Non-invasive exclusion of fetal aneuploidy in an at-risk couple with a balanced translocation

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A pregnant woman who was a carrier for a balanced chromosome translocation [46,XX, t(1;6) (p31;q14)] and who had had six miscarriages, declined invasive testing but agreed to non-invasive prenatal diagnosis by analysis of fetal cells in maternal blood. Monoconal antibody (Mab) against the zeta (z) and gamma (γ) chains of embryonic and fetal haemoglobin were used to identify fetal nucleated erythrocytes (FNRBC). There were no FNRBC detected at 7 weeks, one anti-z-positive FNRBC was detected at 11 weeks, and 12 anti-γ-positive FNRBC were detected at 20 weeks. Fluorescent in-situ hybridization was performed using probes for chromosomes X, Y, 1 and 6 to identify fetal gender and the presence of an unbalanced chromosomal translocation. A tentative prenatal diagnosis was made of a female fetus disomic for chromosomes 1 and 6. A female infant with a 46,XX karyotype was born at term. This is the first attempt of exclusion of a chromosome translocation using fetal cells isolated from maternal blood. There is an advantage of using fetal cells isolated from maternal blood for non-invasive prenatal diagnosis in couples who have a history of multiple miscarriages due to a parental translocation, and who decline invasive testing in a pregnancy that continues to the second trimester.

Key words: chromosomal translocation/fetal cells in maternal blood/fetal nucleated erythrocytes/FISH/non-invasive prenatal genetic diagnosis

Introduction

Reciprocal translocations are the most common form of chromosome abnormality, occurring in ~1/500 live births (Jacobs, 1981). Although the carriers of these translocations are nearly always phenotypically normal since no loss of genetic material is involved, they have an increased risk of infertility, recurrent pregnancy loss, and chromosomally unbalanced offspring (Scriven et al., 1998).

The standard prenatal recommendation for the detection of unbalanced translocations in translocation carriers is in a fetus by amniocentesis or chorionic villous sampling (CVS). Both amniocentesis and CVS are invasive procedures, with a 0.5–1% respective incidence of miscarriage (Goldberg, 1997), a 1.5–2.4/10 000 live birth incidence of limb defects as a result of CVS (Firth, 1997), and a 1.7% incidence of club foot as a result of early amniocentesis (Sundberg et al., 1997). Many couples, in which one member carries a balanced translocation, have been ascertained through a history of multiple miscarriages. When a pregnancy continues past the first trimester, frequently couples are unwilling to undertake these procedures because of the associated risks. An alternative is preimplantation genetic diagnosis (PGD) (Conn et al., 1998). However, this involves prior in-vitro fertilization (IVF). For couples who are unwilling to undergo IVF and who decline invasive procedures, fetal aneuploidy diagnosis through the analysis of fetal cells in maternal blood is a potential option.

The frequency of fetal cells in the circulation of pregnant women is very low, with an average of 1.2 cells/ml maternal blood as detected by a quantitative polymerase chain reaction technique (Bianchi et al., 1997). Because of this, many different anti-fetal cell antibodies have been used to identify fetal cells in maternal blood, and a variety of enrichment techniques have been used to separate fetal from maternal cells (reviewed in Bianchi, 1999). Fetal nucleated erythrocytes (FNRBC) have been successfully used to diagnose fetal gender, aneuploidy, and single gene disorders (Bianchi et al., 1990; Elias et al., 1992; Ganshirt-Ahlert et al., 1993; Sekizawa et al., 1996).

This study was designed to attempt the first non-invasive diagnosis of an unbalanced chromosomal translocation in a fetus from a mother with a known chromosomal translocation, 46,XX,t(1;6)(p31;q14), and a history of six miscarriages, including one following IVF, who declined invasive prenatal diagnosis after a pregnancy that was conceived naturally, but agreed to participate in fetal cell research.

Materials and methods

Samples

The study subject was a G7P0Sab6 healthy female with a known 46,XX,t(1;6)(p31;q14) karyotype. Peripheral blood samples (10–20 ml) were obtained at 7, 11 and 20 weeks of gestation with informed consent from the patient. Samples were collected into glass tubes containing acid–citrate–dextrose solution A (ACDA) (Becton-Dickinson, Rutherford, NJ, USA) and processed immediately.

FNRBC preparation

FNRBC were prepared according to a previously optimized protocol for fluorescence-activated cell sorting (FACS) (DeMaria et al., 1996).
The blood was diluted 1:2 with Ca$^{2+}$- and Mg$^{2+}$-free Dulbecco’s phosphate-buffered saline (PBS) (Sigma Chemical Co., St Louis, MO, USA). Diluted blood was overlaid onto a single density gradient of Histopaque 1.083 and centrifuged at 400 g for 30 min at room temperature. The mononuclear cell layer was isolated from the interface of Histopaque with diluted blood. The isolated mononuclear cells (MNC) were fixed with 4% of ultrapure formaldehyde (Biosciences Inc.) in PBS at 37°C for 60 min, and permeabilized with cold methanol–acetone solution (1:1 ratio) at 4°C for 2 h. The MNC were then stained with either anti-z-Mab conjugated with

Figure 1.

Figure 2.
fluorescein isothiocyanate (anti-z-FITC) (Isolabs, Akron, OH) for samples from 7 and 11 weeks or anti-γ-Mab conjugated with phycoerythrin (anti-γ-PE) (Cortex Biochem, San Leandro, CA, USA) for the sample at 20 weeks at 4°C for 30 min. The MNC were then counterstained with Hoechst 33342. Nucleated anti-z-FITC or anti-γ-PE positive cells were flow-sorted onto microscope slides coated with 1% BSA and analysed using a Zeiss fluorescence microscope. The location of FNRBC on the microscope slide was recorded (Figure 1a and d).

Metaphase chromosome preparation
Metaphase chromosomes were prepared from maternal blood and newborn umbilical cord blood using standard techniques to identify the presence or absence of the translocation. Chromosome specific probes for X, Y, 1 and 6 were first tested on prepared maternal metaphase chromosomes and the maternal chromosomal translocation was confirmed.

Fluorescence in-situ hybridization
Poly-FISH (Zhen et al., 1998) was performed on both the isolated FNRBC and metaphase chromosomes to identify fetal gender and the number of copies of chromosomes 1 and 6. The slides were first hybridized to directly labelled X (DXZ1) and Y (PHY10) probes to identify the gender of the fetus. The slides were then denatured to remove previously hybridized probes and rehybridized to indirectly labelled 1p36 (D1Z2) and 6cen (D6Z1) probes (Oncor) to identify the presence or absence of the unbalanced chromosomal translocation.

Results
Interphase analysis of fetal cells in the maternal blood
There were no anti-z-FITC positive FNRBC detected in the 7 week sample. There was 1 anti-z-FITC positive FNRBC detected at 11 weeks (Figure 1a), and 12 anti-γ-PE positive FNRBC were detected at 20 weeks (Figure 1d). First round FISH was performed using probes for chromosomes X and Y, and all the antibody positive cells showed two copies of X (Figure 1b and e). Second round FISH was performed using probes for the short arm of chromosome 1 and for the centromere of chromosome 6, and all the antibody positive cells showed two copies of each signal (Figure 1c and f). Two of the anti-γ-PE positive cells were lost after the first round of FISH.

Metaphase analysis of maternal and newborn lymphocytes
Metaphase analysis of maternal and newborn lymphocytes confirmed the presence of a maternal translocation between 1 and 6 (Figure 2a), and showed that the newborn’s chromosomes had no translocation (Figure 2c). Second round FISH was performed using probes for chromosomes X and Y, and showed two copies of X signals on both maternal and newborn metaphase chromosomes (Figure 2b and d).

Discussion
Traditional cytogenetics, e.g. analysis of fetal metaphase chromosomes obtained by amniocentesis or CVS, has been the most common strategy to accurately diagnose chromosomal translocations prenatally. Recent advances in molecular cytogenetic techniques (Luke and Shepelsky, 1998), such as whole chromosome painting, reverse-chromosome painting technique, and multicolour FISH, combined with traditional cytogenetics have made it possible to diagnose very subtle chromosome structural abnormalities that might be missed by traditional cytogenetics. Detection of chromosomal translocations in an interphase nucleus, however, remains a diagnostic challenge. FISH detection of translocations in an interphase nucleus has been successfully performed in tumours (Kluin and Schuuring, 1997) and in PGD (Munné et al., 1998; Conn et al., 1999), but to date, there have been no cases where attempts to prenatally diagnose a chromosome translocation using FNRBC isolated from the blood of pregnant women have been made.

Thus far, two approaches for diagnosis of translocation on a single interphase nucleus have been described. One is to develop breakpoint-specific probes that cross or are located very close to the breakpoints. These probes are the most accurate and able to differentiate the normal chromosome from the balanced translocation, and have been routinely used in cancer molecular cytogenetic cases because of the availability of commercialized probes. For most translocation cases in prenatal genetics, however, there are no commercial probes available and to develop such probes for each translocation would be time consuming and expensive. The second approach is to use chromosome-specific probes that are distal to but flank the two breakpoints, which can differentiate the balanced from the unbalanced translocation, but not the balanced translocation from the normal chromosome. There were no breakpoint-specific probes available for our patient, so the chromosome-specific probes of 1q36 and 6cen that flank the two breakpoints were used. These would enable us to distinguish a normal karyotype or balanced translocation from an unbalanced translocation; assuming 2:2 segregation. The unlikely event of 3:1 segregation of both copies of chromosome 1 with chromosome 6 would not be detected. However, since
there is a loss of a large region of chromosome 1 and duplication of 6q, it is expected that a fetus with this karyotype would either be miscarried or would have major anomalies that would be detected on sonographic examination.

Although there is presently no ideal antibody that recognizes 100% of circulating fetal cells and 0% of maternal cells, anti-γ-Mab and anti-z-Mab are the most specific markers currently available for identification of FNRBC (Zheng et al., 1993, 1999; Cheung et al., 1996; DeMaria et al., 1996). Human γ-globin chains are fetal haemoglobinos that are normally present in the fetus, but can be detected in the adult (F-cell) with a low frequency (Wood et al., 1975; Popat et al., 1997). Anti-γ-Mab cannot differentiate between FNRBC and adult F-cells. Human zeta (ζ) globin chains are embryonic haemoglobinos that are normally present in the fetus during the first 3 months of gestation, but can also be detected in an adult with α-thalassaemia (Chung et al., 1984; Chui et al., 1989). Both anti-ζ-Mab and anti-γ-Mab positive FNRBC were identified in our maternal sample at different gestational ages.

Our study confirmed that the fetus did not have an unbalanced translocation as a result of missegregation of the maternal t(1;6), though we would not have been able to determine whether the fetus carried the translocation or not. In our laboratory we are also currently developing micromanipulation techniques that will permit amplification of highly polymorphic regions in the genome, which will allow definitive identification of the cells as fetal in the case of female gender. This study demonstrates the feasibility of this technique. This is the first attempt to exclude a chromosomal translocation using fetal cells isolated from maternal blood. Our report demonstrates the potential advantage of using fetal cells isolated from maternal blood for non-invasive prenatal diagnosis in couples who have a history of recurrent pregnancy loss and who do not want to take the risk of miscarriage due to an invasive procedure.

The non-invasive exclusion of aneuploidy is not meant to replace the traditional metaphase karyotype, as obtained from amniotic fluid or chorionic villous cells. An additional chromosomal abnormality may be missed, as only probes for the chromosomes involved in the parental translocation are used. Similarly, mosaicism could be missed, as only a very few cells are studied that are haematopoietic in origin. The predicted accuracy of this test is at present unknown.

Acknowledgements
This study was supported in part by NIH contract N01-HD-4-3204, NIH grant P01-HD-18658, a grant from Genzyme Genetics, and support for the Molecular Cytogenetics Core Facility from New England Medical Center.

References

Received on June 25, 1999; accepted on November 12, 1999