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SHORT REPORT

Prenatal Diagnosis of a Trisomy 13 Case Associated with Holoprosencephaly by Ultrasonography and Quantitative Fluorescent PCR

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Trisomy 13, first described by Patau in 1960 (1), occurs in 1/5000 of births and is the most severe of the autosomal trisomies (2). Common features of trisomy 13 include holoprosencephaly with midfacial defects (3). Holoprosencephaly arises from incomplete cleavage of the embryonic forebrain. Cytogenetic abnormalities comprise some 24% to 41% cases of holoprosencephaly (4,5). Trisomy 13 accounts for 75% of such abnormalities (6) and the median survival for trisomy 13 is 2.5 days (2). Eighty-two percent of newborns with trisomy 13 die in the first month and only 5% survive the first 6 months (2). Survivors have severe mental defects, often have seizures, and they fail to thrive. Because the prognosis of the syndrome is very poor, early prenatal diagnosis is important. In the past, only a cytogenetic diagnosis from cultured amniocytes or fetal blood samples was performed on the basis of maternal age and/or maternal serum screening. Fluorescence in-situ hybridization (FISH) has also been used since 1992 for the rapid detection of numerical aberrations of chromosomes X, Y, 13, 18 and 21 on uncultured amniocytes of pregnancies at risk (7). However, both ultrasonography and QF-PCR utilizing fetal DNA isolated from uncultured amniocytes are useful tools for rapid prenatal diagnosis of structural central nervous system abnormalities, such as holoprosencephaly (3) and chromosome aneuploidies (7), respectively. In this case report, we present the successful prenatal diagnosis of a trisomy 13 case by ultrasonography and QF-PCR.

Case Report

A 24-year-old nulliparous woman was admitted to Pamukkale University Hospital's obstetrics clinic in the 16th week of her pregnancy. The woman and her husband were not genetically related and there was no history of anomalous children in their families. She had not been exposed to any known teratogenic agent during her pregnancy. Ultrasonography showed growth retardation together with structural central nervous system abnormalities, i.e. the presence of holoprosencephaly and agenesis of the corpus callosum (Figure 1). An amniotic

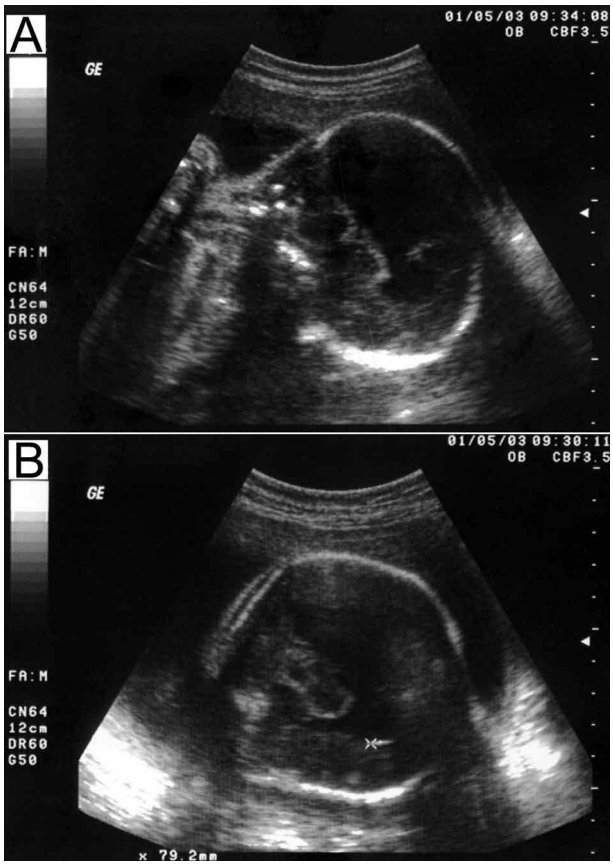

Figure 1. Ultrasonographic images, A, sagittal and B, coronal view, confirming the alobar holoprosencephaly and agenesis of the corpus callosum.

fluid sample and a fetal cord blood sample were obtained for chromosome analysis during the termination of this pregnancy.

QF-PCR and cytogenetic analysis

A rapid QF-PCR screening for common chromosome aneuploidies, i.e. aneuploidies of chromosomes X, Y, 13, 18 and 21, from uncultured amniocytes was followed by postmortem cytogenetic analysis of a fetal cord blood sample. Genomic DNA was extracted from uncultured amniocytes using the QIAamp blood kit (Qiagen). For sex determination the unique sequence in the first intron of the X/Y homologous gene amelogenin was amplified (Table) (7). For the analysis of sex chromosomes and common autosomal aneuploidies selected STR markers were used per chromosome as listed in the Table (7). In each primer pair for the different STR markers the forward primer was labeled with FAM, a fluorescent dye (Iontek), to enable the visualization and analysis of the PCR product. PCR amplification was performed in a total volume of 50 µl containing genomic DNA (10 µl of the extracted DNA), 4–20 pmol of each primer, 25 µl of HotStarTaq Master Mix (containing 2.5 units of HotStarTaq DNA polymerase, 1x PCR Buffer with 1.5 mM $MgCl_2$, and 200 µM of each dNTP; Qiagen, Hilden, Germany). After the initial activation of HotStarTaq DNA polymerase at 94 °C for 15 min, 31 cycles of PCR amplification followed (1 min denaturation at 94 °C, 1

Table. Primers and short tandem repeat markers (STRs) used for sexing and for the detection of sex chromosome and common autosomal aneuploidies.

Chromosome	Marker primer sequence (5'-3')	Expected fragment size (bp)
X chromosome marker		
DXS8337-F-FAM	FAM-CACTTCATGGCTTACCACAG	203-245
DXS8337-R	GACCTTTGGAAAGCTAGTGT	
Chromosome 13 marker		
D13S258-F-FAM	FAM-ACCTGCCAAATTTTACCAGG	180-296
D13S258-R	GACAGAGAGAGGGAATAAACC	
Chromosome 18 marker		
D18S1002-F-FAM	FAM-CAAAGAGTGAATGCTGTACAAACAGC	286-318
D18S1002-R	CAAGATGTGAGTGTGCTTTTCAGGAG	
Chromosome 21 marker		
D21S1411-F-FAM	FAM-ATGATGAATGCATAGATGGATG	283-313
D21S1411-R	AATGTGTGTCCTTCCAGGC	
Amelogenin locus		
HUMAMG-F-FAM	FAM-CCCTGGGCTCTGTAAAGAATAGTG	106X;112Y
HUMAMG-R	ATCAGAGCTTAAACTGGGAAGCTG	

F = forward primer; R = reverse primer.

min annealing at 60 °C, 1 min extension at 72 °C, final extension for 5 min at 72 °C) performed in a Hybaid PCR Sprint Temperature Cycling System. The amplified allelic fragments were resolved on a denaturing polyacrylamide gel using a DNA sequencer and the GeneScan software was employed for the analysis and calculation of these products (Iontek). The results of QF-PCR screening obtained within 24-48 h of amniocentesis (Figure 2B-F) were in complete concordance with those of postmortem cytogenetic analysis (Figure 3), which revealed a (47,XX,+13) karyotype. QF-PCR analysis of maternal DNA isolated from maternal leukocytes for chromosome 13 using the same STR marker primers showed 2 allelic fragments (Figure 2A) completely overlapping with 2 out of the 3 allelic fragments observed in the fetus for chromosome 13 (Figure 2B), suggesting the nondisjunction of maternal chromosome 13 homologues at meiosis I as the cause of trisomy 13 in this fetus.

Autopsy report

A complete autopsy was performed, revealing a 68 g female fetus, with a crown-rump length of 10 cm, and a crown-heel length of 15 cm. Macroscopic examination of the head and face revealed hypotelorism, a proboscis-like single nostril, a short philtrum, and low auricles (Figure 4A). The extremities, hands, feet, fingers and toes were all normal. Bilateral simian lines were present. Craniotomy revealed alobar holoprosencephaly and the absence of cerebellum (Figure 4B). Cerebral weight was 3.4 g. The pons, medulla oblongata and medulla spinalis were normal in appearance. Dissection of the cerebral tissue revealed the absence of corpus callosum, and a single dilated ventricle (Figure 4C). All organs within the chest and abdominal cavities were normal, except for the fact that no uterus, uterine tubes, or ovaries were observed. The plasenta and its associated membranes were normal; however, the umblical cord contained 2 blood vessels, i.e. a single artery and a single vein. Thus, the autopsy findings were supportive of the trisomy 13 karyotype and confirmed the findings of the ultrasonography report.

The presented growth retarded fetus with holoprosencephaly and agenesis of the corpus callosum is a classical example showing the importance of traditional mid-second trimester obstetric ultrasound scanning during pregnancy. The common ultrasonographic features of trisomy 13 include holoprosencephaly, facial

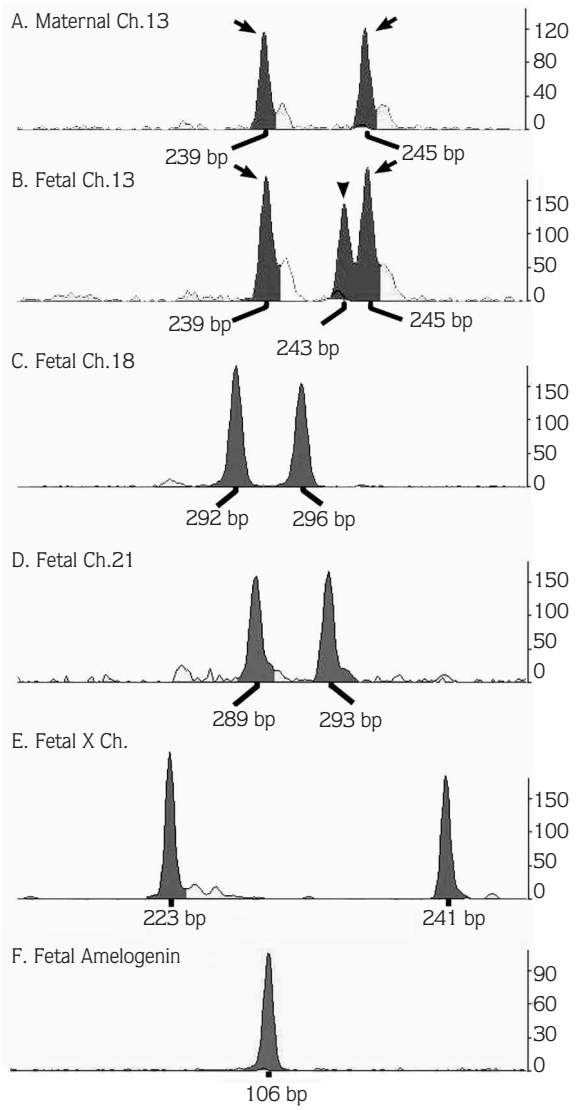

Figure 2. Analysis of chromosomes 13, 18, 21, X and Y by QF-PCR utilizing DNA extracted from uncultured amniocytes revealed a female fetus with trisomy 13 (B-F). QF-PCR analysis of maternal DNA isolated from maternal leukocytes for chromosome 13 using the same STR marker primer pair showed 2 allelic fragments (A) completely overlapping with 2 out of 3 allelic fragments observed in the fetus for chromosome 13 (B, overlapping fragments are indicated with arrows and the extra third fragment is indicated with an arrow head). Numbers on x-axes represent fragment length in base pairs (bp), whereas numbers on y-axes are arbitrary units.

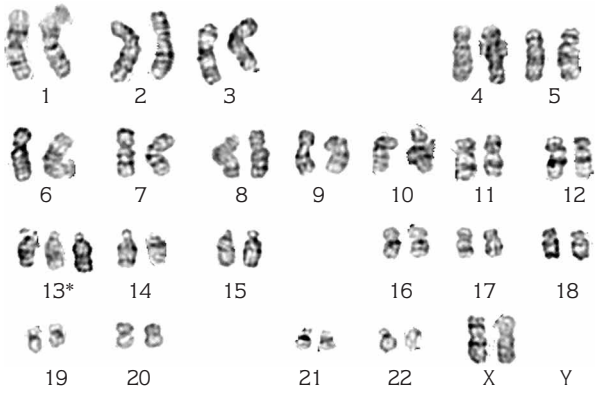

Figure 3. Cytogenetic analysis results of the fetus confirming the (47, XX, +13) karyotype.

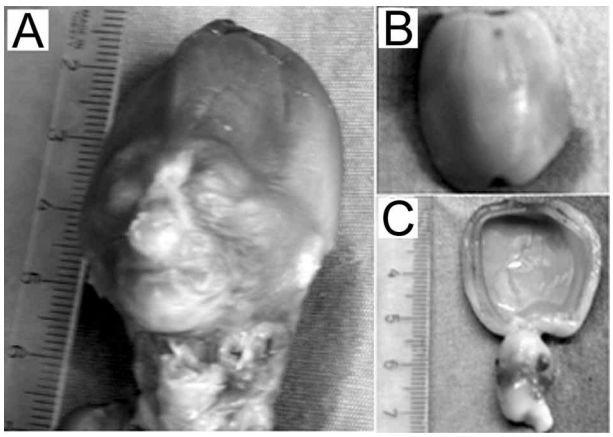

Figure 4. Autopsy images of the fetus. Macroscopic examination of the head and face revealed hypotelorism, a proboscis-like single nostril, a short philtrum, and low auricles (A). Craniotomy revealed alobar holoprosencephaly and the absence of cerebellum (B). Dissection of the cerebral tissue revealed the absence of corpus callosum, and a single dilated ventricle (C).

clefts, cardiac defects, intrauterine growth retardation, microcephaly, neural tube defects, omphalocele, polycystic kidneys, and polydactyly (3). Intracranial anomalies include abnormal posterior fossa, agenesis of the corpus callosum, and ventriculomegaly (3). Thus, chromosomal analysis has to be recommended in all cases with such ultrasonographic features diagnosed prenatally, helping to determine the etiology of these defects and providing accurate recurrence risks for the families in subsequent pregnancies.

Karyotyping, the traditional "gold standard" method, is carried out on cultured cells at the metaphase stage of the cell cycle when the chromosomes are optimally condensed and this technique identifies a wide range of chromosomal abnormalities, including aneuploidies, translocations and inversions. However, the long waiting period for karyotype results (about 2 weeks) may cause a great deal of stress for the parents during the prenatal diagnosis of common chromosome disorders and this has been one of the main reasons for the initiation of molecular methods (8-11). The 2 most common molecular methods for prenatal diagnosis of chromosome disorders are FISH and QF-PCR, which are applied to fetal nondividing cells obtained by amniocentesis and/or CVS and the results can be delivered within 1-2 days. These methods allow rapid and simple yet reliable prenatal diagnosis of targeted fetal chromosome disorders including trisomy 13, 18, 21 and some sex chromosome abnormalities (9). FISH employs hybridization of selected chromosome-specific DNA sequences that have been labeled with fluorescent dyes to chromosome preparations, which are visualized under the fluorescence microscope. It requires 1.0-1.5 ml of amniotic fluid for the aneuploidy diagnosis and spot counting is the most time-consuming part of the interphase FISH procedure (about 30 min per sample). On the other hand, QF-PCR involves DNA isolation from the sample (0.5-1.0 ml) and the amplification of chromosome-specific, repeated DNA sequences named STRs using fluorescent primers by PCR and the products are visualized/quantified using an automated DNA sequencer with the GeneScan software. The QF-PCR analysis carried out by GeneScan takes about 5 min per sample and it lends itself more easily to automation than does FISH. The most important step of the QF-PCR setup concerns the optimization of the primers used. The risk of misdiagnosis with QF-PCR and FISH has been reported as acceptably low (8-14). One of the disadvantages of FISH is that maternal and fetal XX cells per se are indistinguishable by FISH, rendering maternal cell contamination undetectable from female fetuses. In contrast, maternal cell contamination is easily detected by QF–PCR, showing a characteristic pattern with extra alleles or skewed ratios between peaks for the target chromosomes (8-14).

The importance of this case was the successful use of QF-PCR for the rapid and accurate diagnosis of trisomy 13 in conjunction with cytogenetic analysis. In cases like this with features suggesting a common chromosomal aneuploidy, QF-PCR provides a rapid, cost efficient, and dependable choice for prenatal testing (8-14). Although QF-PCR has been used as a preamble to full chromosome

analysis by microscopy or for pure research purposes, there is a debate over whether to apply this test as a 'stand-alone' test for women who are offered amniocentesis due to advanced maternal age and/or maternal serum screening results (8-14). These women comprise the majority of subjects who are at risk of having a baby with a common aneuploidy (8). If introduced on a larger scale, the use of QF-PCR would lead to substantial time, labor, and financial savings in the prenatal testing of suggested risk groups (9-14). In addition, QF-PCR analysis of maternal DNA in comparison to that of fetal DNA for chromosome 13 suggested that the possible cause of trisomy 13 in this fetus was the nondisjunction of maternal chromosome 13 homologues at meiosis I. Thus, the case presented here represents an excellent pilot study of how QF-PCR can be utilized in the rapid prenatal diagnosis of common aneuploidies in a clinical setting.

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