Severe factor XI deficiency in the Abruzzo region of Italy is associated to different *FXI* gene mutations

Coagulation factor XI (FXI) is a glycoprotein of 160 kDa, which, upon thrombin-mediated activation, activates factor XII or FXI itself, catalyzing the conversion of factor IX (FIX) to activated FIX in the consolidation phase of blood coagulation.[1] FXI deficiency is a rare autosomal recessive bleeding disorder which is however particularly common among Ashkenazi Jews with a heterozygote frequency of 9%.[2]

Bleeding tendency in FXI-deficient patients seems to poorly correlate with plasma FXI levels and hemorrhagic episodes are usually associated with injury or surgery.[1,5] However, even patients with severe deficiency may not suffer from significant bleeding.

More than 150 FXI gene mutations have so far been reported and a founder effect has been demonstrated in some populations (*see http://www.med.unc.edu/isth/mutations-databases/FactorXI_2007.html*, also *http://www.wienkav.at/kav/kar/texte_anzeigen.asp?ID=7137*, and *http://www.factorxi.com*).

In this paper, we report the genetic basis of FXI deficiency in 6 patients from the Abruzzo Region in Italy, confirming the allele heterogeneity of the disease.

Six unrelated patients (3 males and 3 females, age 18–60 years) with inherited severe FXI deficiency were investigated. All these patients had been living in the Abruzzo Region, in Central Italy, for at least three generations. All subjects gave their informed consent according to the Declaration of Helsinki before blood withdrawal. Venous blood was collected in 1:10 volume of 0.11 M trisodium citrate, pH 7.3, and centrifuged at 2,500 g for 15 mins. FXI activity (FXI:C) and antigen (FXI:Ag) were measured as previously described.[6] The detection limits of the FXI:C and FXI:Ag assays were 0.5% and 2% respectively.

Genomic DNA was purified as previously described.[6] The coding region, intron-exon boundaries and 5′ and 3′ untranslated regions of the *FXI* gene (GeneBank accession number NT_022792) were PCR-amplified. The primers used, PCR conditions and sequencing procedures are available upon request. All novel sequence changes were confirmed in both DNA strands. Fifty normal subjects, half of whom were from Abruzzo, were used for screening of the novel identified sequence variants.

The main laboratory and genotypic results in the investigated patients are summarized in Table 1. All patients had FXI:C < 2%, while FXI:Ag showed a more heterogeneous pattern, but always with severely reduced levels. In addition to assay variability, the measurable FXI:Ag compared with FXI:C in patient 1 suggests that some dysfunctional FXI could be synthesized and secreted. All the patients were identified by pre-surgical or routine laboratory evaluation. Minor bleeding, not requiring substitutive treatment, was reported for patients 1, 3 and 4. None of the heterozygous relatives suffered from significant bleeding. Sequence analysis identified candidate mutations in all the 12 affected chromosomes. A total of 8 different candidate mutations were identified, 3 of which were novel (Figure 1). Only one patient (patient #5) resulted homozygous for the type II mutation E117X. The remaining 5 were compound heterozygotes. Patient 1 was heterozygote for the E117X mutation in combination with a c.419G>A transition in exon 5, predicting the C122Y amino acid change in the Apple 2 domain. Patient #2 was heterozygous for the E117X mutation and for a novel transversion in exon 9 (c. 981 C>A), resulting in the C309X nonsense mutation in the Apple 4 domain. Patient #3 had the heterozygous nonsense mutation Q116X and a novel candidate mutation in exon 5 (c.422 C>T), resulting in a T123M amino acid substitution in the Apple 2 domain. Patient #4 showed compound heterozygosity for A91T and F283L (type III mutation). Patient #6 was heterozygous for the novel nonsense mutation C309X and for a novel mutation in exon 15 (c.1786 G>T), predicting the gain of a cysteine in the catalytic domain (G578C). All the novel mutations were absent in 100 normal alleles (*data not shown*). The total population of the Abruzzo Region is approximately 1,250,000 (see *http://www.regione.abruzzo.it/portale/index.asp*). Therefore, the prevalence of index cases with severe deficiency is 6/1,250,000 or 4.8/1,000,000.

Table 1. Main phenotypic and genotypic results in the investigated patients.

Patient, sex and year of birth	*FXI:C[#] (%)*	*FXI :Ag[#] (%)*	*Reason for referral*	*Bleeding symptoms*	*Mutation (exon, nucleotide, amino acid)*
1–F 1989	<0.5	10	Pre-surgical screening	Bleeding after tooth extraction	Ex 5 c.403G>T; E117X Ex 5 c.419G>A; C122Y
2–M 1974	<0.5	4	Routine laboratory evaluation	None	Ex 5 c.403G>T; E117X **Ex 9 c.981C>A; *C309X***
3–M 1947	0.9	4	Routine laboratory evaluation	Bleeding after knee arthroscopy	Ex 5 c.400C>T ; Q116X **Ex 5 c.422C>T; T123M**
4–F 1955	1.6	6	Routine laboratory evaluation	Menorrhagia	Ex 4 c.325G>A; A91T Ex 9 c.901T>C ; F283L
5–M 1974	<0.5	< 2	Pre-surgical screening	None	Ex 5 c.403G>T; E117X homozygous
6–F 1965	1	< 2	Routine laboratory evaluation	None	**Ex 9 c.981C>A; C309X** **Ex 15 c.1786G>T ; G578C**

Ex: exon; bold: novel mutations [#]: the results are the mean of two separate measurements.

Hereditary FXI deficiency (MIM+264900) is an autosomal inherited bleeding disorder first reported by Rosenthal *et al*., in 1953.[7] Spontaneous bleeding is rare even in patients with severe FXI deficiency,[8] and bleeding usually occurs only after trauma or surgery, particularly at sites where there is local fibrinolysis.[1] Furthermore, bleeding manifestations are not well correlated with the plasma levels of FXI:C and can vary widely among patients with similar FXI levels.[9] Although the disease is rare in most populations (estimated prevalence 1:10[6]), it is frequent in Ashkenazi Jews and severe deficiency (FXI:C <20 U/dL) was esti-

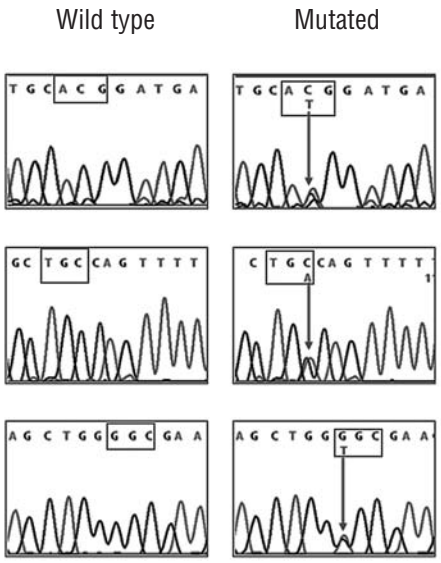

Figure 1. Identification of the 3 novel FXI mutations by sequencing (see text).

mated to occur in 1 in every 450 individuals.[2] Two mutations, designated as type II (E117X) and type III (F283L), account for 98% of alleles in this population.[1,2] However, a significantly higher allelic heterogeneity has been reported in patients belonging to other ethnic groups. Remarkable exceptions are represented by a few populations in whom prevalent ancestral mutations were found (C38R in French Basques,[10] Q88X in French patients from Nantes,[11] and C128X in English patients).[12]

In this study, we identified 6 apparently unrelated index cases with severe FXI deficiency in the Abruzzo Region in whom 8 different mutations were identified (Table 1). All these patients were detected through presurgical or routine screening, since only minor bleeding episodes had been observed in some of them. The E117X (type II mutation) was identified in homozygosity in one patient and in compound heterozygosity in 2 other index cases, whereas only one patient was compound heterozygous for the F283L (type III) mutation. These observations reinforce the results of our recent findings in a series of 31 Italian FXI-deficient patients, in whom the type II mutation (present in 3 homozygous and in 8 heterozygous individuals, allele frequency 30.4%) was more frequent than the type III mutation (found only in one compound heterozygote type II/III, allele frequency 2.2%).[6] However, in an unselected population of 3,879 apparently healthy Italian individuals the frequencies of the type II and type III mutations (allele frequency 0.00064 and 0.00051 respectively) were indeed similar.[6] In addition, 3 previously reported (A91T, Q116X and C122Y) and 3 novel candidate mutations were found in the Abruzzo patients. The T123M mutation, near C122, causes the introduction of a hydrophobic sulfur atom, with loss of highly hydrophilic hydroxyl group within the Threonine. C309X occurs in the Apple 4 domain, with the disruption of C309-C303 pairing in the inner loop. G578C causes the introduction of a sulfhydryl group in the catalytic domain of FXI.

In conclusion, inherited FXI deficiency in the Abruzzo Region has a heterogeneous genetic background and no single predominant mutation could be identified.

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