Disorders of Hemostasis

Homozygosity for a Thr575Met missense mutation in the catalytic domain associated with factor XI deficiency

In this study we investigated an asymptomatic 55-year-old Lebanese woman with factor XI deficiency. The *F11* gene was analyzed and a cross reacting material positive (CRM⁺) variant, Thr575Met, was identified in homozygosity in the proband, and in heterozygosity in four of her siblings.

The human *F11* gene is located on chromosome 4 (4q35) and consists of 15 exons. To date, more than fifty mutations responsible for factor XI deficiency have been identified.[1] We studied 10 Lebanese patients with factor XI deficiency for mutations in the *F11* gene by single-strand conformational analysis (SSCA) and sequencing. The majority of patients were homozygous (6/10) or heterozygous (2/10) for the common type II Glu117stop mutation while one patient was homozygous for a missense mutation (Trp501Cys, exon 13) in the catalytic domain.[2]

A 55-year old woman with no personal or family history of bleeding was admitted for kidney donation to her brother. She was found to have a prolonged activated partial thromboplastin time (aPTT) (47s, normal range 25-31s). The proband's parents are first cousins. Blood was obtained after informed consent and routine coagulation tests were performed. Factor XI coagulant activity was measured by a one-stage method based on a modified partial thromboplastin time using lyophilized immunodepleted human plasma deficient of FXI (Hemoliance, UT, USA). FXI antigen was assayed by an in-house enzyme-linked immunosorbent assay (ELISA) based on goat anti-human FXI polyclonal antibodies and peroxidase-conjugated IgG (Affinity Biological Inc., Ontario, Canada). The normal pool was constructed by mixing equal volumes of plasma from 30 healthy control subjects (reference range 70-130%). The coefficient of variation of 15 measurements of this pool was 3.7. APTT, factor XI:c and antigen levels are shown in Table 1 and Figure 1A. The *F11* gene was analyzed by polymerase chain reaction (PCR) amplification of all 15 exons, including intron-exon junctions, followed by SSCA, as previously described.[3] *F11* oligonucleotides for exons 1-15 were as described by Alhaq *et al.*[4] DNA corresponding to SSCA variants were purified and directly sequenced. Carrier detection in the family and population screening of the Thr575Met mutation on 165 DNA samples from individuals of the same geographical region was performed by restriction fragment length polymorphism (RFLP) analysis. The Thr575Met mutation (ACG→ATG) creates a BspHI restriction site (details of this analysis are available on request). PCR-SSCA for *F11* exon 15 revealed a variant DNA fragment for the patient (Figure 1B). Sequencing identified a homozygous missense mutation Thr575Met (ACG→ATG; g.163C→T according to Genbank accession number M20218.1). Following this initial identification of the Thr575Met by SSCA, the remaining *F11* exons and

Table 1. Phenotype and genotype of the family.

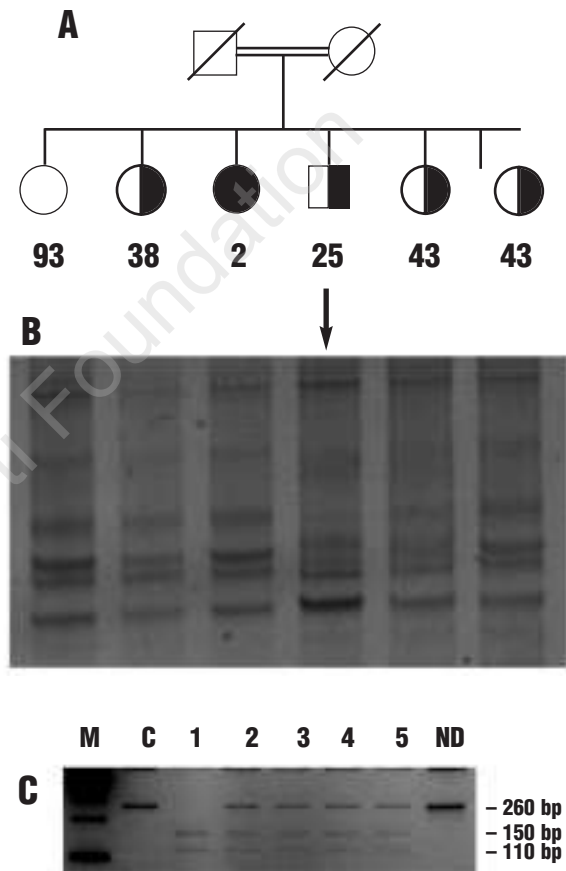| *Identification* N. sex	APTT(s)	FXI:c (U/dL)	FXI:Ag (U/dL)	*Genotype*
1 f	24	93	99	wt/wt
2 f	32.5	38	83	Thr575Met /wt
3 f	46.6	2	105	Thr575Met /Thr575Met
4 m	35.2	25	67	Thr575Met /wt
5 f	33.8	43	106	Thr575Met /wt
6 f	33.8	43	112	Thr575Met /wt

Figure 1. Pedigree of the family with factor XI deficiency with the identification of the Thr575Met mutation in the catalytic domain of factor XI. A. Factor XI level (FXI:c; U/dL) is indicated for each individual. The double horizontal line indicates consanguinity (parents are first cousins). B. Single-strand conformational analysis (SSCA) of *F11* exon 15. The variant pattern obtained for the proband's sample is indicated by the arrow, surrounded by five samples from individuals normal for exon 15. C. BspHI restriction fragment length polymorphism (RFLP) analysis for the five family members. The normal 260 bp product remains undigested while the mutant yields two bands (150 bp and 110 bp). The same procedure was used to screen 165 control DNA samples from the same geographical region. M: marker, C: control wt/wt DNA sample, 1: patient homozygous for Thr575Met, 2-5: heterozygous sibs, ND: non-digested PCR product.

intron-exon junctions were sequenced. No other mutation was identified. BspHI RFLP analysis confirmed homozygosity for this mutation in the proband and heterozygosity in her four siblings available for analysis (Figure 1C, Table 1). No such mutation was identified in the 165 control individuals.

Although in the absence of functional studies one cannot formally exclude that this amino acid change is a rare polymorphism, several observations indicate that the mutation we found is responsible for the patient's factor XI deficiency. First, this was the only change noticed after having sequenced the complete *F11* coding sequence and intron-exon junctions. Second, the mutation occurs in a highly conserved portion of the FXI serine protease catalytic domain. Indeed, comparisons of sequences from human (SwissProt accession number P03951), mouse (SwissProt accession number Q91Y47), rabbit (Genbank accession number AF395821), rat (Genbank accession number XM224872) and cow (TrEMBL accession number P79131), show conservation of the Thr575 residue. Although Thr and Val residues can be found at this position in the catalytic domains of other serine proteases, no Met has been reported. Other mutations in the active site have previously been shown to cause factor XI deficiency, including a mutation in the immediately adjacent residue: Ser576Arg.[5] Third we found that controls did not have the mutation. Finally, the proband's elder sister with normal values of factor XI did not have the mutation.

Interestingly the Thr575Met mutation was identified earlier this year in compound heterozygosity associated with a much more severe early truncating mutation (Q88X).[6] No functional analysis or population screening for the Thr575Met was performed in this study. Our finding of the same mutation is additional evidence that it is indeed responsible for the defect. To date the majority of mutations identified in the factor XI gene seem to prevent or greatly reduce protein expression. In the present case the factor XI antigen level was 105 U/dL, and this therefore represents a dysfunctional CRM+ variant.[7-9] A discrepancy between antigen level and activity level was also observed for the French patient heterozygous for Thr575Met (FXI:Ag 42 U/dL).[6]

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Disorders of Hemostasis

Plasma soluble fibrin monomer complex is a useful predictor of disseminated intravascular coagulation in neonatal sepsis

Disseminated intravascular coagulation (DIC) is a major factor influencing mortality in neonatal sepsis. Clinical trials have supported the use of antithrombin and activated protein C supplementation in DIC associated with sepsis.

Disseminated intravascular coagulation (DIC) is a major factor influencing mortality in neonatal sepsis.[1] Clinical trials have supported the use of antithrombin and activated protein C supplementation in DIC associated with sepsis. The outcome of therapy was poorer with increasing DIC scores, suggesting that therapy could be more effective if initiated in the pre-DIC state than in established DIC.[2] Wada *et al.* emphasized the usefulness of plasma soluble fibrin monomer complex (SFMC) in the diagnosis of DIC and pre-DIC state in adults.[3] The present study was designed to assess the accuracy of plasma SFMC for early diagnosis of DIC in septic neonates.

Thirty-three neonates were prospectively enrolled in this study after obtaining informed consent from their parents. They were categorized into three groups: group I was formed of 10 healthy neonates as controls; group II comprised 13 neonates suffering from neonatal sepsis; and group III was formed of 10 septic neonates with overt DIC consecutively enrolled from those neonates of group II who satisfied the criteria proposed by the Scientific Subcommittee on DIC of the International Society on Thrombosis and Hemostasis for overt DIC during their clinical course.[4]

Plasma SFMC was measured according to the method previously reported by Wiman and Ranby[5] using the Berichrome FM Kit from Dade Behring Marburg GmbH (Germany). The Kruskal Wallis H test was used to test for