PRENATAL DIAGNOSIS, VOL. 15: 51–55 (1995)

SHORT COMMUNICATION

RETROSPECTIVE STUDY OF TRISOMY 18 IN CHORIONIC VILLI WITH FLUORESCENT *IN SITU* HYBRIDIZATION ON ARCHIVAL DIRECT PREPARATIONS

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Received 2 February 1994
Revised 18 July 1994
Accepted 27 July 1994

SUMMARY

Trisomy 18 in direct chorionic villus preparations needs further investigation since the chromosome abnormality may be confined to the placenta and may not represent the actual fetal karyotype. We performed, retrospectively, fluorescent *in situ* hybridization (FISH) with the chromosome 18 centromere probe (L1.84) on interphase nuclei of destained slides of all cases of full trisomy 18 ($n=22$) and mosaic trisomy 18 ($n=8$) detected among 7600 first-trimester chorionic villus samples during an 8-year period (1985–1992). More nuclei displaying three signals were encountered in cases of full and mosaic trisomy 18 confirmed in fetal tissue than in non-confirmed cases. FISH can be useful for the verification of trisomy 18 in direct chorionic villus preparations.

KEY WORDS—Trisomy 18, chorionic villi, confined placental mosaicism, fluorescent *in situ* hybridization.

INTRODUCTION

Trisomy 18 in non-mosaic, as well as mosaic, appearance in direct preparations of placental chorionic villi may not represent the chromosomal status of the fetus (Simoni *et al.*, 1985; Wirtz *et al.*, 1991). Confirmatory studies of long-term villus cultures are used as one of the means of verification. However, the culturing of chorionic villi adds significantly to the reporting time, while contamination of the sample with maternal tissue can interfere with the accurate interpretation of the results (Vejerslev and Mikkelsen, 1989; Kalousek *et al.*, 1992). Moreover, discrepancies between the karyotype of cultured villi and fetal tissue have been reported (Hogge *et al.*, 1986; Wang *et al.*, 1993).

We performed, retrospectively, fluorescent *in situ* hybridization (FISH) with a chromosome 18-specific probe on interphase nuclei in destained archival direct villus preparations of 30 trisomy 18 cases, to investigate whether this technique can be used as a possible quick and accurate method of verification of trisomy 18 in chorionic villus direct preparations.

MATERIALS AND METHODS

Chorionic villus samples and slide preparations

Thirty cases of trisomy 18 (eight mosaic and 22 non-mosaic) were encountered in 7600 consecutive

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CCC 0197–3851/95/010051–05

first-trimester chorionic villus samples over an 8-year period (1985–1992). Sampling was performed transcervically in ten cases and transabdominally in the remaining cases as described previously (Jahoda *et al.*, 1989, 1990). Indications for prenatal diagnosis were advanced maternal age and/or ultrasound abnormalities. Karyotyping was performed on trypsin–giemsa stained direct preparations (Simoni *et al.*, 1983; Gibas *et al.*, 1987). A mean of 20 metaphases (range 5–50) in the non-mosaic and a mean of 29 metaphases (range 9–50) in the mosaic cases were analysed. Preparations of long-term villus cultures (Smidt-Jensen *et al.*, 1989) were karyotyped in some mosaic cases.

Giemsa-stained archival direct villus preparations of the 30 trisomy 18 cases and of 30 control cases with a normal karyotype, matched for maternal age, gestational age, fetal gender, and storage time, were destained prior to hybridization (Klever *et al.*, 1991).

DNA probe and labelling

The 18 centromere probe L1.84 (Devilee *et al.*, 1986) was used for detection of the chromosome 18 copy number in metaphases and interphase nuclei. The probe was labelled with biotin-11-dUTP by nick translation with the BioNick system (BRL, Gaithersburg, U.S.A.).

Fluorescent in situ *hybridization (FISH) and probe detection*

The centromere probe L1.84 (40 ng in 10 μl of 60 per cent formamide/2 × SSC) and chromosomal DNA were denatured simultaneously for 3 min at 80°C. Hybridization was allowed to proceed overnight at 37°C. After hybridization the slides were washed three times in 50 per cent formamide/2 × SSC at 42°C for 5 min, followed by three changes of 2 × SSC, twice at 42°C and once at 65°C, respectively.

The probe was visualized by alternating layers of fluoresceinated avidin and biotinylated goat anti-avidin (Vector Lab, Burlingame, U.S.A.). The slides were mounted in anti-fade medium containing the fluorescent counterstains propidium iodide (0·06 μg/ml) and DAPI (0·6 μg/ml). Slides were examined under a Leitz Aristoplan fluorescence microscope and cells were photographed on Kodak Ektachrome 400 ASA daylight film.

Samples were analysed in a blind fashion on coded slides. For each case, the number of fluorescent spots was counted in 200 hybridized intact non-overlapping and non-clumped interphase nuclei. The specificity of probe hybridization was checked in metaphases present on each slide.

RESULTS

Cytogenetic analysis

Twenty-two of the 30 cases with trisomy 18 in direct villus preparations revealed a non-mosaic trisomy 18 karyotype. The pregnancies were terminated at the parents' request; one pregnancy resulted in intrauterine fetal death within a week of sampling. The diagnosis of trisomy 18 was confirmed in the fetus by karyotyping skin fibroblasts in 15 of the 22 cases. In one early case, we could not confirm the trisomy 18 in fetal cells, which showed a normal 46,XY karyotype. In the six remaining cases, cytogenetic confirmatory studies could not be carried out or failed.

Eight of the 30 cases with trisomy 18 displayed a mosaic trisomy 18 (Table I). Five pregnancies were terminated at the parents' request and the trisomy 18 was confirmed in fetal tissue in only three instances. In case 26, showing a double trisomy of chromosomes 18 (mosaic) and 21 (non-mosaic) in chorionic villi, only a full trisomy 21 was recovered in skin fibroblasts and the cytogenetic confirmation in case 23 failed. Three pregnancies continued after extensive follow-up studies, including a long-term villus culture (LTC) and amniocentesis. They resulted in the birth of healthy children. In two out of five mosaic cases, in which LTC was performed, the culture showed a mosaic trisomy 18 while fetal skin fibroblasts did not exhibit this chromosome aberration (cases 26 and 30).

FISH interphase analysis

Fluorescent *in situ* hybridization (FISH) with the 18 centromere probe L1.84 was successfully applied to destained archival slides of all but two of the trisomy 18 cases and to the corresponding normal controls. The mean percentages of nuclei showing one, two, and three signals in the 30 normal cases were 7, 92, and 1 per cent, respectively. Figure 1 shows the percentage of nuclei with two and three signals in individual cases of full and mosaic trisomy 18. The mean percentage of nuclei showing one signal was 2 per cent (range 0–4·5 per cent) in the non-mosaic and 3 per cent (range 0–9 per cent) in the mosaic trisomy 18 cases. In the

Table I—Eight cases of mosaic trisomy 18 in first-trimester chorionic villi

Case no.	CVS		Follow-up	Outcome
	DP	LTC		
23	46,XX/47,XX,+18(2/7)	—	—	TOP
24	46,XX/47,XX,+18(3/47)	—	F: 47,XX,+18(45)	TOP
25	46,XX/47,XX,+18(1/31)	46,XX(7)	A: 46,XX(15)	Healthy girl; 3200 g
26	47,XX,+21/48,XX, +18,+21(1/19)	47,XX,+21/48,XX, +18,+21(28/23)	F: 47,XX,+21(16)	TOP
27	46,XY/47,XY,+18(12/23)	47,XY,+18(32)	F: 46,XY/47,XY,+18(2/36)	TOP
28	46,XX/47,XX,+18(19/19)	46,XX(27)	A: 46,XX(8)	Healthy girl; 3375 g
29	46,XX/47,XX,+18(1/15)	—	F: 47,XX,+18(16)	TOP
30	46,XX/47,XX,+18(28/2)	46,XX/47,XX,+18(17/13)	A: 46,XX(19)	Healthy girl; 2750 g

CVS=Chorionic villus sample; DP=direct villus preparations; LTC=long-term villus culture; A=amniocentesis; F=fetal fibroblast culture; TOP=termination of pregnancy. The numbers in parentheses denote the number of cells analysed.

series with non-mosaic trisomy 18, the FISH results on interphase nuclei closely matched the cytogenetic findings in the direct preparations (Fig. 1A). The confirmed cases were found to express three fluorescent signals in more than 83 per cent of their nuclei (mean 87·5 per cent). In the only non-confirmed case (No. 8), the percentage of nuclei with three signals was 72 per cent, which is far outside the 95 per cent confidence interval of the confirmed cases. In the series with mosaic trisomy 18, a broad range of signal distributions was found which did not always match the cytogenetic analysis of the direct preparations (Fig. 1B); four mosaic cases (Nos 24, 25, 26, and 29) with more than 90 per cent of abnormal cells in GTG metaphase analysis showed three fluorescent signals in 76, 66, 44, and 77 per cent of the interphase nuclei, respectively. In general, cases that were confirmed as being trisomy 18 in amniotic fluid and/or fetal cells (Nos 24, 27, and 29) showed a higher percentage of three signal-containing nuclei (76, 66, and 77 per cent, respectively) than did the non-confirmed cases 25, 26, 28, and 30 (66, 44, 18, and 13 per cent, respectively).

DISCUSSION

The diagnosis of trisomy 18 (mosaic and non-mosaic) in direct chorionic villus preparations in the first trimester of pregnancy is complicated by the occurrence of false-positive (Sachs *et al.*, 1990; Breed *et al.*, 1990; Ledbetter *et al.*, 1992) and false-negative results (Leschot *et al.*, 1988; Kalousek *et al.*, 1989). Confirmatory studies of long-term villus cultures have been proposed as a means of verification, as mesenchymal cells in the villus core are suggested to have a closer ontogenetic relation to the fetal cells than the trophoblast cells (Crane and Cheung, 1988). Our own results, as well as various earlier reports, argue against the use of long-term villus cultures (LTC) as the sole and sufficient independent confirmation (Wirtz *et al.*, 1991; Miny *et al.*, 1991; Ledbetter *et al.*, 1992). Cytogenetic analysis of a subsequent amniotic fluid sample seems the most reliable procedure for verification of trisomy 18 in chorionic villi. We studied the usefulness of interphase FISH as a possible quick and accurate method of further investigation of trisomy 18 in chorionic villus direct preparations. It was shown that FISH with a chromosome 18-specific probe, applied to interphase nuclei in direct villus preparations of non-mosaic trisomy 18 cases, has a strong predictive value for the chromosomal status of the fetus and contributes significantly to the results of the classical cytogenetic metaphase analysis. The non-confirmed case of full trisomy 18 had a significantly smaller number of interphase nuclei displaying three signals than the real, confirmed cases of trisomy 18.

In cases of mosaic trisomy 18, the application of FISH also adds to the classical cytogenetic analysis: higher levels of three signal-containing nuclei were found in the three confirmed cases as compared with the four non-confirmed cases. If the percentage of nuclei with three signals was lower than 66 per cent, trisomy 18 was not confirmed in

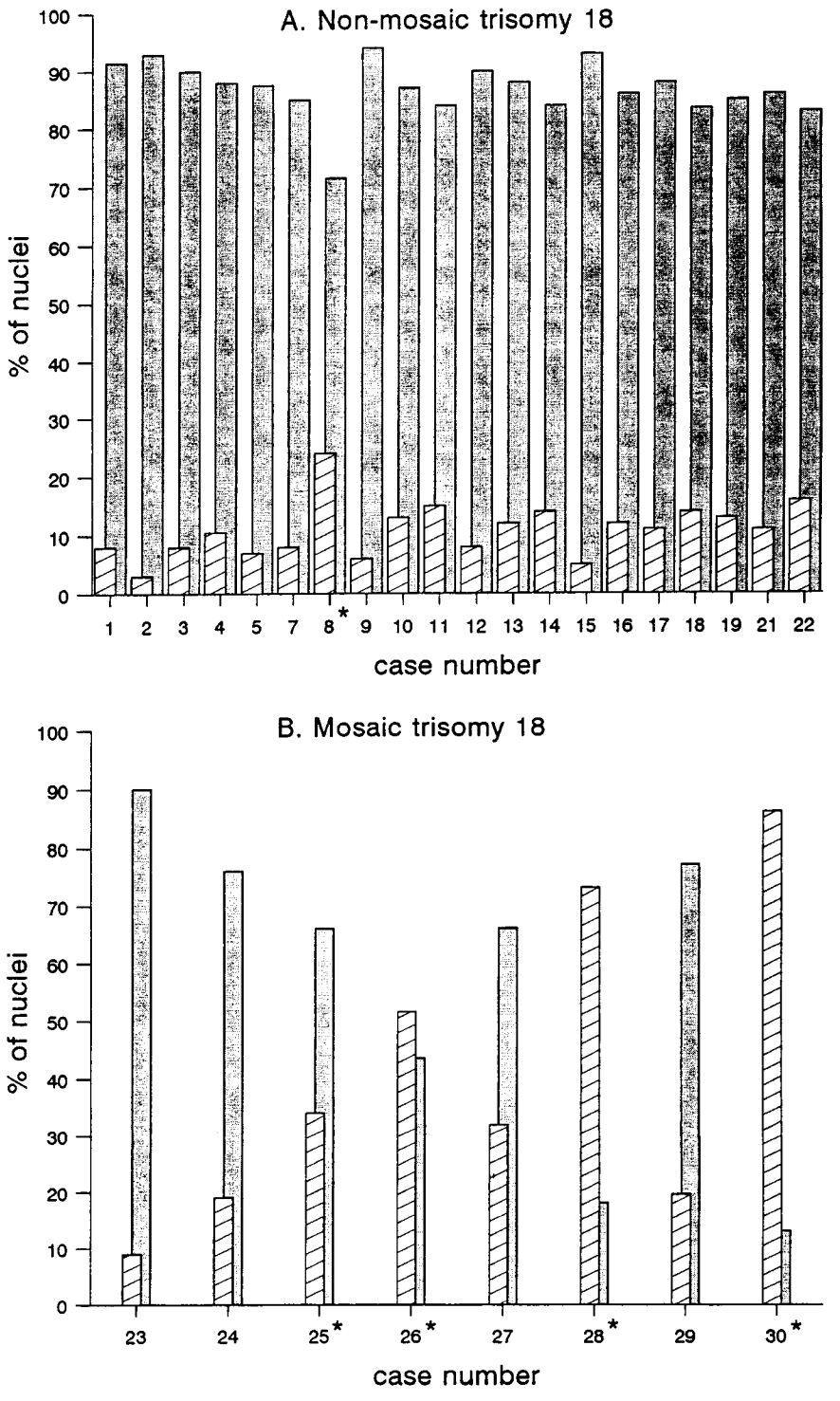

Fig. 1—Percentage of interphase nuclei showing three (closed bars) and two (hatched bars) fluorescent signals after *in situ* hybridization with an 18-specific probe on direct villus preparations of cases with non-mosaic (A) and mosaic (B) trisomy 18 (*non-confirmed case).

fetal cells. In general, the FISH data were better able to predict the fetal outcome than the classical cytogenetic analysis of the direct preparations. However, FISH yielded ambivalent results in some cases; an intermediate level (66–83 per cent in our series) of three-signal nuclei could correspond to either a true mosaic, a non-mosaic trisomy 18 in the fetus, or a false-positive result.

We have shown that the application of FISH on chorionic villus direct preparations in which a trisomy 18 karyotype is found, has a predictive value for the true fetal chromosome constitution and therefore can aid in the counselling procedures. However, a final and reliable result on which irreversible clinical decisions can be based, can only be achieved by karyotyping a subsequent amniotic fluid sample.

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