mutation. An analysis of the sequence of the exons and the intron–exon boundaries in clone CL3-6 led to the identification of a stop codon (type-II mutation) in exon 5, where GAA coding for Glu-117 was
Fig. 2. Analysis of type-I mutation in six Ashkenazi patients (CL1-CL6) with factor XI deficiency. (A) The DNA segment of the factor XI gene that was amplified, including part of exon 14 (open box) and part of intron N (hatched box). Positions of the Mae III sites are indicated by the vertical arrows, and the synthetic oligonucleotide primers (PCL1 and PCL2) used for the PCR (21) are shown by solid horizontal arrows. The nucleotide sequences of the PCL1 and PCL2 primers complementary to opposite strands of DNA are also shown. The amplified fragment of 162 bp yielded 99-, 60-, and 3-bp DNA fragments after Mae III cleavage of the normal fragment and 159- and 3-bp fragments from the mutant fragment. (B) An agarose gel stained with ethidium bromide showing the PCR products before (lanes) and after (+ lanes) cleavage using the cloned phage DNA of CL3 and genomic DNA of the six patients. Lanes: 1, DNA from normal control; 2, DNA from phage clone CL3-6; 3, DNA from phage clone CL3-2; 4, genomic DNA from CL3; 5 and 6, genomic DNA from CL1 and CL2; 7-9, genomic DNA from CL4-CL6. The outer two lanes show DNA standards and size of these bands.

changed to a stop codon of TAA (Fig. 1B). Premature polypeptide termination may result in a truncated and presumably unstable factor XI protein from this allele. It may also lead to other abnormalities, such as factor XI mRNA deficiency, without changing cytoplasmic factor XI mRNA stability, as described for the β2-39 thalassemia gene (22).

A change of G to A at Glu-117 also abolishes a recognition site for Bsm I (GAATGC to TAATGC) (Fig. 3A). Accordingly, a separate PCR analysis was developed by using a second set of oligonucleotide primers (PCE1 and PCE2, Fig. 3A). This procedure resulted in 125- and 301-bp fragments from normal DNA (Fig. 3B, lane 1) and a 426-bp fragment from mutant DNA after digestion with Bsm I (Fig. 3B, lane 3). Furthermore, the type-I and type-II mutations in CL3 were each present in a separate allele (Fig. 3B, lane 2 vs. lane 3), as illustrated in Fig. 1A. This result was confirmed by a DNA sequence analysis of the genomic subclones CL3-2 and CL3-6. These experiments showed that the sequence for codon 117 was normal in CL3-2 and carried the type-I mutation. Also, the sequence of the intron N junction was normal in CL3-6 that carried the type-II mutation. Although only a single clone of the 5' half of the factor XI gene was analyzed, the two mutations in the 3' half of the gene can account for the deficiency in CL3. Furthermore, an analysis of the parents of CL3 by the PCR-Bsm I assay and the PCR-Mae III assay showed that the type-I mutation was present in the father, whereas the type-II mutation originated from the mother (data not shown). The application of this test to the genomic DNA of the remaining five factor XI-deficient patients indicated that CL1, CL2, CL5, and CL6 also carried the type-II mutation and, in all cases, in the heterozygous state (Fig. 3B, lanes 5, 6, 8, and 9), whereas it was absent in CL4 (Fig. 3B, lane 7). The results for CL1 were somewhat surprising because this individual resulted from a consanguineous marriage.

Type-III Mutation (Amino Acid Substitution). Mutations in CL4 were studied by direct PCR amplification and sequence analysis. A third point mutation (type III) was identified in CL4. This mutation was located in exon 9, where TTC coding for Phe-283 was changed to CTC coding for leucine (Fig. 4). The type-III mutation was not an artifact due to misincorporation of dNTP by the Thermus aquaticus (Taq) polymerase because it was seen in all the amplified clones from exon 9 and was also present in four other patients, as described below. The single base change of T to C generated a new Sau3AI site (GATC) at the mutation site, making it possible to study the 711-bp PCR product that includes exons 8-10 by Sau3AI digestion (Fig. 5A). Normal DNA gives bands of 581 and 130 bp in length (Fig. 5B, lane 1), whereas in CL4 the 581-bp band was converted to fragments of 330 and 251 bp, and the 130-bp fragment remained unchanged (Fig. 5B, lane 2). Furthermore, CL4 was homozygous for this mutation. PCR amplification and Sau3AI cleavage analysis of the genomic DNA from the remaining five factor XI-deficient patients showed that the type III mutation was also present in one of the alleles of CL1, CL2, CL5, and CL6 (Fig. 5B, lanes 4, 5, 7, and 8).

Fifty-three apparently normal, unrelated Ashkenazi Jews from Cuyahoga County in northern Ohio were also analyzed to test whether the type-III mutation resulted from polymorphism. The selection of individuals with normal factor XI levels was difficult, however, due to the high frequency of heterozygotes in this population and the absence of bleeding
had 55% and 79% of normal factor XI coagulant activity (data not shown). These latter two values fall within the range for heterozygotes. These experiments are consistent with the concept that the type-III abnormality is not due to polymorphism. In addition, analysis of 42 normal Caucasians from the Seattle area indicated the absence of a type-III mutation, further suggesting that this mutation was not due to polymorphism.

The detection of 2 heterozygotes with type-III mutations among 53 apparently normal Ashkenazi Jews is consistent with the homozygote prevalence of 24 in 80,000 Ashkenazi Jews in Cuyahoga County and a calculated value of 1 in 29 heterozygotes (unpublished observations).

The genotype for the six factor XI-deficient patients from Cleveland is shown in Table 1. Gene cloning and sequence analysis on the type-I and type-II mutations clearly indicate that alleles carrying each of these mutations do not carry the type-III mutation on the same allele. Therefore, the type-III mutation leading to the substitution of Phe-283 by leucine in the fourth tandem repeat of the amino acid sequence is directly responsible for the factor XI-deficient phenotype in these patients as well as in CL4, who was homozygous for type III. The type-III mutation may interrupt disulfide bond formation in the fourth tandem repeat, leading to a destabilization of the protein and its rapid turnover. This explanation would be analogous to phenylketonuria resulting from an amino acid conversion at a specific codon to tryptophan (24). It is also possible, however, that the type-III mutation is linked to another mutation yet to be identified. In either case, the conversion of Phe-283 to leucine in five of the six patients indicates a factor XI abnormality. Site-directed mutagenesis and expression of a type-III cDNA in cell culture will be necessary to confirm whether this mutation directly causes factor XI deficiency.

**Clinical and Genetic Implications.** Patients with factor XI deficiency show considerable variability in bleeding tendencies. Also, there is little or no correlation between the circulating level of the plasma protein and a bleeding tendency for these patients (5, 8). The presence of a dysfunctional factor XI protein in plasma has been suggested as a cause of variability in the bleeding tendency among different patients, but significant cross-reacting antigen has been detected in the plasma of only three individuals with factor XI deficiency (8, 25). In addition, the bleeding tendency is not consistent for a given patient in response to a different hemostatic challenge (2, 5). The reason for these clinical variations is unclear. Analysis of the bleeding histories of the six patients in the present study after surgery suggested a classification into four general bleeding groups: (i) moderate, including those with recurrent episodes of moderately severe bleeding, sometimes requiring a blood transfusion; (ii) mild, including those with a few episodes of mild but significant bleeding; (iii) trivial, those with a few mild but insignificant bleeding episodes; and (iv) no bleeding, including those with no hemorrhagic symptoms despite multiple surgeries (Table 1). The four patients with type II/III genotype (CL1, CL2, CL5, and CL6) fall either into category iv (CL1 and CL2) or category i (CL5 and CL6). These data indicate that the type II/III genotype shows no apparent correlation with the degree of bleeding in these six patients with factor XI deficiency. Additional studies are necessary, however, to clarify this possibility.

Ashkenazi Jewry grew out of Oriental Jewry during the time of the Roman Empire and settled in present-day France and Germany in the Middle Ages (26). Subsequently, this group spread throughout eastern Europe, Israel, and the United States. Factor XI deficiency is found in high frequency only in Ashkenazi Jews and is seldom found in Oriental and Sephardic Jews (7). Mutations that cause factor XI deficiency probably occurred after the establishment of
the three major Jewish communities (Ashkenazi, Oriental, and Sephardic). Unlike Tay–Sachs disease and Gaucher disease, factor XI deficiency has not been traced to a specific geographic origin (7). The identification of more than one type of genetic defect in the present study is inconsistent with the hypothesis that a single mutation for factor XI deficiency in Ashkenazi Jews has spread in this population by a typical founder effect, as discussed by Diamond and Rotter (27, 28).

It is worthwhile to mention that three lipid-storage diseases caused by defects of different lysosomal hydrolases are also found in high frequency in the subgroup of Ashkenazi Jews of eastern European descent, a finding that supports the role of a founder effect in these disorders (29). Recent DNA studies of individuals with Tay–Sachs disease (18–20, 30, 31) and Gaucher disease (32), however, showed the existence of at least three different mutations being responsible for each of these disorders. For infantile Tay–Sachs disease in the Ashkenazi Jews, the splice junction mutation accounted for ≈30% of the patients and a 4-bp insertion accounted for the remaining 70%. With factor XI deficiency, type-II mutation accounted for 42% of the abnormal alleles, and type-III mutation accounted for 50% of the abnormal alleles in the present six patients. Some selective advantage (28) probably exists in maintaining the high frequency of these mutations in this population. On the other hand, the importance of genetic drift is recognized in the various “Ashkenazi diseases” because there are geographical aggregations of ancestral origins for many of these diseases (29).

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