Systemic causes of heavy menstrual bleeding
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Chapter 7: Identification of a novel factor XI gene mutational event in a Dutch Caucasian family with inherited factor XI deficiency

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Dear Sirs,

Factor XI (FXI) deficiency is an autosomal recessive disorder and is predominantly present in Ashkenazi Jews \(^1,2\). To achieve sufficient haemostasis the haemostatic level of FXI needs to be between 15-20%; however, the correlation between FXI level and bleeding tendency is weak and inconsistent \(^3,4\). FXI gene contains 15 exons and 14 introns located on the long arm of chromosome 4 (4q35) \(^1,5\) and more than 240 mutations have been reported (http://www.factorxi.org). We performed an analysis in order to determine the molecular background of FXI deficiency in a Dutch Caucasian family. FXI activity levels were determined by an one-stage clotting assay (Siemens, Marburg, Germany), which was standardized using a home-made normal plasma pool. The reference interval, based on a locally performed reference range, was 65-150 U/dl. The assay was performed using a Sysmex CA-7000 (Siemens). Direct sequencing analysis of all 15 exons and flanking introns of FXI gene was performed to detect mutations. The proband was a 70-year-old woman of Dutch Caucasian origin (Figure 1 A; II:4). She had several bleeding episodes following surgery (dacryocystorhinostomy and hip replacement), spontaneous haematomas and postpartum haemorrhage. Her total bleeding score according to Tosetto was 7 \(^6\). Although the Tosetto bleeding score was originally developed as a tool to quantify bleeding tendency in patients with type \(1\) M von Willebrand disease, the score is now also used in patients who are analysed for increased bleeding tendency \(^7\). It has been suggested that a score below 3 is normal \(^8\). The proband's FXI activity level was 30 U/dl. In addition, her two sisters, two daughters and one niece were tested (Figure 1 A). Her affected dizygotic twin sister (II:5) underwent a considerable number of surgical procedures, only a lumpectomy because of breast cancer was complicated by bleeding (bleeding score 2, FXI activity 45 U/dl). The affected niece (III:3) had a single surgery (tonsillectomy) with bleeding and spontaneous haematomas; she used combined oral contraceptives for menorrhagia (bleeding score 6, FXI activity 27 U/dl). Sequencing analysis showed in all three subjects with FXI deficiency the presence of two novel heterozygous mutations in sequential nucleotides in exon 3 of the FXI gene: a silent mutation (NM_000128.3 (FXI):c.177C>T) that preserves amino acid Phenyldalanine at position 59.
and a potential missense mutation (NM_000128.3 (FXI):c.178A>C) (Figure 1 B). These mutations were not found in another sister (II:7 with a bleedingscore of -2) and two daughters of the proband (III:1 with a bleedingscore of 3; and III:2 with no bleeding score assessed) with normal levels of FXI. Although it is very unusual to observe two mutations in sequential nucleotides, absence of the mutations in the proband’s daughters excluded compound heterozygosity. It is therefore likely that only one mutational event occurred, possibly by slippage yielding two symmetric repeats TTTC/CTTT. NM_000128.3(FXI):c.178A>C may lead to a conversion of hydrophilic and median sized Threonine at position 60 to hydrophilic large Proline. Threonine is buried in the region of apple 1 domain between two Phenylalanine residues. Multiple alignment analysis of the human FXI gene sequence with sequences of other vertebrates was performed using OMA browser (http://omabrowser.org/cgi-bin/gateway.pl?f=DisplayTRGroup&p1=389460&p2=Vertebrata). The alignment showed that Threonine is highly conserved among other species (Figure 1 C). To evaluate the possible effect of Threonine to Proline substitution on the molecular structure of FXI, we used a computerbased model (2f83) in Swiss PDB Viewer (http://spdbv.vital-it.ch/). Threonine 60 interacts with Phenylalanine 30, Serine 96, and Valine 77 through strong hydrogen bonds (Figure 1 D-a). After substitution, only an interaction remains with Valine 77 (Figure 1 D-b). Furthermore, due to its biochemical properties, Proline might result in steric hindrance with Phenylalanine 59. To prove that the novel mutated nucleotides are not polymorphisms, we also performed sequence analysis in 100 unrelated healthy volunteers. No healthy volunteer carried these mutations. Additionally, these mutated nucleotides may inactivate exon splicing enhancers (ESEs), leading to failure of splicing and exon skipping or inclusion of intron segments in mRNA. For exons with weak splice sites, like exon 3 of FXI gene, ESEs are essential for normal splicing \(^{10,11}\). ESEs are binding sites for specific serine/ arginine-rich (SR) proteins. Through binding of SR proteins to ESEs inclusion of exons is promoted. To test the possibility that these mutated nucleotides cause a splicing defect, we used the motif-scoring matrices in a web-based program called ESEfinder (release 2.0: http://rulai.cshl.edu/tools/ESE2/). Twentyseven ESEs were identified in normal exon 3 of FXI gene. The two novel mutated nucleotides did not result in an additional or
Taking our data together, it is likely that the presence of these two novel heterozygous mutated nucleotides may modify the FXI function and are associated with FXI deficiency in a Dutch Caucasian family. Moreover, the FXI activity levels in the affected subjects ranged from 27 to 45 U/dl, although all three were heterozygous. Perhaps these mutations, and in particular the potential missense mutation (NM_000128.3(FXI):c.178A>C) has a dominant negative effect, as was previously shown by Kravtsov et al. However, in order to prove the actual mechanism by which this mutation affects the patient, it needs to be expressed in cells and present a similar effect as in the patient.
Figure 1: Novel FXI gene mutational event.

**A**

- C
- T
- T
- C
- T
- T
- C
- A

**B**

- Factor XI activity level
- Level has been measured elsewhere
- Proband

**Human**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Chimpanzee**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Orangutan**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Gorilla**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Giant Panda**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Mouse**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Cow**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Cat**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Hedgehog**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Rabbits**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Tree shrew**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Guinea pig**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Gray mouse lemur**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**American pika**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Horse**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Bottle-nose dolphin**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Flying fox**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Guansko**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Kangaroo rat**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Philippine Tarsier**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Two-toed sloth**
- PRCL:FEPFASEPSEPFTWFTCVKLD

**Common Marmoset**
- PRCL:FEPFASEPSEPFTWFTCVKLD

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Figure 1: Novel FXI gene mutational event. **A** Pedigree. **B** Two novel heterogenous mutated nucleotides in exon 3 of FXI gene. A = adenine; C = cytosine; G = guanine; T = thymine. Positions of the nucleotide alterations are indicated: C>T at nucleotide 177 and A>C at nucleotide 178. C) Multiple alignment of part of FXI sequence in different vertebrates.
Identification of a novel factor XI gene mutational event in a Dutch Caucasian family with inherited factor XI deficiency

A) Pedigree. B) Two novel heterozygous mutated nucleotides in exon 3 of FXI gene. A = adenine; C = cytosine; G = guanine; T = thymine. Positions of the nucleotide alterations are indicated: C>T at nucleotide 177 and A>C at nucleotide 178. C) Multiple alignment of part of FXI sequence in different vertebrates. Threonine at position 60 is underlined. D) Three-dimensional structure of FXI molecule with indicated Threonine at position 60 (red arrow) buried in the apple 1 domain (a). Below, the molecular structure is shown for Threonine (b) and after Proline substitution (c). Threonine forms strong hydrogen bonds with Phenylalanine at position 30, Valine at position 77, and Serine at position 96 (black dotted lines) and 1 weak hydrogen bond (grey dotted line). After substitution only the interaction with Valine at position 77 remains. In addition, a clash between Proline at position 60 and Phenylalanine at position 59 occurs (pink dotted line). Furthermore, the figure is colour-coded as follows: red areas represent oxygen atoms, blue areas represent nitrogen atoms, yellow areas represent sulphate atoms, and grey areas represent others.
Chapter 7

References
