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Disorders of Hemostasis

Factor XIII deficiency: new nonsense and deletion mutations in the human factor XIIIA gene

We identified five disease-causing mutations in six factor XIII deficient patients from four unrelated families: two novel nonsense mutations (nucleotide 979C→T corresponding to Arg326Stop; and nucleotide 2075G→A corresponding to Trp691 Stop), one novel deletion of a single nucleotide (nucleotide 708G or 709G), one previously reported missense mutation (nucleotide 888C→G corresponding to Ser295Arg), and a previously reported splice site mutation (nucleotide 319G→T at the last position of exon 3). The phenotypic consequences of these mutations are discussed.

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Coagulation factor XIII (FXIII) circulates as a tetramer of two FXIIIA and two FXIIIB subunits. The active catalytic FXIIIA' subunit covalently cross-links fibrin monomers in the final stages of blood clotting to enhance the structural integrity of the fibrin clot. FXIII deficiency results in a lifelong bleeding condition, problems with wound healing and spontaneous miscarriage in FXIII-deficient females.[1] Inherited FXIII deficiency arises from mutations in the FXIIIA or FXIIIB subunit.[2] Around fifty mutations are known in the FXIIIA subunit, and four in the FXIIIB subunit (see FXIII gene mutation databases at *http://www.hgmd.cf.ac.uk* and *http://www.med.unc.edu/isth/mutations-databases*).

Six patients, from four unrelated families, were studied. All families gave informed consent to the investigations. Patients 1, 2, 5 and 6 had each presented with bleeding from the umbilicus. Investigations revealed the absence of plasma FXIIIA in each individual. All patients are currently being treated with prophylactic FXIII concentrate (Fibrogammin). There have been no reported episodes of severe bleeding in any of the six patients since commencing therapy. All data are presented in Table 1.

Family A. Both patients 1 and 2 were homozygous for a CGA→TGA sequence change at codon 326 of the FXIIIA gene. The parents were each heterozygous for this mutation. To date, this is the second sequence change to be found at FXIIIA codon 326. The previously reported change was a CGA→CAA missense mutation corresponding to Arg326Gln.[3] The CGA→TGA change reported here is a nonsense mutation that would lead to early termination of mRNA translation. A truncated FXIIIA polypeptide that lacks most of the catalytic core domain, as well as all of barrels 1 and 2 of the molecule is predicted.[4] This truncated polypeptide is likely to be unstable.

Family B. Patients 3 and 4 were each homozygous for an AGC→AGG change at codon 295 of the FXIIIA gene. The parents in this family were each heterozygous for this mutation. This AGC→AGG mutation corresponds to a missense serine to arginine change at residue 295, which is located in the core domain of the FXIIIA molecule.[4] We have previously reported this mutation, and detailed its effects, in another FXIII deficient family.[5] Serine at position 295 is conserved in all known transglutaminases and a FXIII polypeptide containing Arg at position 295 is predicted to fold incorrectly and will probably be unstable.[5]

Family C. Patient 5 was found to be a compound heterozygote. A deletion of a single G nucleotide, segregating through the paternal line, at either nucleotide 708 or 709 (numbering with A of initiator ATG as +1) was identified. This single nucleotide deletion is the first to be reported in FXIIIA exon 6. Previously reported single nucleotide deletions have been in FXIIIA exons 2, 5 and 14.[3,6] The deletion described here results in a frame-shift whereby mRNA translation would terminate five codons later (^{234}ATC_^{235}CTG _ACA_CTT_GCC_TGT_ATG_TGA. This truncated polypeptide would lack most of the core domain as well as barrels 1 and 2, and is likely to be highly unstable. The second mutation in this patient, inherited through the maternal line, was a TGG→TAG change in codon 691 in FXIIIA exon 15. This is a nonsense mutation that would result in early termination of mRNA translation. The truncated FXIIIA polypeptide corresponding to this allele would lack only the final 41 residues, or the end of barrel 2 domain.[4] To date, the most terminal FXIIIA nonsense mutation reported is at codon 661, and this was found to result in a significant reduction in the steady-state FXIIIA mRNA level.[7]

Family D. Patient 6 carried a known heterozygous G→T mutation, inherited through the paternal line, at the last position of exon 3. Although mRNA was not available for analysis from this patient, we have previously demonstrated that this G→T mutation results in skipping of exon 3, with perfect splicing together of exons 2 and 4 during RNA splicing.[8] The truncated FXIIIA mRNA transcript, lacking 189bp corresponding to the exon 3 sequence, would produce a truncated polypeptide, with an in-frame deletion, lacking amino acid residues 43 to 105 inclusive. Despite analysis of all fifteen FXIIIA gene exons in both orientations, a second disease-causing mutation, segregating through the maternal line was not found in this family. We also analyzed over 1000bp of

Table 1. Clinical, genotype and phenotype data.

Family*	Origin	Patient	Sex	Age (yrs.)	Clinical symptoms	DNA Mutation†	Mutation Type	Effects of mutation
A‡	Pakistan	1	F	8	Prolonged umbilical stump bleeding, intracranial bleed. hematomas, deep vein thrombosis, repeated subduro-peritoneal shunt infections, persistent bleeding from cranial wound site	Homozygous CGA→TGA (codon 326 in *F13A* exon 8)	Nonsense	Early translation termination resulting in truncated FXIIIA polypeptide
		2	M	6	Prolonged umbilical stump bleeding		As for patient 1	
B§	Pakistan	3	F	20		Homozygous AGC→AGG (codon 295 in *F13A* exon 7)	Missense	Incorrect folding resulting in an unstable FXIIIA polypeptide
		4	F	17			As for patient 3	
C¶	UK	5	F	5	Persistent bleeding from umbilical stump	Heterozygous TGG→TAG (codon 691 in *F13A* exon 15)	Nonsense	Early translation termination resulting in truncated FXIIIA polypeptide
						Heterozygous deletion of 708G or 709G (in *F13A* exon 6)	Deletion	Frame-shift resulting in truncated FXIIIA polypeptide
D¶	UK	6	M	7	Persistent umbilical bleeding	Heterozygous 319G→T (last base of *F13A* exon 3)	Splicing	Exon 3 skipped during RNA splicing

**There is consanguinity in families A and B, with the parents being first cousins. †Genomic DNA was purified from peripheral blood using standard techniques. All fifteen exons of the FXIIIA gene (F13A) were amplified by the polymerase chain reaction (PCR) and PCR products were directly sequenced in both orientations as detailed previously.[9] Any mutations identified were confirmed, in both orientations, in new PCR products. F13A gene exons that appeared to carry minor deletions and/or insertions were further analyzed by TA-cloning. PCR products were subcloned into pGEM-T vector (Promega). Following ligation and transformation into competent bacteria DH5· cells (Promega), recombinant clones were selected and plasmid DNA was purified using the Wizard miniprep kit (Promega). Plasmid clones were sequenced as before. RNA was not available for analysis from any of the patients, or their family members. ‡Family A: The case history of patient 1 has been described previously.[10] The diagnosis of FXIIIA deficiency in this patient was delayed due to an unusual clinical course, and this individual was eventually diagnosed as FXIII deficient at the age of 3.5 years. Her brother (patient 2) was diagnosed with FXIII deficiency at the age of 11 months. Both patients 1 and 2 have since been maintained on prophylactic FXIII supplementation. §Family B: Patients 3 and 4, also have an elder sister (aged 21 years) who is phenotypically normal. This individual is homozygous normal (AGC) at the F13A codon 295 gene locus. ¶Family C: Patient 5 presented at 7 days of age with persistent bleeding from the umbilical stump, 24 hours after the residual umbilical cord separated. She was admitted to the intensive care unit, for 7 days, following severe blood loss for volume and blood product replacement. Bleeding was resolved by suturing umbilical vessels. The PT, APTT and factors VIII, IX and XI levels were within normal limits. Fibrinogen level was normal, but the clot solubility test was abnormal. Plasma FXIIIA analysis revealed absence of FXIIIA subunits. She has since been maintained on prophylactic factor XIII infusions and has suffered no significant bleeding episodes. ¶Family D: Patient 6 developed persistent umbilical bleeding at 5 days of age. Investigations revealed normal PT and APTT, but the absence of plasma FXIII activity. He was treated with factor XIII concentrate. Since receiving prophylaxis from early infancy, this patient has not had any further bleeding symptoms. This patient is also homozygous for a previously reported silent CCA→CCC sequence change at codon 331 in exon 8 of the F13A gene.[8]*

sequence upstream of FXIIIA exon 1 in genomic DNA from this patient and his parents, in the event that a promoter mutation may affect expression levels from the FXIIIA maternal allele. No sequence changes were identified in the FXIIIA promoter region in this family. The second disease-causing mutation in this family may be a large deletion that would be most effectively studied through mRNA analysis.

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