

Prenatal diagnosis in factor XIII-A deficiency

Caroline J Killick, Carol J Barton, Shazia Aslam, Graham Standen

Abstract

Congenital factor XIII deficiency is a severe bleeding disorder that is inherited as an autosomal recessive trait. The condition is commonly due to absence of the factor XIII-A subunit protein in the plasma. The case of a baby is reported who showed typical clinical features of factor XIII-A deficiency, including recurrent bleeding from the umbilical stump and a life threatening haemorrhage after circumcision. Family studies were performed and molecular analysis, using a Short Tandem Repeat (STR) marker closely linked to the A subunit gene, allowed antenatal exclusion diagnosis to be undertaken in a subsequent pregnancy. The case highlights the importance of seeking a family history of bleeding disorders before surgery in the neonatal period, particularly if the parents are consanguineous.

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Congenital factor XIII deficiency is a rare inherited bleeding disorder that commonly occurs in neonates.¹ We report a case of a baby with this disorder who developed a large sub-aponeurotic haemorrhage when 2 days old, with recurrent bleeding from the umbilical stump and a life-threatening haemorrhage after a circumcision on day 28. After the coagulation defect had been diagnosed, genetic studies were undertaken which ensured that a successful prenatal diagnosis was performed in a subsequent pregnancy.

Case history

A baby was born at 39 weeks of gestation to a 21 year old primigravid woman of Pakistani origin. The parents were second cousins. One of the mother's siblings had died in infancy as a result of superficial haemorrhaging from the penis following circumcision. Two further cousins were receiving treatment for factor XIII deficiency. There was also a family history of congenital adrenal hyperplasia.

The infant was delivered by spontaneous vaginal delivery and had received 500 mcg of intramuscular vitamin K immediately after birth. At 24 hours of age he had a large sub-aponeurotic haematoma which had extended by day 3 to involve much of the left side of the face. The blood picture showed that haemoglobin was 95 g/l, with a platelet count of $170 \times 10^9/l$; International Normalised Ratio (INR) 1.0, APTR 1.04. An x-ray of the skull showed no evidence of fracture and a cranial ultrasound scan revealed no intracranial haem-

orrhage. The infant received a blood transfusion and a second dose of vitamin K 500 mcg. He was discharged home at 6 days of age.

At 18 days of age, he was referred with umbilical bleeding, thought to be due to an umbilical granuloma. His clotting studies were again normal. At 28 days a circumcision was performed by a General Practitioner. Over the next 48 hours the wound intermittently haemorrhaged, the clot separating each time the dressings were changed. On admission at 30 days old, physical examination revealed a pale, well grown infant with a tachycardia of 160 beats per minute. No other abnormalities were detected. Investigations showed that his haemoglobin was 40 g/l, INR 1.18, and APTR 1.33. Clotting assays revealed a factor VIII value of 2130 U/l and a factor IX concentration of 390 U/l (both normal for age). Initial testing using the 1% monochloroacetic acid test for clot stability suggested factor XIII deficiency. A factor XIII-A activity of 4.5% was later confirmed by chromogenic assay (normal neonatal range: 50-60%). The activity detected was probably due to transfusion in the neonatal period. The mother's factor XIII-A value was 66% and the father's 69% (normal range in adults: 70-140%). The infant's cranial ultrasound appearances remained normal. He received a further blood transfusion and monthly prophylactic treatment with factor XIII concentrate. This has successfully prevented further haemorrhaging and his development to date is normal.

Molecular genetic studies were performed in this family to allow first trimester antenatal exclusion diagnosis to be undertaken in a subsequent pregnancy. A highly polymorphic Short Tandem Repeat (STR) sequence, HUMF13A01 (AAAG)_n, in the immediate 5' untranslated region of the A subunit gene, was detected using polymerase chain reaction (PCR).²

Leucocyte DNA was isolated from the parents and the proband; a chorionic villus sample taken at 11 weeks was used as a source of fetal DNA. The oligonucleotide primers described by Polymeropoulos and coworkers³ were used: sense strand 5' GAGGT TGCCTC-CAGCCTTT 3' and antisense strand 5' AT-GCCATGCAGATTAGAAA 3'. The PCR conditions used were denaturation 94°C for 1 minute, annealing at 50°C for 1 minute, and primer extension at 73°C for 1 minute. After 30 cycles of amplification and a final extension step of 73°C for 10 minutes, the products were run on a 6% polyacrylamide gel (1 × TBE buffer) and visualised under ultraviolet light after staining with ethidium bromide.

The results of the analysis are shown in fig 1. The father (I₁) is homozygous L1/L1 for the

Department of
Paediatrics,
Royal Berkshire
Hospital,
Reading,
Berkshire RG1 5AN
C J Killick

Department of
Haematology
C J Barton

Molecular
Haematology Unit,
Bristol Royal
Infirmary,
Bristol BS2 8HW
S Aslam
G R Standen

Correspondence to:
Dr G R Standen.

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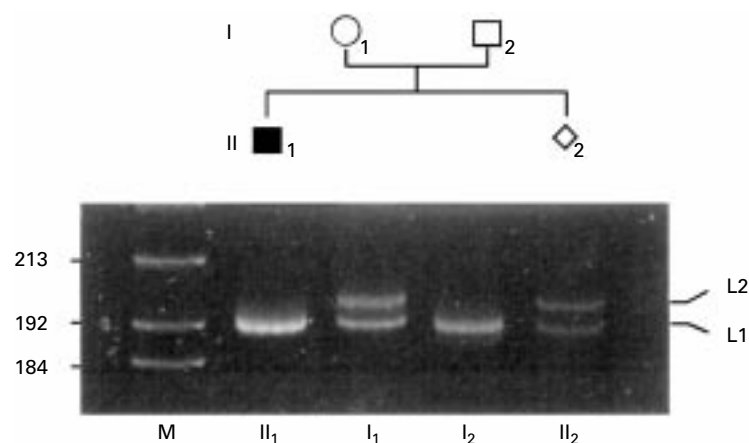


Figure 1 Antenatal exclusion diagnosis of factor XIII-A deficiency using the STR sequence HUMF13A01. M: molecular size markers (base pair) pBR322 digested with *Hae*III.

STR and the mother (I₁) is heterozygous L1/L2. The affected proband (II₁) is L1/L1 whereas the fetus (II₂) is L1/L2—meaning that the mother's normal allele has been inherited and the fetus is unaffected. A healthy boy was delivered at term and a normal factor XIII-A value of 94% was confirmed.

Discussion

Factor XIII deficiency is a severe bleeding disorder associated with impaired wound healing and an increased risk of spontaneous abortion in women.¹ Twenty seven patients are currently registered with the haemophilia service in the United Kingdom, indicating a prevalence of about one case per two million of the population. Eighty per cent of affected infants present with recurrent bleeding from the umbilical stump. In the absence of replacement therapy, patients in later life frequently sustain superficial bruising, epistaxes, haematoma in muscles and joints, and intracranial haemorrhage. However, lifelong prophylactic treatment with plasma derived factor XIII concentrate prevents haemorrhagic complications. The half life of factor XIII is around 10 days and the concentrate is administered on a monthly basis at a dose of 10–15 units/kg.

Factor XIII circulates in plasma as a heterotetramer of two catalytic A subunits and two B subunits.⁴ Following activation by thrombin, the dimers disassociate and the A subunit catalyses the formation of covalent bonds between fibrin monomers and also between fibrin and other proteins including collagen, fibronectin, and α_2 plasmin inhibitor. This cross linking generates a clot of high tensile strength which is more resistant to fibrinolysis.

In factor XIII deficiency the A subunit protein is usually absent and the B subunit deficiency has only been described occasionally.⁵ The A subunit gene is located on chromosome 6 at p24-25, contains 15 exons, and encodes a protein of 731 amino acids.⁶ Ideally, for early prenatal diagnosis, the specific mutation in an affected family should be identified. However, recent molecular studies in these patients indicate that it is genetically heterogeneous and in

most cases the mutation is family specific.⁷ Missense mutations are most frequently detected with most amino acid substitutions located in the highly conserved catalytic core domain of the A subunit protein.⁸ Other defects, including microdeletions, frameshift, and nonsense mutations, are randomly distributed through the genomic sequence.^{9,10} If the location of the specific mutation is unknown, we have shown that the closely linked polymorphic marker HUMF13A01 can provide a valuable tool for gene tracking and accurate prenatal diagnosis. The size of this (AAAG)_n tetra nucleotide repeat sequence can be rapidly and simply determined using PCR amplification. Previous studies have shown that the heterozygosity rate is around 75% in several ethnic groups and because it is located in the immediate 5' region of the gene, the risk of meiotic recombination is negligible. Kangsadalampai *et al.*¹¹ have recently studied the segregation of the STR alleles in seven families with A subunit deficiency and showed that, in all cases, the inheritance of the mutated allele could be unequivocally linked to the STR marker.

This case highlights the importance of seeking a medical and family history of bleeding disorder in neonates before surgery. Haemophilia A and B are the commonest inherited coagulation disorders which may present with a severe haemorrhagic tendency in early life. However, rare autosomal recessive conditions including factor XIII deficiency, type III von Willebrand disease, and congenital afibrinogenaemia may also be encountered, particularly in the offspring of consanguineous parents.

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