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Circulating cell-free fetal DNA in maternal serum appears to originate from cyto- and syncytio-trophoblastic cells. Case report

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Circulating cell-free fetal DNA in maternal serum offers an early and non-invasive method for prenatal diagnosis, but the origin of this DNA is still unknown. We report the absence of the *SRY* gene in maternal serum of a pregnant woman despite male genitalia at ultrasound. The karyotype was 45,X after direct trophoblast analysis and 45,X/46,Xidic(Yp) after culture and in all fetal tissues studied. Due to the absence of the *SRY* sequence in maternal blood and in the cytotrophoblast, we presume that free fetal DNA in this case originates from trophoblastic cells. As the case presented here is exceptional, it only has a minor impact on the accuracy of fetal sex determination by maternal serum analysis, but highlights the importance of and the necessity for the complementary ultrasonographic control.

Key words: fetal DNA/maternal serum/trophoblastic cells/case report

Introduction

Since its first demonstration (Lo *et al*., 1997), cell-free fetal DNA is known to be present in blood of pregnant women, but the source of this DNA remains unclear. It has been suggested recently that cell types other than fetal erythroblasts are responsible for the release of fetal DNA into the maternal circulation (Zhong *et al*., 2002). Furthermore, many reports strongly suggest that placental cells are the main source of cell-free fetal DNA in maternal serum. However, there is no direct proof to date to support this hypothesis (Guibert *et al*., 2003).

Case report

In the case reported here, a pregnant woman carrier of severe haemophilia A was referred for prenatal diagnosis. As fetal sex determination can be achieved efficiently by maternal serum analysis early in pregnancy (Costa *et al*., 2001), this new strategy (Costa *et al*., 2002) was proposed to the parents who gave written informed consent for the procedure. Given that no *SRY* gene was detected in the maternal serum at 13 weeks' gestation, the fetus was identified as probably female. However, 3 weeks later, ultrasonographic examination revealed male genitalia, and chorionic villus sampling (CVS) was performed. Direct chromosomal analysis showed a 45,X karyotype on all the 100 analysed mitoses whereas, following CVS culture, a 45,X (51 mitoses)/46,XY (11 mitoses) mosaic karyotype with a non-fluorescent Y chromosome was identified. After fluorescent *in situ* hybridization (FISH) using a specific *SRY* probe, the abnormal Y was identified as an isodicentric (Yp) chromosome. The parents were informed that the fetus in all likelihood was affected by haemophilia. They opted for termination of pregnancy (TOP). A second maternal serum sample was tested before TOP, and the absence of the *SRY* sequence was confirmed. However, the *SRY* DNA sequence was detected in fetal blood, amniotic cells and chorionic villi obtained following TOP, and a technical problem could thus be eliminated. Fetal karyotypes established on *in situ* cultured amniocytes and blood lymphocytes confirmed the 45,X/46,Xidic(Yp) mosaicism, which was more complex in blood, with up to six cell lines observed (Table I). Fetal examination confirmed that genitalia were unambiguously male.

The discrepancy between cytotrophoblast direct analysis (finding a 45,X karyotype only) and the other fetal tissues examined [showing mosaicisms including an isodic(Yp) cell line] can be explained by the embryological derivation of fetal and placental tissues. In the zygote, the inner cell mass (ICM) forming the extraembryonic mesenchyme (analysed by cultured chorionic villi), the embryo and the amnion is distinguished early from the trophoblast which results in the syncytiotrophoblast and cytotrophoblast (analysed by direct analysis). The mosaicism observed can be explained by two successive events (Kalousek *et al*., 1992): first a confined placental mosaicism due to early anaphase lag in a diploid

Table I. Results of chromosome and FISH analyses on *in situ* cultured amniotic cells and fetal blood lymphocytes

Cultured amniotic cells	
Karyotype (45 clones)	45,X (31 clones)/46,Xder(Y) (13 clones)/47,Xder(Y),+der(Y) (1 clone)
Fetal blood lymphocytes	
Karyotype (50 mitoses)	46,Xder(Y) (31)/45,X (15)/47,Xder(Y),+mar(Y)(2)/46,Xmar(Y) (1)/47,Xmar(Y),+mar(Y) (1)
FISH (160 mitoses)	46,Xidic(Yp) (114)/45,X (26)/46,Xmar(Y) (8)/47,Xidic(Yp),+mar(Y) (8)/47,Xidic(Yp),+idic(Yp) (3)/48,Xidic(Yp),+idic(Yp),+mar(Y) (1)

Der(Y) has been identified as an isodicentric (Yp) [idic(Yp)] and mar(Y) (derived Y marker) by FISH with SRY, DYZ3 and Tel Yp probes.

zygote carrying an idic(Yp) leads to a cytotrophoblast non-mosaic 45,X karyotype; secondly, a 45,X/46,Xidic(Yp) mosaicism occurred in all tissues derived from the ICM due to the unstable nature of the abnormal Y chromosome. As Y chromosome material was present in all fetal tissues examined but not in cytotrophoblastic cells and in maternal serum, it may be hypothesized that circulating non-cellular fetal DNA mainly originates from cytotrophoblastic cells. These cells might be discharged in the maternal circulation, trapped by her lungs and degraded by alveolar macrophages, or trophoblast might actively release DNA into maternal blood.

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