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Rapid detection of chromosome aneuploidies in fetal blood and chorionic villi by fluorescence in situ hybridisation

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ABSTRACT

Objective Evaluation of fluorescence *in situ* hybridisation in the detection of numerical aberrations involving chromosomes X, Y, 13, 18 and 21.

Setting Harris Birthright Research Centre for Fetal Medicine.

Subjects and methods Chorionic villi ($n = 45$) or fetal blood ($n = 34$) were obtained from 79 pregnancies undergoing fetal karyotyping at 10 to 39 weeks of gestation because of ultrasonographic markers of fetal chromosomal abnormality. Karyotyping was performed by both traditional cytogenetics and fluorescence *in situ* hybridisation, using commercially available kits which utilise a heterochromatic Y probe and the alpha satellite repeat probes for chromosomes X, 18, and 13/21. The frequency distributions of the number of signals obtained by fluorescence *in situ* hybridisation in the chromosomally normal and abnormal fetuses were compared.

Results Traditional cytogenetic analysis demonstrated that the fetal karyotype was normal in 47 cases and abnormal in 32 (including 24 with trisomies 21, 18 or 13, three with triploidy, one with Turners syndrome and four with translocations, deletions or mosaicism). With fluorescence *in situ* hybridisation it was possible to obtain accurate diagnosis of trisomy 18, Turners or triploidy within six hours of sampling; signal distributions with these chromosomal abnormalities were very different from those of normals. However, for trisomies 21 and 13 there was an overlap in values with those from normals.

Conclusions In detection of fetal numerical chromosomal abnormalities the use of the combined 13/21 probe cannot provide sufficiently accurate results to justify abandonment of traditional cytogenetics in favour of fluorescence *in situ* hybridisation.

Screening for fetal chromosomal abnormalities on the basis of maternal age has not resulted in a substantial fall in the proportion of infants born with an abnormal karyotype (Cuckle *et al.* 1991). This has led to the introduction of new approaches to screening based on maternal serum biochemistry (Wald *et al.* 1992) and examination of the fetal anatomy by ultrasonography (Nicolaides *et al.* 1992a, b). Since these methods of screening are directed at the identification of fetuses at risk for specific chromosomal abnormalities, it may well be appropriate to utilise laboratory methods aiming to detect or to exclude these specific chromosomal abnormalities. This can now be done by fluorescence *in situ* hybridisation, a technique whereby deoxyribonucleic (DNA) probes are used to bind to chromosomes in the interphase nucleus (Cremer *et al.* 1986; Lichter *et al.* 1988; Pinkel *et al.* 1988). Commercially available kits for fluorescence *in situ* hybridisation can be used to diagnose or exclude certain chromosomal aneuploidies within a few hours of sampling; this compares favourably with traditional cytogenetics which is labour intensive and requires highly trained personnel.

The aim of this study is to evaluate the application of fluorescence *in situ* hybridisation for karyotyping chorionic villi and fetal blood from fetuses with ultrasonographic markers of chromosomal abnormalities.

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Subjects and methods

Fetal karyotyping was performed in 79 pregnancies with ultrasonographically detectable markers of fetal chromosomal abnormality. In 45 of these pregnancies there was fetal nuchal translucency of 3 mm or more thickness at 10 to 14 weeks of gestation, and transabdominal chorion villus sampling was performed (Nicolaides *et al.* 1992a). In another 34 cases, fetal blood samples were obtained by cordocentesis at 16 to 39 weeks of gestation because ultrasound examination demonstrated a wide range of fetal malformations, growth retardation, or both (Nicolaides *et al.* 1992b).

Traditional cytogenetic techniques were used for analysis of chorionic villi and stimulated blood lymphocytes; in addition, samples were processed for fluorescence *in situ* hybridisation. The villi were incubated in hypotonic potassium chloride at 37 °C for 10 min. They were then placed in fresh fixative (3:1 v/v methanol:glacial acetic acid), stored at −20 °C for 10 min, and this step was repeated. Subsequently, the villi were dissociated with 60 % acetic acid and cytocentrifuged (Cytospin 3, Shandon Scientific Ltd, Cheshire, UK) onto microscope slides. Fetal blood (50 μl) was mixed with 20 μl of isotonic edetic acid solution (0·5 mmol/l in 0·15 mmol/l sodium chloride) and 5 μl drops were then placed on individual glass slides to produce smears.

The slides were hybridised using the α satellite repeat probes for chromosomes X, 18 and a combined 13/21 probe and the heterochromatic Y probe (Cytocell Ltd, Oxon, UK). The fluorescence *in situ* hybridisation kits incorporate fluorescein-labelled DNA probes which are coated onto a glass coverslip, thereby avoiding probe handling and excluding the need for post-hybridisation signal amplification.

The slides were examined by fluorescence microscopy (Nikon Optiphot 2, Nikon UK Ltd, Surrey, UK). For each slide, 100 hybridised nuclei were counted, and the number displaying zero, one, two, three, four, five, or six hybridisation signals was recorded. Hybridisation efficiency was calculated by dividing the number of nuclei without any signals by the number of nuclei with at least one signal. In addition, the percentage of cells displaying different number of signals was calculated. Statistical analyses using ANOVA were applied to determine if there were significant differences in results obtained from chorionic villi and fetal blood, in hybridisation efficiencies of the different probes frequencies, and to determine if there were differences in the signal distribution between chromosomally normal and abnormal groups.

Results

Results from fluorescence *in situ* hybridisation analysis were available within 6 h of sampling. With traditional cytogenetics, results were available after 10 days for fetal blood; for chorionic villi, results were available after 72 h with direct preparations and after 21 days with long term cultures.

Traditional cytogenetic analysis demonstrated that the

Table 1. Results from traditional cytogenetic analysis of chorionic villi and fetal blood.

Karyotype	Chorionic villi	Fetal blood
46 XX	12	7
46 XY	17	11
47 XX+18	5	5
47 XY+18	2	2
47 XX+21	4	1
47 XY+21	3	1
47 XY+13	0	1
69 XXY	2	0
69 XXX	0	1
45 X	0	1
46 XX t(6; 21)	0	1
46 XX t(5; 18)	0	1
46 XX del(4) (p15.2)	0	1
46XX/47 XX+mar (13/21)	0	1
TOTAL	45	34

Table 2. Hybridisation efficiency for the different DNA (chromosomes (Chrom.) X, Y, 18 and 13/21) probes using chorionic villi and fetal blood; values are shown as median and range percentages. The efficiency of the 13/21 probe was significantly lower than all the other probes (X, $z = 3{\cdot}99$, $P < 0{\cdot}0001$; Y, $z = 4{\cdot}41$, $P < 0{\cdot}0001$; 18, $z = 3{\cdot}95$, $P < 0{\cdot}0001$).

DNA probe	Chorionic villi ($n = 45$)	Fetal blood ($n = 34$)	Combined ($n = 79$)
Chrom. X	86 (64–96)	90 (60–96)	87 (60–96)
Chrom. Y	88 (72–98)	86 (70–98)	88 (70–98)
Chrom. 18	89 (56–98)	86 (78–96)	88 (56–98)
Chrom. 13/21	76 (50–94)	70 (56–92)	76 (50–94)

Table 3. Percentage (median and range) of cells with different number of signals after hybridisation with the various probes.

	Chromosome 18 probe	
No. of signals	Non-trisomy 18 ($n = 65$)	Trisomy 18 ($n = 14$)
2	82 (64–96)	16 (8–24)
3	6 (0–18)	75 (66–92)
	Chromosomes 13/21 probe	
No. of signals	Non-trisomy 13 or 21 ($n = 70$)	Trisomy 13 or 21 ($n = 9$)
4	54 (34–80)	29 (14–56)
5	12 (2–28)	47 (24–62)
	Chromosome X probe	
No. of signals	Male ($n = 37$)	Female ($n = 38$)
1	80 (80–96)	11 (2–24)
2	10 (4–20)	88 (74–96)
	Chromosome Y probe	
No. of signals	Male ($n = 37$)	Female ($n = 38$)
0	12 (2–30)	92 (80–98)
1	88 (70–98)	8 (2–20)

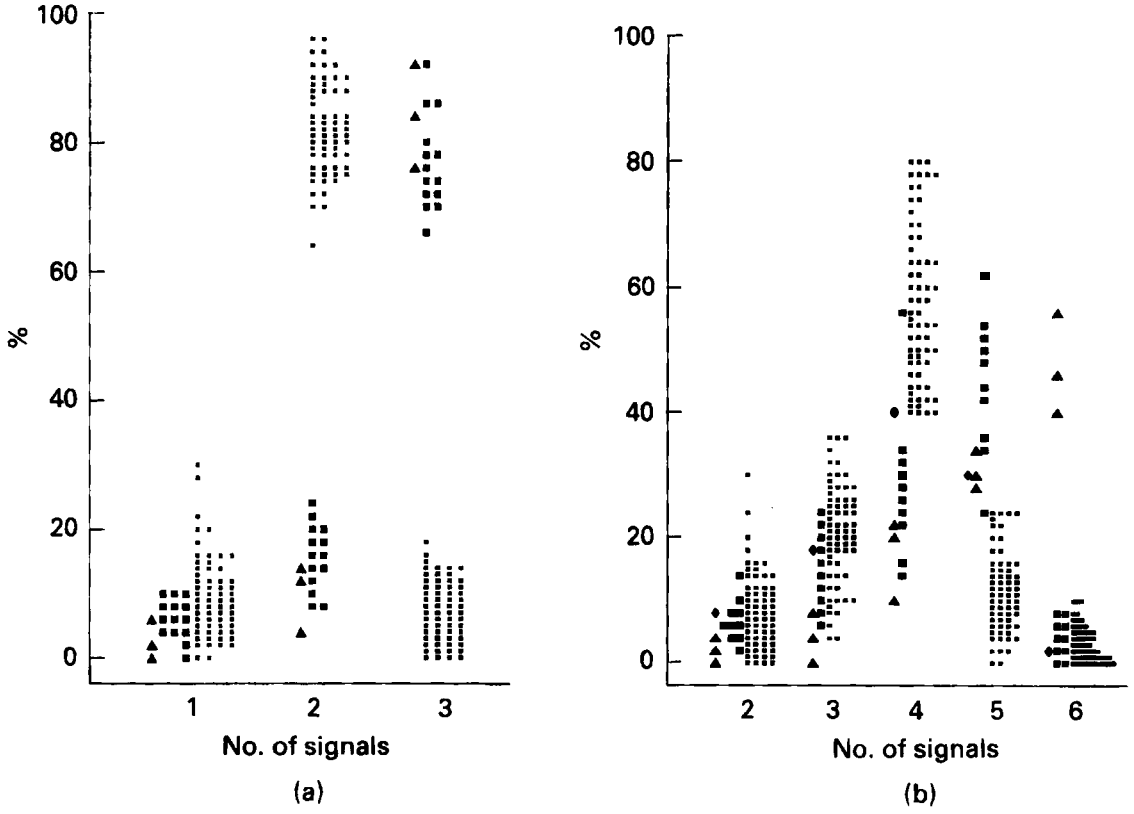

Fig. 1. Frequency distribution of number of signals with chromosome 18 probe (a) and the chromosome 13/21 probe (b) in fetuses with normal karyotype (■), trisomy 18 (■), trisomy 13/21 (■), triploidy (▲) and mosaicism (◆).

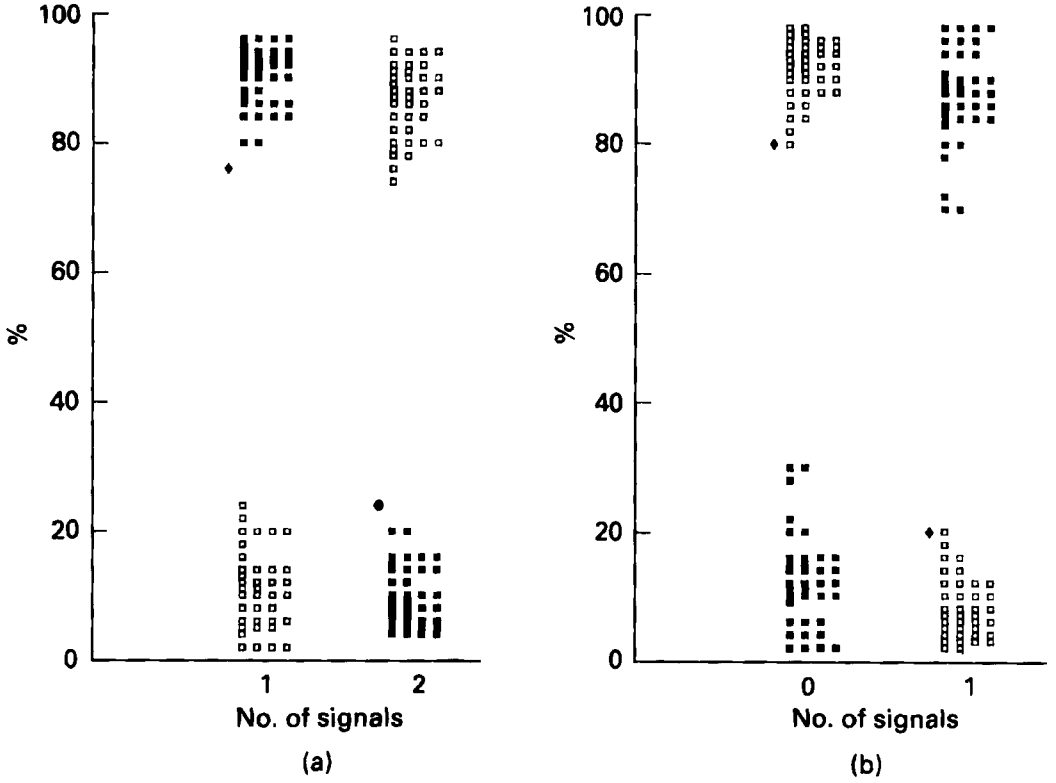

Fig. 2. Frequency distribution of number of signals with the chromosome X probe (a) and chromosome Y probe (b) in male (■), female (□) and Turner (◆) fetuses.

fetal karyotype was normal in 47 cases and abnormal in 32 (Table 1). The data obtained from fluorescence *in situ* hybridisation analysis, including the percentages of cells demonstrating at least one signal (hybridisation efficiency) and the frequency distribution of number of signals with the various probes are shown in Tables 2 and 3 and Figs 1 and 2. There was no significant difference in hybridisation efficiency between chorionic villi and fetal blood for any of the probes. However, hybridisation efficiency was significantly lower with the 13/21 than with the other probes.

The percentage of cells with each number of signals was significantly different (with no overlap in values) in male (one signal with the X probe and one with the Y probe) compared with female fetuses (two signals with the X probe and zero with the Y probe), and in fetuses with trisomy 18 (three signals with the 18 probe), triploidy (three signals with the 18-probe and six signals with the 13/21 probe) or Turners syndrome (one signal with the X-probe and zero with the Y probe) compared with those without these chromosomal abnormalities (Table 3, Figs 1 and 2). Although for the 13/21 probe there were also significant differences between affected (five signals) and non-affected (four signals) fetuses, there was an overlap in values. For example, in trisomy 13 or 21 fetuses 14 to 56% (median 29%) of cells had four signals and 24 to 62% (median 47%) had five signals; in non-trisomy 13 or 21 fetuses, the respective values were 34 to 80% (median 54%) and 2 to 28% (median 12%). If the upper limit of normal for five signals was taken to be 28%, one of the 10 fetuses with trisomy 21 or 13 would have been misdiagnosed as non-affected.

The one case of mosaicism (46 XX/47XX, mar 13/21 *de novo*) involved a centromeric piece from chromosome 13 or 21, displayed 40% disomic signals and 30% trisomic signals with the α satellite probe. As expected, the three cases of structural chromosomal abnormalities were not detected by fluorescence *in situ* hybridisation.

Discussion

The data of this study indicate that fluorescence *in situ* hybridisation, using currently available kits, can provide accurate diagnosis of fetal sex, trisomy 18, Turners and triploidy within 6 h of sampling either chorionic villi or fetal blood. This has advantages over traditional cytogenetic analysis which requires *in vitro* culture of cells, is labour intensive and can take three to four weeks to complete for amniocytes and chorionic villi and up to 10 days for fetal blood.

Most fetuses with major cytogenetic abnormalities have either external or internal defects (Jones 1988) which can be recognised by detailed ultrasonographic examination. When a fetal defect is detected and fetal tissue sampling is undertaken, the application of user-friendly fluorescence *in situ* hybridisation kits can provide rapid results which can be particularly useful when urgent decisions on perinatal management will depend on the knowledge of fetal karyotype. For example, trisomy 18 and triploidy will commonly present with severe intrauterine growth retardation in the late second trimester of pregnancy. Knowledge that the fetus is affected with these lethal chromosomal abnormalities may spare the mother an emergency caesarean section for fetal distress.

At the other end of the gestational age spectrum, fetal nuchal translucency at 10 to 13 weeks has a high association with trisomies 21, 18 and 13 (Nicolaides 1992a). The detection of nuchal translucency generates tremendous anxiety for the parents, especially in the younger age group who had not perceived the possible risk of chromosomal defects. The theoretical advantage of fluorescence *in situ* hybridisation is that within 6 h of chorion villus sampling the parents can be reassured that the fetus is not affected by one of the common major trisomies. Alternatively, if the fetus is affected and the parents request termination of pregnancy, this can be undertaken within 24 to 48 h of the ultrasonographic examination.

Despite these theoretical advantages of fluorescence *in situ* hybridisation, the findings of the present study demonstrate that in the detection of fetal numerical chromosomal abnormalities the use of the combined 13/21 probe cannot provide sufficiently accurate results to justify abandonment of traditional cytogenetics in favour of fluorescence *in situ* hybridisation. The 13/21 probe used in our study binds to a portion of the chromosome (repeat α satellite sequence) that shares homologous sequences in both chromosomes 21 and 13 and therefore cannot distinguish between them. Furthermore, there are practical difficulties in visualising four or five fluorescent signals as opposed to two or three signals with the other probes. In addition, the use of this probe may fail to diagnose trisomy 21 because in chromosome 21 the repeat α satellite sequence is polymorphic, and in some cases it is too small for binding to the probe (Verma & Luke 1992). This also can account for our findings that hybridisation efficiency was significantly higher with the X, Y, and 18 probes (86–88%) than with the 13/21 probe (76%).

In order to optimise detection of trisomy 21 and 13, it is necessary to use chromosome specific probes, such as those cloned in cosmids (Evans *et al.* 1992; Klinger *et al.* 1992; Zheng *et al.* 1992). However, these probes necessitate well-trained personnel, they are labour intensive, and they take 48 to 72 h to provide results. In these respects they are not dissimilar to direct preparations of chorionic villi that have the advantage of providing a more complete karyotype.

Even if fluorescence *in situ* hybridisation utilises highly specific probes and the process becomes fully automated, a major contention will remain concerning its failure to provide a complete karyotype. For example, data from series on amniocentesis and chorion villus sampling for advanced maternal age indicate that 12 to 35% of clinically significant chromosomal abnormalities cannot be diagnosed by probes for chromosomes X, Y, 13, 18 and 21 (Schreinemachers *et al.* 1982; Ferguson-Smith & Yates 1984; Lebo *et al.* 1992; Clark *et al.* 1993; Snijders *et al.* 1994). Similarly, in a series of 2086 patients with ultrasonographic markers, 28/301 (9·3%) abnormal karyotypes would not have been diagnosed if fluorescence *in situ* hybridisation had been used (Nicolaides 1992b); the same

is true in 3/32 cases in the present study. Ward *et al.* (1993) have used region-specific cosmid probes to chromosomes 13, 18, 21, X, and Y for examination of 4500 amniotic fluid samples in routine clinical practice. Of the 165 clinically significant chromosomal abnormalities, they could have potentially identified 146 (88%), but the actual detection rate was 65% (107/165).

This study has shown that in the investigation of fetuses with ultrasonographic markers, fluorescence *in situ* hybridisation provides a rapid tool for the detection of numerical aberrations of chromosomes X, Y, 13, 18, and 21. However, at present fluorescence *in situ* hybridisation can be considered as an adjunct, rather than replacement, of traditional cytogenetic analysis.

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