Disorders of Hemostasis

A type II mutation (Glu117stop), induction of allele-specific mRNA degradation and factor XI deficiency

The Glu117stop mutation in the factor XI (FXI) gene is the most common cause of FXI deficiency and might cause the disease either by poor secretion/stability of the truncated protein or by decreased mRNA levels. Platelet- and lymphocyte-derived mRNA from three Glu117stop heterozygotes were analyzed by reverse-transcriptase polymerase chain reaction and sequencing, demonstrating allele-specific reduction of FXI Glu117stop mRNA.

FXI deficiency is a bleeding disorder characterized by hemorrhagic symptoms mainly associated with injury or surgery and generally inherited as an autosomal recessive trait (MIM *264900). However, cases of dominant transmission of the disease were recently reported. The bleeding tendency in FXI-deficient patients is highly variable and is often independent of circulating FXI levels. The disease has been described in several populations (prevalence 1:1000 but it is particularly common in Jews, among whom a heterozygote frequency of 9% has been reported.

The genetic bases of this rare coagulation disorder are mutations within the FXI gene (F11), which is located on chromosome 4q35.2 and consists of 15 exons. Among known genetic defects in F11, the type II mutation (Glu117stop, located in F11 exon 5) is highly frequent in Ashkenazi and Iraqi Jews, and has also been repeatedly found in other populations, always associated with the same founder haplotype. The pathogenic mechanisms by which Glu117stop leads to FXI deficiency have not yet been clarified and may include poor secretion/stability of the truncated protein and decreased synthesis/stability of the mutant mRNA. Transfection of BHK cells with FXI cDNA with the Glu117stop mutation yielded unmeasurable FXI protein, thus suggesting an effect at the protein level. On the other hand, a recent real-time reverse-transcriptase polymerase chain reaction (RT-PCR) analysis supports an effect at the mRNA level, since no detectable FXI mRNA was found in platelets and lymphocytes of a Glu117stop homozygous individual. Normal levels were found in Glu117stop heterozygotes, but the monoallelic expression of the wild-type FXI was not checked.

Mutations introducing premature termination codons in eukaryotes often signal mRNA degradation by the nonsense-mediated mRNA decay pathway. About half (12 nonsense, 7 frameshift, and 9 splicing mutations) of the more than 60 published mutations in F11 are predicted to cause the formation of premature termination codons. However, no clear-cut data on the effect of such premature termination codons on FXI mRNA stability have yet been provided. To address this issue, we studied three Italian patients affected by mild FXI deficiency, whose main demographic and clinical characteristics are summarized in Table 1. DNA sequencing of F11 revealed that they were all heterozygous for the Glu117stop mutation in exon 5.

A F11 region spanning from exon 3 to exon 8 was amplified by RT-PCR from platelet- and lymphocyte-derived mRNA of each patient. In two cases a nested PCR (with primers located in exons 4 and 6) was necessary to obtain a detectable PCR product, confirming that platelets and lymphocytes contain low levels of FXI mRNA. Direct sequencing of RT-PCR products demonstrated the complete absence of the mutant transcript in all cases (Figure 1), suggesting the selective degradation of mRNA molecules carrying the premature termination codon, even though decreased mRNA synthesis cannot be ruled out. Sequence analysis at a polymorphic site in exon 5, associated with the type II mutation (430T→C), confirmed the presence of only the wild-type FXI mRNA (data not shown).

In addition to triggering nonsense-mediated mRNA...
Decay, premature termination codons may also cause nonsense-associated altered splicing, a surveillance mechanism upregulating alternatively spliced transcripts that have skipped exons containing premature termination codons. To confirm that the type II mutation does not cause nonsense-associated altered splicing, we set up a two-step RT-PCR assay with primers designed to exclusively amplify FXI transcripts lacking exon 5. In particular, a first PCR using primers located in exons 2 and 8 was followed by a nested PCR with a forward primer crossing exon 4 and 6 (5′-ATAAGGTAACATTGTGTCTAC-3′) coupled with a reverse primer in exon 8. No products were obtained in any of the analyzed patients confirming that there is no evidence an alternative splicing of FXI exons 5 and that the Glu117stop mutation does not trigger nonsense-associated alternative splicing (data not shown). A synthetic 99-base oligonucleotide spanning the exon 4/6 junction was used as a positive control of the exon-5-skipped amplification.

In conclusion, the data reported here suggest that Glu117stop causes a quantitative deficiency of FXI by reducing mRNA levels. Nonsense-mediated mRNA decay might be responsible for this allele-specific degradation, and could play an important role in the pathogenesis of FXI deficiencies caused by mutations introducing premature termination codons.

Giulia Solda,* Rosanna Asselta,* Rossella Ghietto,° Maria Luisa Tenchini,* Giancarlo Castaman,° Stefano Duga*

*Department of Biology and Genetics for Medical Sciences, University of Milan, Milan, Italy; °Department of Hematology and Hemophilia and Thrombosis Center, San Bortolo Hospital, Vicenza, Italy

Funding: the financial support of Telethon, Italy (Grant no. GGP030261) is gratefully acknowledged.

Acknowledgments: we are indebted to Prof. Massimo Malcovati (Department of Biology and Genetics for Medical Sciences, University of Milan) for critically reading the manuscript.

Key words: coagulation factor XI, factor XI deficiency, type II mutation, nonsense-mediated mRNA decay, mRNA stability.

Correspondence: Dr. Stefano Duga, University of Milan, Department of Biology and Genetics for Medical Sciences, Via Venezia, 3/5, 20133 Milan, Italy. Phone: international +39.02.50315823. Fax: international +39.02.50315864. E-mail: stefano.duga@unimi.it

Table 1. Characteristics of the patients with FXI deficiency.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>City of origin</td>
<td>Bari</td>
</tr>
<tr>
<td>Gender</td>
<td>M</td>
</tr>
<tr>
<td>Present age, years</td>
<td>12</td>
</tr>
<tr>
<td>FXI activity levels, %*</td>
<td>35</td>
</tr>
<tr>
<td>Main symptoms</td>
<td>Recurrent epistaxis</td>
</tr>
<tr>
<td>Mutation, state</td>
<td>Glu117stop, heterozygous</td>
</tr>
</tbody>
</table>

All patients were referred because of an isolated prolonged activated partial thromboplastin time (APTT) associated with a mild bleeding diathesis. Plasma FXI level was evaluated by a functional assay based on a modified APTT and by an enzyme-linked immunosorbent assay (ELISA). FXI levels in both tests are expressed as percentages of values in pooled normal plasma from 30 normal individuals. The detection limits of the FXI functional and immunologic assays were 1% and 0.1%, respectively. Measured levels and normal ranges for both tests confirmed the diagnosis of FXI deficiency;*normal range: 8-136%; †normal range: 63-129%.

Figure 1. Effect of the Glu117stop mutation on FXI mRNA metabolism. Sequence analysis of both genomic DNA (top) and mRNA (bottom) of FXI-deficient patients (P1-P3) heterozygous for the Glu117stop mutation in exon 5. A FXI region spanning from exons 3 to 8 is schematically depicted (middle); nucleotides surrounding the mutations are also indicated. Primers used for PCR amplification from genomic DNA are shown by black arrows (FXI-In5F 5′-AAAGGATGAGTCAGGAGGGA-3′, and FXI-In5R 5′-TCTGGCATGGTATGCG-3′), while those used in the RT-PCR experiments are indicated by white (first amplification, FXI-Ex3F 5′-TGTGACTCAGTTGTTGAAGG-3′, and FXI-Ex6R 5′-GAAATGGTACAGCTGTT-3′) or gray (nested amplification, FXI-ex4F 5′-GAAACACTGCCAAGAGTGAA-3′, and FXI-ex8R 5′-CCAATCCACGACAGAC-3′) arrows. Exons (boxes) and introns (lines) are not drawn to scale. PCR conditions are available on request.
References