Deficiency of Coagulation Factor XIII A Subunit Caused by the Dinucleotide Deletion at the 5′ End of Exon III

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Abstract

A congenital deficiency of the coagulation Factor XIII A subunit (F XIII A) is a rare autosomal recessive disorder that is characterized by a life-long bleeding tendency complicated by a difficulty in healing. Thus far, no molecular genetic analysis of this disorder has been reported. In this study, we demonstrate the molecular abnormalities in a family with this disorder. We performed Northern blot analysis of peripheral blood monocytes obtained from the propositus and found a 4-kb single band of F XIII A mRNA whose size was identical with that of normal subjects. Exons II–XV, which encode all the amino acids, were individually amplified by a polymerase chain reaction (PCR). All PCR products from the propositus had lengths indistinguishable from those of the wild type on agarose gel, suggesting that this defect results from either a point mutation or a short deletion/insertion. The sequencing of F XIII A cDNA from the propositus revealed a deletion of the dinucleotide AG within the AGAG repeat at the position of 210 to 213. Concerning the genomic sequence, a deletion of dinucleotide AG was also demonstrated in the intron B–exon III boundary. This deletion appeared to cause a frameshift mutation making a new stop codon shortly thereafter, and leading to a deficiency of plasma F XIII A. The heterozygosity of the F XIII A deficiency in the patient's offspring was documented by the nucleotide sequences of their exon III. (*J. Clin. Invest.* 1992. 90:315–319.) Key words: coagulation Factor XIII • deficiency of Factor XIII A • gene analysis • deletion of gene • PCR

Introduction

Coagulation Factor XIII (F XIII)[1] is a transglutaminase that crosslinks a fibrin monomer into a stable clot in the final stages of blood coagulation (1). In addition to that fibrin monomer, substance such as von Willebrand factor, α_2-plasmin inhibitor, thrombospondin, and fibronectin are also crosslinked into a fibrin clot by this enzyme. Such crosslinking leads to an increased resistance to digestion by plasmin (2, 3), as well as to an increased mechanical strength of the clot (4, 5). Factor XIII circulates in the plasma as a tetramer (A_2B_2, mol wt 320,000) consisting of two A subunits (mol wt 75,000) and two B subunits (mol wt 80,000) (6–8). The A subunit (F XIII A) is the catalytically active subunit, while the B subunit (F XIII B) may primarily play a role of a carrier protein protecting the circulating A subunit (6–8).

The A and B subunits are separately synthesized by different cells and form a tetramer in the plasma. The A subunit has been reported to be synthesized mainly by platelets/megakaryocytes, monocytes/macrophages, and the placenta, and to be localized in the cytoplasm of those cells as dimers (6–17).

The hereditary deficiency of F XIII is a rare autosomal recessive disorder, and this frequency has been reported to be one in 2×10^6 (18). This disorder was first reported in 1960 by Duckert et al. (19). Since then, about 160 patients with a F XIII deficiency have been reported, characterized by life-threatening bleeding, difficulty in healing, and a high risk of miscarriage (18, 19). Although a complete absence of the A subunit was demonstrated in most cases, a few instances of a deficiency of the B subunit have also been reported (20, 21).

The primary structure of human F XIII A has been elucidated from the amino acid sequence (22, 23) and cDNA sequence obtained from the human placental library (24). F XIII A cDNA consists of 3,902 bases, which include 2,196 bases of coding region for 732 amino acids, 81 bases of 5′, and 1,625 bases of 3′ nontranslated regions (22–24). The F XIII A gene is mapped at chromosome 6p24-p25 (25, 26). The gene structure has recently been demonstrated as showing that the gene extends to more than 160 kb and is separated into 15 exons by 14 introns (27).

The elucidation of the gene structure of F XIII A prompted us to investigate the genetic abnormality of F XIII A deficiency. We herein report the results of the molecular analysis of a family with congenital F XIII A deficiency.

Methods

Case report. The propositus was a 52-yr-old Japanese man who had suffered from a bleeding tendency since birth. At 27 yr of age, he experienced intracranial bleeding and was diagnosed as having a deficiency of F XIII based on the solubility of a fibrin clot in 5 M urea and 2% monochloracetic acid. Since then, he has been treated with F XIII A concentrate. As shown in Fig. 1, consanguinity was found in his parents (first cousins). The patient's younger brother has a similar bleeding problem as well.

Materials. F XIII A cDNA clone (λ gt10–12) from the human placental library was kindly provided by Dr. U. Grundmann, Department of Molecular Biology, Research Institutes, Germany (24). Re-

Data on the patient described in this paper have been previously published (1972. *Diagnosis and Treatment.* 46:906–910).

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1. *Abbreviations used in this paper:* F XIII, coagulation Factor XIII; F XIII A, coagulation Factor XIII A subunit; PCR, polymerase chain reaction.

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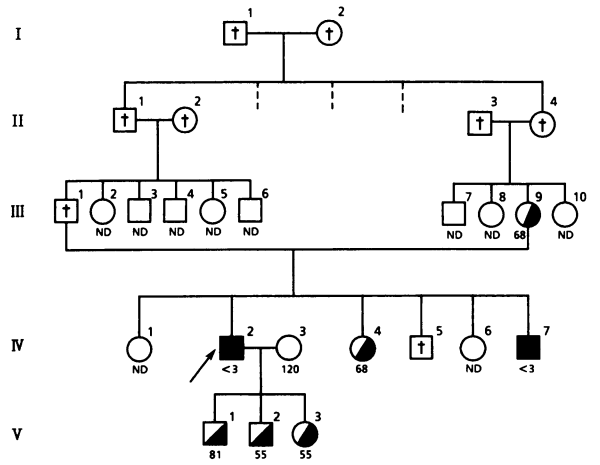

Figure 1. Pedigree of the family with F XIII A deficiency. The proband is indicated by an arrow. The parents of the proband are first cousins. F XIII activity (%) is shown below each member of the family. ■, homozygote; ◪◓, heterozygote; †, deceased; ND, not determined.

striction endonucleases were purchased from Takara Shuzo Corp., Osaka, Japan. Plasmid vector pUC19, *Escherichia coli* strain DH5α, X-gal, ampicillin, T4 DNA ligase, calf intestinal phosphatase, and M13 sequencing primers were obtained from Toyobo Corp., Osaka, Japan. Deoxycytidine 5'-[α-^{32}P]triphosphate ([α-^{32}P]dCTP), deoxycytidine 5'-[α-^{35}S]thiotriphosphate [α-^{35}S]dCTP), and a megaprime DNA labeling kit were obtained from Amersham Corp., Arlington Heights, IL. *Taq* DNA polymerase (Amplitaq) and a GeneAmp RNA polymerase chain reaction (PCR) Kit were obtained from Perkin Elmer-Cetus Instrs., Norwalk, CT. Agarose (type II, Medium EEO) and ethidium bromide were obtained from Sigma Chemical Co., St. Louis, MO. Nytran nylon membrane was obtained from Schleicher & Schuell Inc., Keene, NH. The Sequenase Version 2 Deaza-dCTP kit was obtained from U.S. Biochemical Corp., Cleveland, OH. Hydrolink (Long Ranger, 50% concentrate) was obtained from AT Biochemical, Malvern, PA. Oligonucleotides were synthesized using a Cyclone Plus DNA Synthesizer; Millipore Corp., Bedford, MA. A PCR was performed with the Program Temp. Control System PC-600, Astec Corp., Fukuoka, Japan.

Determination of plasma F XIII A levels. The activity of plasma F XIII A was determined with a fluorescence assay using dansylcadaverine (28). The antigen levels of F XIII A and B were measured by Laurell's method (29).

Isolation of genomic DNA and RNA. 30 ml of peripheral blood was obtained from the patient and his family members, using sodium heparin as an anticoagulant. Genomic DNA from leukocytes was isolated according to the standard method (30). DNA was dissolved in 1 ml of 10 mmol/liter of Tris-HCl, 1 mmol/l EDTA, pH 8 (TE buffer) and stored at 4°C until used. The total RNA was isolated from the PBMC by guanidinium thiocyanate extraction (31), and were then purified by ultracentrifugation on a cesium chloride gradient. The RNA was dissolved in H_2O and stored at −70°C until used.

Northern and Southern blot analysis. 10 μg of total RNA was separated on a 1% formaldehyde agarose gel and then was transferred to a Nytran Nylon membrane. After endonuclease (HindIII, PvuII, and BamHI) digestion, 10 μg of leukocyte DNA was electrophoresed on a 0.8% agarose gel and was transferred. These membranes were hybridized with the F XIII A cDNA probe radiolabeled with [α-^{32}P]dCTP using the random primer method.

PCR amplification of DNA. Exons II–XV were individually amplified from 0.4 μg of genomic DNA by 30 cycles of PCR according to the method of Saeki et al. (32) using 14 pairs of oligonucleotide primers designed from the normal genomic sequence (Table I). Each cycle consisted of 1 min at 94°C, 2 min at 55°C, and 1 min at 72°C. The final extension was carried out for 7 min at 72°C. The amplified PCR products were electrophoresed on a 2% agarose gel and were stained with ethidium bromide.

Table I. Oligonucleotide PCR primers of F X III A gene

Primer		Direction	Sequence*
Exon II	1	Sense	5'-ACATGCCTTTTCTGTTGTCT-3'
	2	Antisense	5'-CCCAGAGTGGTGGGGAAGGGG-3'
Exon III	1	Sense	5'-GATTATTTTCTTCAACCCTTG-3'
	2	Antisense	5'-TCAAGACTGGAGCTTGCACA-3'
Exon IV	1	Sense	5'-TCAACCTAACAGAGTATTTC-3'
	2	Antisense	5'-GAGAAAACTAAATGTCTGCC-3'
Exon V	1	Sense	5'-GTCTGGTTTGGTAATAGTCAC-3'
	2	Antisense	5'-AATGTCCTTGACAATAAC-3'
Exon VI	1	Sense	5'-GCAGAGTGAACACTAGTTTC-3'
	2	Antisense	5'-CAGGTGTAACAGATTTTAGG-3'
Exon VII	1	Sense	5'-CTTCCTTCTCACTTCTCACG-3'
	2	Antisense	5'-GTCTTAGAGTGAAGTTTCCT-3'
Exon VIII	1	Sense	5'-TGTTTAGCTGTGGTCTGTCC-3'
	2	Antisence	5'-TCAGCCAATGCCATTGTCAA-3'
Exon IX	1	Sense	5'-TTAACCTTTCTGGGCTTGTG-3'
	2	Antisense	5'-ATGAAGCAAGTTCCCAGAGG-3'
Exon X	1	Sense	5'-AACTCACACTGCCCTTCCTC-3'
	2	Antisense	5'-AACAGCACTTTCCTCCAGCT-3'
Exon XI	1	Sense	5'-ATGGCTAATGCTCTCCTCTC-3'
	2	Antisense	5'-GTACTCAATGGACTTGGGCA-3'
Exon XII	1	Sense	5'-TGCCTGTGATTATCTCTGGA-3'
	2	Antisense	5'-AGCGAGTCTCACAAAGAACC-3'
Exon XIII	1	Sense	5'-GATGCCAGGCCTGTGTGTGT-3'
	2	Antisense	5'-TCTGTTCCAGGATGAGACGC-3'
Exon XIV	1	Sense	5'-GCTGCTAATGACCTGCATTC-3'
	2	Antisense	5'-AAGCTTCCCACAGCTCTGCA-3'
Exon XV	1	Sense	5'-GAACCTCTCCTCTCTTTTCC-3'
	1	Antisense	5'-CTCTTATGAGCTATGAGAGC-3'

* Primer sequences refer to reference 27.

Synthesis of cDNA and PCR amplification of cDNA (reverse transcriptase [RT]-PCR). First-strand cDNA was generated from 1 μg of total RNA using oligo (dT) primers and reverse transcriptase. Four pairs of PCR primers were designed according to the normal cDNA sequence (Table II). The sequences of the primers were partially modified to make a restriction enzyme site near their 5' ends. The nucleotide numbers 19 to 2352 of cDNA, which cover the entire amino acid coding region, were divided into four overlapping fragments named A, B, C, and D, which included nucleotides 19-766, 596-1275, 1158-1695, and 1630-2352, respectively (Fig. 2). Each fragment was amplified under the same conditions as described above. To confirm that these products had the predicted sequence, they were digested by restriction endonucleases that recognized the known sites as shown in Fig. 2, and, in addition, they were electrophoresed on a 2% agarose gel and stained with ethidium bromide.

Subcloning and DNA sequence of PCR products. The PCR products were subcloned using pUC19 and competent cells derived from *E. coli* strain DH5α. Plasmids were prepared from the transformed *E. coli* by the method of Maniatis (33). Following the alkaline degeneration of plasmids, inserts were sequenced from both sides with the dideoxynucleotide chain-termination method (34). The reaction mixtures for sequencing were electrophoresed at 45 W on a 5% long ranger gel. The gel was then dried and exposed on Fuji RX Film for 24 h at room temperature.

Table II. Oligonucleotide PCR Primers of F XIII A cDNA

Primer	Direction	Sequence*	Position‡
A 1	Sense	5′-AGAGCGAGCTCACGCGAGGGCACCT-3′	19–43
A 2	Antisense	5′-AGCTCAAGCTTCTGGTCTTGATGTC-3′	742–766
B 1	Sense	5′-AACCAGTCGACACCCAGAAACAGAC-3′	596–620
B 2	Antisense	5′-GTCCAAAGCTTGCCAGCCTCCAAAT-3′	1248–1272
C 1	Sense	5′-AACGGAATTCCAACTCACCA-3′	1158–1178
C 2	Antisense	5′-GATGGAAAGCTTGAAGTCTT-3′	1676–1695
D 1	Sense	5′-CCAACGTCGACATGGACTTTGAAG-3′	1630–1653
D 2	Antisense	5′-TTTGCTCTAGAATTTCCTTAGCCAAGA-3′	2326–2352

*‡ Primer sequences and positions are derived from reference 27.

Results

The propositus showed a complete absence of both functional and immunodetectable F XIII A in the plasma. The antigen level of F XIII B was reduced to 29% of the normal concentration. Therefore, the propositus was considered to have a homozygous deficiency of F XIII A. As shown in Fig. 1, it was suggested that one sibling (IV-7) had a homozygous deficiency. His mother (III-9), another sibling (IV-6), and three offspring (V-1, 2, 3) were all considered to be heterozygotes.

With Northern blot analysis, a 4-kb single band of F XIII A mRNA was detected in PBMC from the patient, identical in size to those from a normal PBMC and U 937 (monocytic leukemia cell line), as shown in Fig. 3. With Southern blot analysis, no major deletion or rearrangement of the F XIII A gene was found (data not shown).

The PCR products individually amplified from 14 exons (Exon II to XV) of the propositus were indistinguishable from those predicted from a normal gene sequence on a agarose gel (data not shown) (27).

Messenger RNA in PBMC from the propositus was amplified by the RT-PCR method. The lengths and restriction sites of four fragments (A, B, C, and D) were also almost identical to those predicted from normal individuals (data not shown) (27). These observations suggest that either a point mutation or a minor deletion/insertion was responsible for the deficiency.

PCR products of fragments A, B, C, and D were subcloned into pUC19, and the plasmids from two to three independent clones containing each fragment were sequenced. As shown in Fig. 4 *A*, a two-base (A and G) deletion within the AGAG sequence at the nucleotide position of 210 to 213 was found in the PCR fragment A. We could not distinguish whether AG or GA was removed from the AGAG repeat, which is the boundary of exon II and III. In either case, this deletion caused a frameshift mutation and made a stop codon (TAA) at six bases downstream from the deletion site (Fig. 4 *C*). In order to confirm this result, PCR products from exon III, including the adjacent introns, were subcloned into pUC19 and the plasmids from six independent clones were sequenced. In the sequence of the wild type, ---CATAGAGTTT--- is known to be at the boundary of intron B and exon III (27). However, ---CATAGTTT--- was found at this site in all six clones from the propositus, indicating a two-base (A and G) deletion within the AGAG repeat (Fig. 4 *B* and *C*). As with cDNA sequencing, it could not be determined which two nucleotides had been deleted, while three possibilities existed: the first AG, the central GA, and the last AG deletion within the AGAG repeat. In either case, the remaining AG becomes a splice acceptor site of intron B, leading to a deletion of AG at the 212 and 213 position in the mRNA from the propositus.

Analysis of the DNA sequences of exon III from two of the

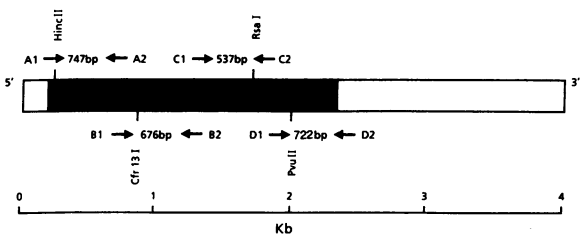

Figure 2. Strategy for PCR amplification and restriction maps. F XIII A cDNA is shown schematically. The location and direction of PCR primers A1, A2, B1, B2, C1, C2, D1, and D2 are indicated by arrows. Four fragments (A, B, C, and D) were amplified with each primer set. A solid box shows the amino acid coding regions of F XIII A cDNA. Open boxes show 5′ and 3′ nontranslated regions of F XIII A cDNA.

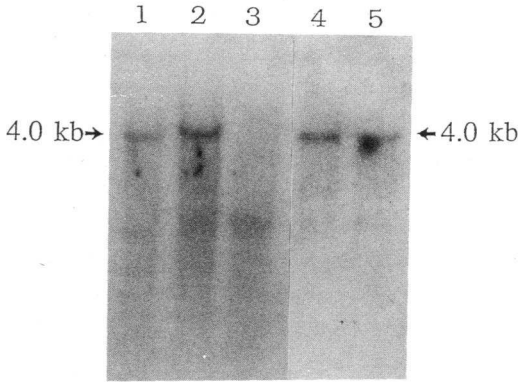

Figure 3. Northern blot analysis. Lane *1*, 10 μg of total RNA in PBMC from propositus; lane *2* and *5*, 10 μg of total RNA in cultured U 937 cells; lane *3*, 10 μg of total RNA in cultured HL 60 cells; lane *4*, 10 μg of total RNA in normal human PBMC. Blotted filters were hybridized by F XIII A cDNA radiolabeled with [α-^{32}P]dCTP. A 4-kb single band of F XIII A mRNA was detected in PBMC from propositus (lane *1*), identical in size to those from normal (lane *4*) and U 937 cells (lanes *2*, *5*).

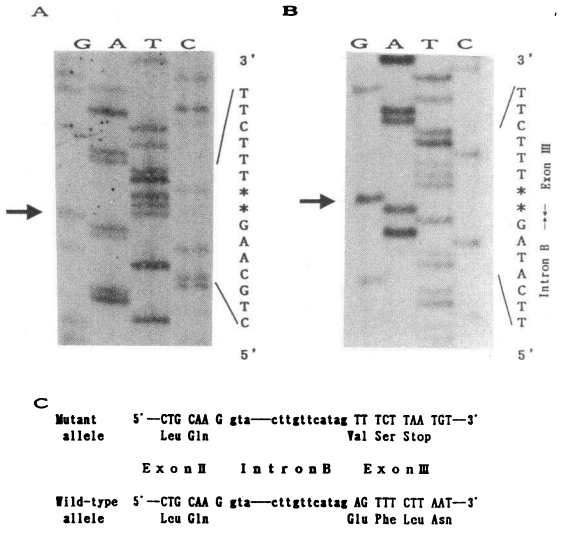

Figure 4. Sequencing of cDNA and the gene from the propositus. (*A*) Sequence analysis of fragment A in the cDNA is shown. * Indicates the deleted bases (AG) and the arrow shows position of the deletion. (*B*) Sequence analysis of exon III including the adjacent introns of the F XIII A gene of the propositus. A two-base deletion of A G at the 5′ end of exon III is shown. (*C*) Comparison of mutant and wild type allele. In the mutant allele, a two base deletion at the site of 212 A and 213 G was found while the stop codon (TAA) is made at six bases downstream from this deletion site. The sequence of the wild type was derived from reference 27. Large and small letters indicate the exon and intron, respectively.

patient's offspring (V-1 and 2) revealed the heterozygosity of the wild type and mutant alleles identical with that of the patient (Fig. 5).

Discussion

Hemostatic analysis of the propositus showed a typical deficiency of F XIII A. The family study revealed the presence of a congenital homozygous F XIII A deficiency in the propositus. The level of the plasma B subunit in this case was reduced to 29% of the normal concentration, as previously mentioned in most patients with F XIII A deficiency (18). The reason is not clear, but it is probably due to a decrease in synthesis and/or a rapid turnover of the B subunit under conditions of the absence of the A subunit in plasma.

According to the sequencing of the cDNA from the propositus, we found the two-base (AG) deletion within AGAG repeat at the position of 210 to 213. This site is the boundary of exon II and III. We next attempted to characterize the genomic sequence. Similarly, we identified the dinucleotide deletion in the intron B–exon III boundary of the F XIII A gene in the propositus. The deletion site in the gene also possesses an AGAG repeat that is the same as that of cDNA. By this deletion, the remaining AG sequence becomes a splice acceptor consensus of intron B, and the mature mRNA transcripted from this abnormal gene lacks the dinucleotide AG at the position of 212 and 213. In this abnormal mRNA, the termination codon TAA is newly made at six bases downstream from the deletion site, and then a short truncated protein, which does not contain an active site, 314 cysteine, of this enzyme (22–24, 27), is assumed to be translated, but unstable. Consequently, this abnormal gene is responsible for the deficiency of F XIII A in the family.

Transmission of the abnormal gene was proven by genomic sequencing in two offspring (V-1 and 2), indicating that they are heterozygotes with the wild type and this mutant.

Alteration at the splice acceptor consensus have been documented in the β-globin gene, the retinoblastoma gene, and the factor IX gene (35–38). In these cases, either a point mutation or a short deletion occurred in the region including the obligatory AG as a 3′ splice acceptor consensus in intron, leading to an elongation of the intron as well as exon skipping. Due to the incidental presence of the AGAG repeat at the intron B–exon III boundary of the F XIII A gene, a major deletion in mRNA was avoided in this case.

Previously, six sites of polymorphism were described in the levels of amino acid, cDNA and gene in F XIII A (22–24, 27). In our case, we found six sites, 77 Arg (CGC), 78 Arg (AGA), 88 Phe (TTC), 564 Pro (CCG), 650 Ile (ATT), and 651 Gln (CAG). No other polymorphisms were newly identified in this study.

This is the first report to characterize the genetic abnormality present in a case of a congenital F XIII A deficiency. As previously found with other genetic disorders, it is possible that the genetic abnormality in this disorder is not identical in all cases. Additional genetic analysis is required, and additional mutations will have to be further identified in this bleeding disorder. Such genetic analyses may ultimately be helpful in future gene therapy applications.

In conclusion, the F XIII A deficiency described in our patient was characterized by dinucleotide (AG) deletion at the 5′ end of exon III.

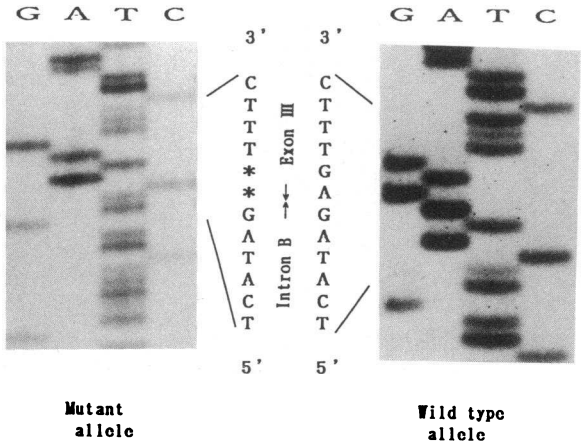

Figure 5. Sequence analysis of exon III and adjacent introns of F XIII A from V-1. Heterozygosity with wild type and mutant alleles is shown. Dinucleotide deletion is indicated by *.

Acknowledgments

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