The bleeding disorder associated with factor XI (fXI) deficiency is typically inherited as an autosomal recessive trait. However, some fXI mutations may be associated with dominant disease transmission. FXI is a homodimer, a feature that could allow certain mutations to exert a dominant-negative effect on wild-type fXI secretion through heterodimer formation. We describe 2 novel fXI mutations (Ser225Phe and Cys398Tyr) that form intracellular dimers, are secreted poorly, and exhibit dominant-negative effects on wild-type fXI secretion in cotransfection experiments. Available data now suggest that mutations associated with cross-reactive material-negative fXI deficiency fall into 1 of 3 mechanistic categories: (1) mutations that reduce or prevent polypeptide synthesis, (2) polypeptides that fail to form intracellular dimers and are retained in cells as monomers, and (3) polypeptides that form dimers that are not secreted. The latter category likely accounts for many cases of dominant disease transmission. (Blood. 2005;105:4671-4673)

Brief report
A classification system for cross-reactive material-negative factor XI deficiency

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The homodimeric plasma protein factor XI (fXI) is the precursor of the coagulation protease fXa. FXI dimer formation, which is unique among coagulation proteases, is a prerequisite for fXI secretion from cells. The noncatalytic portion of the fXI polypeptide contains 4 repeats called apple domains (A1-A4 from the N-terminus). The A4 domain is the primary mediator of dimer formation, and mutations in A4 that interfere with dimerization are associated with retention of fXI monomer within cells and low plasma fXI levels. The dimeric structure has implications for inheritance patterns in congenital fXI deficiency. This disorder is prevalent in Ashkenazi Jews, in whom 2 point mutations, Glu117Stop and Phe283Leu, predominate. These mutations are not clear whether these cases are unusual or represent a common mechanism in fXI deficiency. Prompted by these data, and personal observations of patients with fXI levels in an intermediate range (20%-30% of normal) between those typically seen in homozygous and heterozygous Jewish patients, we analyzed the effects of 2 novel fXI mutations with plasma fXI levels of less than 0.2 U/mL, a range commonly seen in homozygous or compound heterozygous fXI-deficient Jewish patients. In 2 cases, mutations were identified (Gly400Val and Trp569Ser) that form intracellular dimers and are retained in cells as monomers, and (3) polypeptides that form dimers that are not secreted. The latter category likely accounts for many cases of dominant disease transmission.

Introduction

Study design

Patients

Patient 1 is a 34-year-old man with a history of left knee hemarthrosis and frequent epistaxis. His fXI levels range from 22% to 30% of normal. His 2 children have fXI levels of 25% to 37%. All 3 individuals are heterozygous for a C>T change in fXI exon 7, resulting in a Ser225 to Phe substitution.

Patient 2 is a 38-year-old woman with heavy postpartum bleeding, idiopathic hematuria, and low fXI activity (25%-39%). She is heterozygous for a G>A change in exon 11, resulting in a Cys398 to Tyr substitution. A homozygote and 2 heterozygotes for Cys398Tyr were previously reported in unrelated families. Plasma fXI antigen levels for all patients match plasma fXI activity. Procedures for obtaining blood for DNA extraction and factor XI gene analysis were approved by the Institutional Review Boards of Vanderbilt University and the University of North Carolina. Informed consent was obtained according to the Declaration of Helsinki.

Transient transfections

Point mutations for Ser225Phe or Cys398Tyr were introduced into the wild-type fXI (fXI-WT) cDNA in pJVCMV as described. Transfections of 293 fibroblasts, growing in Dulbecco modified Eagle medium (DMEM) with sodium pyruvate, l-glutamine, and 5% fetal bovine serum, were performed in 6-well culture plates using Supercfect reagent (Qiagen, Valencia, CA). Single transfections used 80 ng, and cotransfections 160 ng, of fXI/pJVCMV construct. All transfections included 40 ng Renilla luciferase (pRL-CMV) vector (Promega, Madison, WI) to control for transfection efficiency, and empty pJVCMV to bring total DNA to 2 µg/transfection. Media were collected 67 hours after transfection, and cell lysates were prepared using a Dual Luciferase Reporter kit (Promega). Luciferase activity was measured on a Monolight 2010 luminometer (Analytical Luminescence, San Diego, CA). FXI in media and lysates was measured by enzyme-linked immunosorbent assay (ELISA) using goat anti-human fXI monoclonal antibody (Bentley and Co., La Jolla, CA). A standard curve was established using known concentrations of fXI.

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polycytoplasmic anti-human FXI immunoglobulin G (IgG; Affinity Biologicals, Hamilton, Ontario). Results were corrected for variation in transfection efficiency by luciferase assay. The mean for single FXI-WT transfections was assigned a value of 100%. Lane (1) FXI-WT, (2) FXI-Phe225, (3) FXI-Tyr398, (4) FXI-WT + FXI-WT, (5) FXI-WT + FXI-Phe225, (6) FXI-WT + FXI-Tyr398, (7) pJVCMV without cDNA. Western blots for FXI in lysates of BHK cells transfected with pLg-FXI-IN constructs. Samples from stably transfected cell lines were run on a 7.5% polyacrylamide-SDS gel, followed by Western blotting with goat anti–human FXI polyclonal antibody. (C) Samples are (1) conditioned media from FXI-WT cells and lysates from cells transfected with (2) FXI-WT, (3) FXI-Phe225, (4) FXI-Glu350, and (5) untransfected BHK cells. Positions of dimeric (D) and monomeric (M) FXI are indicated to the right of each panel, and positions of molecular mass standards in kilodaltons are shown on the left. The ratio of dimer to monomer varied between experiments. Note that, with the exception of FXI-Glu350, which does not dimerize, patterns for mutants match the wild-type pattern.

Results and discussion

In single transient transfections, FXI-Phe225 and FXI-Tyr398 levels in media are low (8.8% ± 2.8% and 1.4% ± 1.8%, respectively) compared with FXI-WT (100% ± 11.7%) (Figure 1A), with results for FXI-Tyr398 similar to negative control (1.9% ± 1.7%). This appears to be due to reduced secretion, as FXI-Phe225 and FXI-Tyr398 levels in lysates (99.4% ± 10.3% and 65.7% ± 29.9%) are comparable to FXI-WT (100% ± 18.3%) (Figure 1B). Cotransfection of FXI-WT with FXI-Phe225 or FXI-Tyr398 results in reduced FXI in media (36.6% ± 8.5% and 50.2% ± 6.5%, respectively) compared with FXI-WT control, with little change in intracellular protein (115.3% ± 18.0% and 81.1% ± 8.7%). In comparison, cotransfection of FXI-WT with an additional equal amount of FXI-WT construct results in modest increases in secreted and intracellular antigen (121.1% ± 12.8% and 144.8% ± 18.2%). Stably transfected BHK lines were prepared for FXI-WT, FXI-Phe225, and FXI-Tyr398, as well as the previously reported dominant-negative mutant FXI-Ser569 and the dimerization-defective mutant FXI-Glu350. Only FXI-WT was secreted to a significant extent (data not shown). Western blots of intracellular protein (Figure 1C-D) show that FXI-Phe225, FXI-Tyr398, and FXI-Ser569 form intracellular dimers similar to FXI-WT, indicating that poor secretion of these mutants is not due to a failure to form dimers, as in the case of FXI-Glu350.

Most FXI-deficient patients have proportional decreases in plasma activity and antigen (CRM− deficiency).9,10,16 Observations over the past 40 years suggest that FXI deficiency in Jewish patients is inherited primarily as a recessive condition,9,10,17 and our understanding of the mutations involved is consistent with this interpretation (Figure 2).5,18 However, the multimeric structure of FXI, like those of von Willebrand factor19 and fibrinogen,20 is conductive to CRM− dominant forms of disease through formation of nonsecretable heterotrimers. Hypothetically, any FXI mutation that interferes with protein secretion, but not dimer formation, could inhibit normal FXI polypeptide secretion. If the mutant polypeptide is expressed at a high enough level, the effect on plasma FXI level may be great enough to compromise hemostasis. The data herein indicate that this mechanism, previously proposed...
for Gly400Val and Trp569Ser, applies to Ser225Phe and Csys398Tyr and is, therefore, probably relatively common.

These observations suggest a 3-part mechanistic classification system for CRM− factor XI deficiency (Figure 2). The first category contains mutations that inhibit factor XI activity and include nonsense mutations such as Glu117Stop, frame shifts, deletions, splicing defects, and possibly amino acid substitutions that cause severe polypeptide instability. A second category, represented by the A4 domain substitutions Phe283Leu and Gly350Glu, includes mutations that interfere with dimer formation, resulting in intracellular retention of factor XI as monomer. Mutations in categories 1 and 2 are expected to cause little or no interference with the product of the normal factor XI allele (Figure 2). The third category, which includes Gly400Val and Trp569Ser, and now Ser225Phe and Csys398Tyr, contains mutations that form nonsecretable homodimers and probably nonsecretable heterodimers with wild-type factor XI. Saito et al. noted that factor XI levels tend to be lower in non-Jewish patients heterozygous for factor XI gene mutations compared with their Jewish counterparts. The dominant-negative effect proposed for category 3 explains this observation and would account for some families with apparent autosomal-dominant factor XI deficiency.

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References

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