PRENATAL DIAGNOSIS OF HAEMOPHILIA BM

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Summary   Accurate mid-trimester prenatal diagnosis was achieved
in a Japanese haemophilia BM family.   At 21 weeks of gestation, liver
blood from the male fetus at 50% risk was sampled using a needle guided
by ultrasound.   F.IX (F.IX:C and F.IX:Ag) and ox-brain PT in the fetal
plasma were low and prolonged, but within the normal range of controls
at mid-trimester.   Pregnancy went to term and woman gave birth to an
unaffected male.   Haemophilia BM can be diagnosed in the mid-trimester
of pregnancy by determination of F.IX:C, F.IX:Ag and ox-brain PT in
pure fetal plasma.
Key Words prenatal diagnosis, haemophilia BM, factor IX, ox-brain
prothrombin time, fetal blood sampling

INTRODUCTION

First-trimester prenatal diagnosis of haemophilia B is now possible by analyz-
ing directly DNA in chorionic villi at 8–10 weeks of gestation (Williamson *et al.*,
1981) using restriction fragment length polymorphisms (RFLPs) associated with
the factor IX gene (Graham *et al.*, 1985).   This technique, however, can not be
applied to Japanese haemophilia B relatives, because no RFLPs of the factor IX

gene have been found in normal Japanese DNA samples when using intragenic F.IX probes (Kojima *et al.*, 1987; Mikami *et al.*, 1987).

Another technique of prenatal diagnosis even in Japanese haemophilia B families is determination of the factor IX level in plasma obtained from the fetus at 18–22 weeks of gestation (Mibashan *et al.*, 1979; Holmberg *et al.*, 1980). However, the factor IX antigen (F.IX:Ag) level assayed by current immunological methods is not applicable to fetal diagnosis of haemophilia B$^+$ genetic types characterized by the presence of cross-reacting material (CRM$^+$) (Holmberg and Ljung, 1982).

We describe here the prenatal diagnosis of Japanese haemophilia BM, a haemophilia B$^+$ variant having an abnormal reaction with ox-brain thromboplastin (Hougie and Towmey, 1967). This is the first reported case of prenatal diagnosis of haemophilia BM.

METHODS

Assays of F.IX. Factor IX clotting activity (F.IX:C) was assayed as kaolin-activated partial thromboplastin time (K-PTT) in immuno-depleted F.IX deficient plasma (Nishimura *et al.*, 1986) using 3A6 anti-FIX monoclonal antibody (Yoshioka *et al.*, 1985). Ox-brain prothrombin time (PT) was determined by the method of Quick *et al.* (1935). Laurell's electroimmunoassay (EIA) for factor IX antigen (F.IX:Ag) was performed using a rabbit antibody (Fukui *et al.*, 1981). A two-site solid phase enzyme-linked immunosorbent assay (ELISA) was developed using monoclonal antibody 3A6 to quantitate F.IX:Ag (Yoshioka *et al.*, 1985).

Normal pooled-plasma obtained from 10 normal donors was used as reference material. One unit is the amount of F.IX in 1 ml normal pooled-plasma.

Controls of fetal plasma. Control values of plasma F.IX:C, F.IX:Ag and ox-brain PT were determined in pure fetal blood (19–20 weeks of gestation) from 10 control fetuses, including 3 male fetuses at 50% risk for haemophilia A, aborted for therapeutic reasons because of other genetic disorders.

Case. The propositus, a 3-year-old boy, had easy bruising and epistaxis since his infant period. He had an overt haemorrhage from hard palate after a small injury at the age of 10 months, when a diagnosis of haemophilia B (F.IX:C 5 u/dl) was made at Dokkyo Medical College. At the age of 3 years, haemostatic findings in the patient was re-evaluated at the Department of Paediatrics, Nara Medical College, and the diagnosis of haemophilia BM (Kiryu) was established (Yoshioka *et al.*, 1987) (Table 1).

The 29-year-old mother of the propositus was considered to be a carrier due to low level of F.IX:C (30–40 u/dl) at the same time when the propositus was diagnosed as having haemophilia B. She became pregnant (7 weeks of gestation) and was unwilling to bear a haemophilic son. She visited a genetic counsellor (Dr. A. Matsui) for prenatal diagnosis of the fetus.

The level of F.IX:C and F.IX:Ag in the woman (11 weeks) was 90 u/dl and

Table 1. Haemostatic findings of the propositus and his mother.

	F.IX:C (u/dl)	F.IX:Ag (u/dl)	Ox-brain PT (sec)
Propositus (Haemophilia BM (Kiryu))	2. 5	100	126 (57)[a]
Mother of the propositus			
at non-pregnant state	30-40	ND[b]	ND[b]
at 11 weeks of pregnancy	90	100	79 (57)
6 months after delivery	58	100	72 (53)

[a] ( ), normal control. [b] ND, not determined. Normal range of F.IX:C and F.IX:Ag in normal subjects is 50-200 u/dl.

100 u/dl, respectively. Ox-brain PT was moderately prolonged (Table 1).

Prior to an amniocentesis, Southern bot analysis of the F.IX gene in the family (the propositus and his parents) was carried out (Mikami *et al.*, 1987) using F.IX cDNA (cVII) kindly provided by Dr. G.G. Brownlee, Oxford, UK, and several kinds of endonucleases. Unfortunately, the F.IX RFLPs pattern in the family was not informative.

Then fetal sexing by amniocentesis was performed at 16 weeks of gestation. Chromosome analysis of the cultured fetal fibroblast in the amniotic fluid revealed a male karyotype, 46XY.

Subsequently, fetal blood sampling was also performed at 21 weeks of gestation. Pure fetal blood from an intrahepatic vessel was obtained using a percutaneous transhepatic cholangiography (PTC) needle (15 cm long and 23 gauge) under ultrasound guidance (Koresawa *et al.*, 1987).

RESULTS

Control values of fetal plasma F.IX:C, FIX:Ag and ox-brain PT were determined (Table 2). The significant positive correlation between the levels of F.IX:C and F.IX:Ag supported the validity of the bioassays of these samples. By ultrasound guided percutaneous fetal sampling, a total of 1.5 ml of anti-coagulated (1/10 volume of 3.8% sodium citrate) blood was obtained from the liver. The first 0.5 ml of the whole blood (Sample 1) was over-diluted with anticoagulant and/or amniotic fluid, and had 15% hematocrit (Hct). Sample 2 (1.0 ml) had 35% Hct, which was in the expected range. Sample 1 was analyzed with a combined cell counter and cell size analyzer (Coulter Chanalizer), and found to be free of maternal blood contamination. Because, clear distinction between red cell size distribution curves of fetal blood (MCV 129.9 fl) and maternal control (MCV 94.3 fl) was obtained.

Both F.IX:C and F.IX:Ag in the fetal plasma (Sample 2) were low (3.6 u/dl and 4.8 u/dl, respectively) but remained within the normal range of controls at mid-trimester. Ox-brain PT was within the normal range, whereas ox-brain PT in the propositus was remarkably prolonged.

Table 2.   Haemostatic findings of the fetal plasma.

	Hct (%)	F.IX:C (u/dl)	F.IX:Ag (u/dl)	Ox-brain PT (sec)
Fetal plasma of this case				
Sample 1	15	18.0	2.0	74 (31.5)[a]
Sample 2	35	3.6	4.8	71 (31.5)
Control fetal plasms (n=10)				
Mean	35	6.8	6.2	73.2
Range	33–37	3.5–12.0	3.0–9.5	62–89 (45–57)

[a] ( ), normal control of ox-brain PT.

We speculated that the fetus did not have haemophilia BM because of the normal level of F.IX and normal ox-brain PT.   The woman decided to go to term. At full-term she gave birth to a male whose F.IX:C and F.IX:Ag in the cord were 55 u/dl and 30 u/dl, respectively.   At 10 hr and at 6 months after birth, plasma F.IX and ox-brain PT in the baby was redetermined, and it was confirmed that he did not have haemophilia B.

DISCUSSION

Since haemophilia BM is characterized by CRM+ and prolonged ox-brain PT, the determination of F.IX:Ag alone in the fetal plasma is meaningless.   Moreover, evaluation of the results of F.IX assay and ox-brain PT may be hampered by con-tamination of the sample with maternal blood and/or amniotic fluid, which contains F.IX:Ag and thromboplastin-like material (Holmberg *et al.*, 1980; Bertina *et al.*, 1981; Thompson, 1984).

Table 2 shows the reference values of F.IX:C and F.IX:Ag in mid-trimester in our laboratory.   The mean value was 6.8 u/dl with a range of 3.5–12.0 u/dl and 6.2 u/dl with a range of 3.0–9.5 u/dl, respectively.   These data are similar to those reported by several authors (Mibashan *et al.*, 1979; Holmberg *et al.*, 1980; Terwiel *et al.*, 1980; Bertina *et al.*, 1981; Thompson, 1984).   We successfully obtained 1.5 ml of pure fetal blood by ultrasound guided percutaneous liver sampling using a 23G PTC needle.   Since Sample 1 (Hct 15%) was diluted with anticoagulant and/or amniotic fluid, F.IX:C in the fetal plasma was thought to be estimated higher than it was.   Both F.IX:C and F.IX:Ag in Sample 2, of which Hct (35%) was in the expected range, were low but within normal range.   We considered that F.IX:C in Sample 2 should be below 1 u/dl in case the fetus was affected.   Moreover, ox-brain PT was within the normal range determined in our laboratory.   We speculated that this moderate prolongation of the ox-brain PT in the fetus was due to low levels of factors II, VII and X in the mid trimester but not from abnormal F.IX (Terwiel *et al.*, 1980; Forestier *et al.*, 1985).   From these results, we speculated the fetus did not have haemophilia BM.

The pregnancy went to term uneventfully. The coagulation study confirmed that the newborn infant did not have haemophilia B. Neither any mark of cutaneous puncture nor subcutaneous haematoma was seen. The results of ultrasound tomography of the liver and blood chemistry were normal.

When we started fetal blood sampling through fetal liver, the most popular method of fetal blood sampling had been fetoscopy. When the attempt was failed by fetoscopy, however, we offered both parents another possibility of fetal blood sampling by liver puncture. Since it was the first our experience of this sort of approach, the operational risk could not be estimated. With informed consent and parents' request for fetal blood sampling in spite of its unknown risk, the liver puncture was performed (Koresawa et al., 1987).

Fetoscopic blood sampling did not show prolonged bleeding in utero even if the fetus is affected by haemophilia (Mibashan et al., 1979). The fact suggested that liver puncture would not cause extra haemorrhage in utero. Thereafter, we have detected no prolonged haemorrhage caused by fetal liver puncture even in affected cases (Koresawa et al., 1987).

Prenatal diagnosis of haemophilia B or even haemophilia BM is possible by determinations of F.IX:C, F.IX:Ag and ox-brain PT in pure fetal plasma obtained by percutaneous liver sampling. This is the first report on the prenatal diagnosis in a Japanese haemophilia B family.

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