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Rapid Detection of Chromosome Aneuploidies in Uncultured Amniocytes by Using Fluorescence In Situ Hybridization (FISH)

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Summary

Herein we report the results of the first major prospective study directly comparing aneuploidy detection by fluorescence in situ hybridization of interphase nuclei with the results obtained by cytogenetic analysis. We constructed probes derived from specific subregions of human chromosomes 21, 18, 13, X, and Y that give a single copy–like signal when used in conjunction with suppression hybridization. A total of 526 independent amniotic fluid samples were analyzed in a blind fashion. All five probes were analyzed on 117 samples, while subsets of these five probes were used on the remaining samples (because of insufficient sample size), for a total of over 900 autosomal hybridization reactions and over 400 sex chromosome hybridization reactions. In this blind series, 21 of 21 abnormal samples were correctly identified. The remaining samples were correctly classified as disomic for these five chromosomes. The combination of chromosome-specific probe sets composed primarily of cosmid contigs and optimized hybridization/detection allowed accurate chromosome enumeration in uncultured human amniotic fluid cells, consistent with the results obtained by traditional cytogenetic analysis.

Introduction

Of all the molecular approaches to medical genetics, only in situ hybridization has the power to combine cytogenetic and molecular analyses. Over the past decade, advances in molecular biology have made it possible both to generate probe sets derived from specific chromosomal regions and to chemically modify the probe sets for exquisitely sensitive and specific nonisotopic detection. The application of these molecular tools to cytogenetics has propelled fluorescence in situ hybridization (FISH) into its own as a powerful and important diagnostic technology (Cremer et al. 1986; Landegent et al. 1987; Lichter et al. 1988*b;* Lichter and Ward 1990; Amoldus et al. 1991; McNeil et al. 1991; Tkachuk et al. 1991; Wiegant et al. 1991).

However, clinical applications of FISH are just beginning. Studies published elsewhere have demonstrated the general feasibility of detecting chromosomal abnormalities in interphase nuclei by FISH (Cremer et al. 1986; Julien et al. 1986; Lichter et al. 1988*b;* Pinkel et al. 1988). This technology, often called "interphase cytogenetics," relied on the discovery that each chromosome occupies a distinct focal domain in the interphase nucleus and that, consequently, a discrete hybridization signal is obtained in most nuclei, for each specific chromosome present. For example, a cell exhibiting trisomy 21 yields three chromosome 21–derived hybridization signals in the nucleus, whereas a normal cell displays two. Thus, when a highly chromosome-specific probe is used, in situ hybridization can be used to determine the number of copies of a given chromosome that are present within the nucleus.

A variety of different types of probe sets were used

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in earlier studies, including complex probes composed of the inserts from an entire chromosome library (Cremer et al. 1988; Lichter et al. 1988*a;* Pinkel et al. 1988; Jauch et al. 1990), alpha-satellite repeat probes (Willard and Waye 1987), composite probe sets composed of single-copy subclones (Lichter et al. 1988*b*), and single cosmids (Lichter et al. 1990*a*). While each of these probe types theoretically can be used to enumerate chromosomes, it is clear that different parameters impact the ability of each probe type to detect prenatal chromosomal abnormalities. The complex probes generate a very large diffuse signal, such that the edges of the signal can be difficult to delineate, and overlap of the hybridization domains is common. Overlap of the hybridization signal is exacerbated by the nucleolar organization of the short arms of the D and G group chromosomes, resulting in close proximity of the probes. This results in 10%–50% frequencies for trisomic amniocyte nuclei showing three hybridization domains (Yu et al. 1990; Kuo et al. 1991; Tkachuk et al. 1991). The repetitive alpha-satellite sequences generate a brilliant hybridization signal but present some difficulties in clinical utilization. In general, chromosome specificity of the repetitive probes is very sensitive to hybridization conditions, and the signal size is sensitive to pericentromeric heteromorphisms. Chromosomes 21 and 13 share the same repeat sequences and thus, currently, cannot be differentiated using the alpha-satellite repeat probes. Furthermore, the centromeric position of the probes does not allow identification of Robertsonian translocations, thus limiting detection of Down syndrome. Clustering of acrocentric chromosomes around the nucleolus organizer region may obscure resolution of centromeric probes in interphase nuclei. Plasmid pools have given good signal resolution and specificity, but the signal is often a composite of many smaller signals, which complicates quantitation (Lichter et al. 1990*a;* and authors' unpublished observations). Single cosmids give superb spatial resolution in peripheral lymphocytes (Lichter et al. 1990*a*) but result in weak signals when hybridized to uncultured amniocytes (K. Klinger, D. Shook, G. Landes, and W. Dackowski, unpublished data).

The ability to analyze specific chromosomal regions in interphase nuclei by using FISH has prompted the development of tests specifically designed to allow rapid prenatal identification of the major chromosomal aneuploidies (Klinger et al. 1990; Lichter et al. 1990*a;* Yu et al. 1990; Kuo et al. 1991). Unfortunately, previous studies achieved limited efficiency, because of constraints of probe composition, as well as because of variations in sample preparation and hybridization detection. In practice, this meant that many nuclei did not hybridize and also that, of those nuclei that did hybridize, many had fewer hybridization signals than expected (Klinger et al. 1990; Lichter et al. 1990*a;* Yu et al. 1990; Kuo et al. 1991).

To overcome these limitations, which, until now, had prevented routine clinical application of FISH for prenatal diagnosis of the major chromosomal aneuploidies, we constructed DNA probe sets based on cosmid contigs that were chromosome specific, had high signal-to-noise ratios, had acceptable spatial resolution of the fluorescent signals, and exhibited high hybridization/detection efficiencies. Using these DNA probe sets in a large prospective study, we were able to detect the major chromosomal aneuploidies present in uncultured amniotic fluid samples and thereby were able to demonstrate the clinical utility of FISH for prenatal diagnosis.

Material and Methods

Probe Set Development

Human genomic cosmid libraries were screened with labeled probes derived from single-copy clones, to identify cosmids from the relevant loci. Library screening and subsequent chromosome walking were performed by using standard protocols (Sambrook et al. 1989). We identified, by multiple genetic and physical methods, a starting clone that mapped to each desired chromosomal region, and then, by using standard walking protocols, we used each starting clone to identify overlapping cosmids. Cosmids and/or contigs were generated for multiple loci on each autosome and on the X chromosome (additional loci on chromosome 21 are not shown in fig. 1). For this study, a single probe set was used for each chromosome, as indicated by the heavy bars on the ideogram in figure 1. Contigs were constructed for the chromosome regions shown in figure 1. Starting clones for the contigs used in this study mapped to the following regions: 13q13 (D13S6), 18q22-qter (MBP), and 21q22.3 (D21S71). Cosmid contigs were expanded as necessary, to achieve a total sequence complexity of ≥60 kb. The chromosome 21 probe set is a three-cosmid contig containing 80 kb of nonoverlapping DNA; the chromosome 18 probe set is a three-cosmid contig containing 109 kb of nonoverlapping DNA; and the chromosome 13 probe set is a three-cosmid contig containing ~97 kb of nonoverlapping DNA. The

specificity of the probe sets was verified by FISH to metaphase spreads (fig. 1). Chromosome X–specific cosmids were identified from a cosmid library constructed from DNA of a human-rodent somatic cell hybrid that contained chromosomes 13, 19, and X as the sole human components. A cosmid containing a pericentromeric repeat sequence was used as the X probe in this study. The Y probe used in this study was pDP97, a repetitive clone developed by D. Page (Whitehead Institute of Biomedical Research). The specificity and sensitivity of individual cosmid clones and cosmid contigs were rigorously monitored and evaluated by FISH against both metaphase spreads and interphase nuclei, using short-term blood cultures and uncultured amniotic samples, respectively, during the development of the autosomal probe sets.

Sample Preparation

Metaphase spreads were prepared from short-term blood cultures, using standard cytogenetic protocols. Interphase nuclei were obtained from uncultured amniotic fluid samples in the following manner, which differs from that used in our previous studies (Price et al. 1991): Slides were coated with 2% 3-aminopropyltriethoxysilane (Sigma) in acetone by submersion for 2 min. Slides were rinsed in water for 5 min and air-dried. Uncultured amniotic fluid cells were collected by centrifugation, were resuspended in PBS, and were placed at 37°C on the coated slide, to give $\sim 5 \times 10^3$ cells/slide. Subsequently, the cells were processed in situ by the addition of KCl to total 50 mM and incubated at 37°C for 15–20 min. The hypotonic solution was carefully decanted and was replaced by 100 µl of 30% 3:1 fix (methanol:acetic acid), 70% 75 mM KCl for 5 min at room temperature. The solution was carefully decanted, and fresh 3:1 fix was dropped onto the slide from a height of 2 feet. Excess fix was decanted, and the slides were dried for 5 min at 60°C. Conditions that generated nuclei capable of supporting in situ nick-translation (Manuelidis 1985) generally resulted in good hybridization results. Following ethanol dehydration, the slides were either hybridized or stored at −20°C. Slides were warmed briefly at 60°C prior to hybridization, if they had been stored at −20°C.

Probe labeling, hybridization under suppression conditions, and detection were basically performed according to methods described elsewhere (Cremer et al. 1988; Lichter et al. 1988*a;* Lichter et al. 1990*b*). The concentration of probes used was 5 µg/ml, 2.5 µg/ml, and 1 µg/ml for each autosomal cosmid contig, chromosome X–specific cosmid, and chromosome Y repeat, respectively. Hybridizations were performed in 10 µl of hybridization cocktail containing 6 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate pH 7), 10% (w/v) dextran sulfate, 50% (v/v) formamide, 100 µg sonicated human DNA/ml (except for chromosome Y repeat), 900 µg sonicated salmon DNA/ml. In a small number of samples, human COT 1 DNA (200 µg/ml; GIBCO BRL, Life Technologies, Gaithersburg, MD) was used instead of total human DNA. Probe and target DNA were denatured simultaneously under a sealed coverslip for 8 min at 80°C. Following overnight hybridization at 37°C, slides were washed three times for 5 min each in 50% formamide, 2 × SSC at 42°C, then three times for 5 min each in 0.1 × SSC at 60°C. Slides were blocked in 3% BSA, 4 × SSC at 37°C for 30–60 min. Slides were incubated with avidin-FITC (5 µg/ml) in 4 × SSC, 1% BSA, 0.1% Tween 20 at 37°C for 30 min in a moist chamber. Slides were then washed three times with 4 × SSC, 0.1% Tween 20 for 5 min at 42°C. Occasionally, biotinylated probes were detected with avidin–Texas Red (Vector, Burlingame, CA). Digoxigenin-labeled probes were detected with antidigoxigenin-FITC (Boehringer-Mannheim, Indianapolis), essentially as described above. No signal amplification was used in these studies. All studies comparing probe performance were performed as single-probe hybridizations, using biotinylated probes detected with avidin-FITC. In some experiments, simultaneous hybridization and detection of the X probe (digoxigenin/antidigoxigenin-FITC) and the Y probe (biotin/avidin–Texas Red) was carried out. The slides were mounted in 2.33% DABCO antifade (D2522; Sigma, St. Louis) in 100 mM Tris pH 8.0, 90% (v/v) glycerol prior to analysis and were occasionally counterstained with 4,6-diamidino-2-phenyl-indole (DAPI) or propidium iodide, as indicated in the figure legends. At other times, less stringent suppression conditions were used, to allow repetitive DNA to hybridize, permitting visualization of the nucleus without counterstain. All results were visualized by using a Zeiss Axioplan epifluorescence microscope. A dual band-pass filter (Omega, Brattleboro, VT) was used to visualize FITC and Texas Red simultaneously. Results were photographed directly from the microscope by using a 35 mm camera and Kodak Gold 400 film.

Comparative Analysis

To compare interphase cytogenetics with conventional cytogenetics, samples of amniotic fluid (gener-

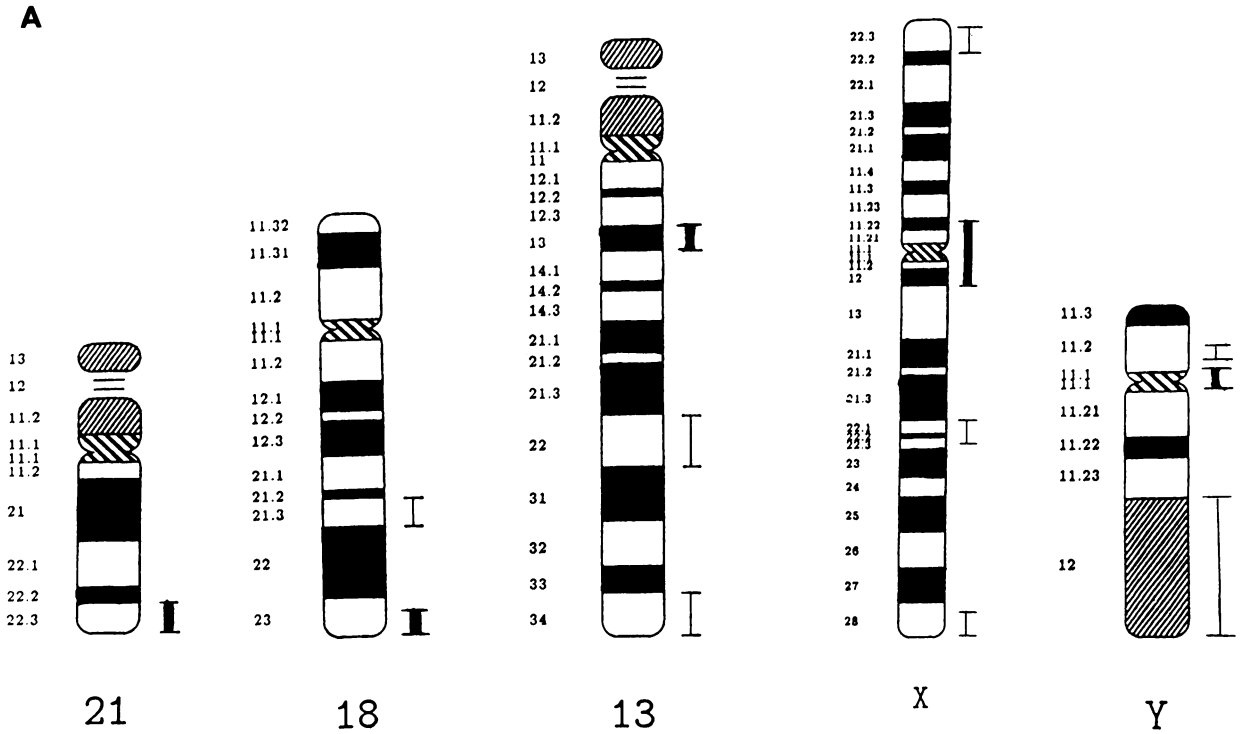
ally 1.5–5 ml) that were identified only by patient identification numbers were obtained from collaborating clinical laboratories. A standard cytogenetic analysis was carried out for each sample, by the collaborating laboratory. On receipt, the samples were further encoded by assignment of an internal Integrated Genetics Laboratories identification number. The latter was the only identifier available to the investigators carrying out this study. Thus, evaluation of probe performance was performed in a blinded manner. No cell culture was performed prior to in situ hybridization. A minimum of 50 hybridized nuclei (range 50–1,296) per probe were counted for each sample, and the number of nuclei displaying one, two, three, or four hybridization signals was recorded. Overlapping or clumped cells were not counted. Each visualized signal was counted as one signal for the purposes of statistical analysis, even if the signals were part of the closely spaced, paired signals characteristic of a G2 nucleus. Samples were then decoded and were categorized as normal or abnormal, as determined by the karyotype analysis.

Statistical Analysis

Statistical analyses of the hybridization results were performed by an independent statistician from Parexel International Corporation (Boston). These analyses were done to determine both whether the results obtained from normal and abnormal samples were significantly different and whether they could be used to define parameters for prospective identification of abnormal samples.

Parametric, univariate discriminate analysis (SAF[fi] Version 6.06) was used to generate optimal cutoff points for normal and aneuploid samples and to minimize the number of indeterminant values (grey zone). The resulting discriminate function was obtained using the pooled covariance matrix and the true prior probabilities of the classifications.

The squared distance from each assay X to its respective classification t is given by $d_t^2(X) = (X - M_t)^1 V_t^{-1}(X - M_t)$, where V_t = pooled covariant matrix. The classification-specific density estimate at each value X from classification t is provided by $F_t(X) = (2\pi)^{-p/2}|V_t|^{-1/2}\exp\ [-0.5d_t^2(X)]$. Applying Bayes' theory, the posterior probability of each value X belonging to classification t, one gets $P(t|X) = [q_tF_t(X)]/[\Sigma_u q_u F_u(X)]$, where t and u are two separate classifications. Thus, the generalized squared distance from value X to classification t was defined as $D_t^2(X) = d_t^2(X) + g(t)$, where $g(t) = -2\log_c(qt)$, because of prior probabilities of each classification being



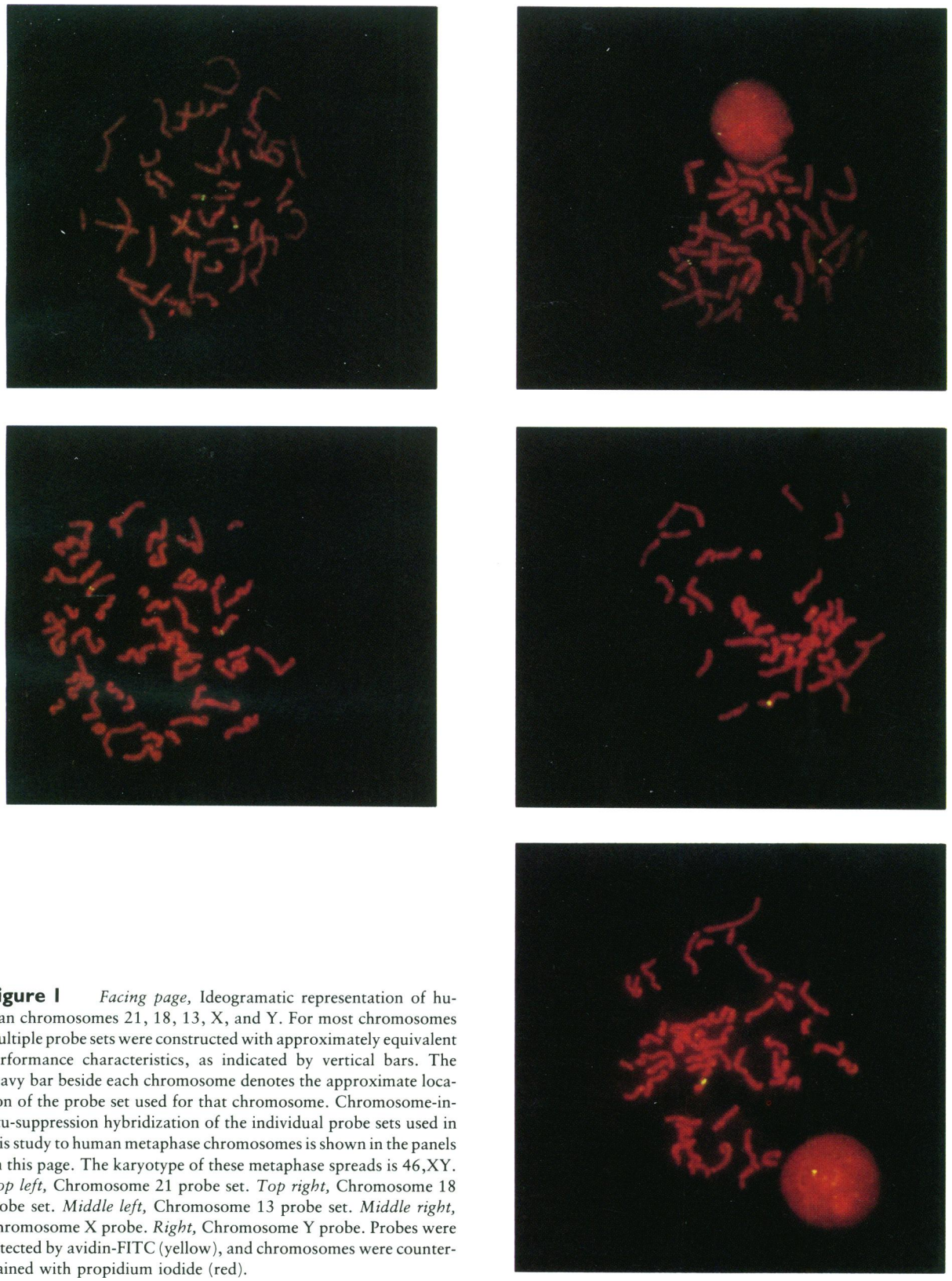
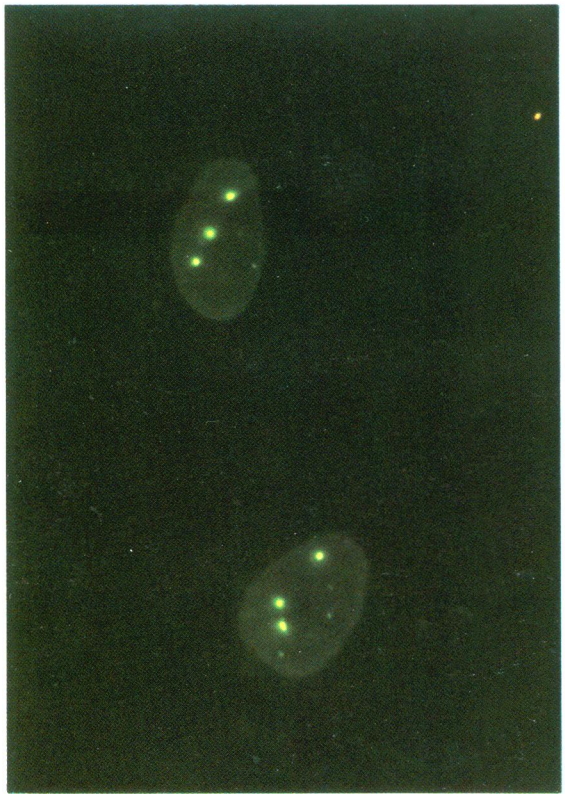
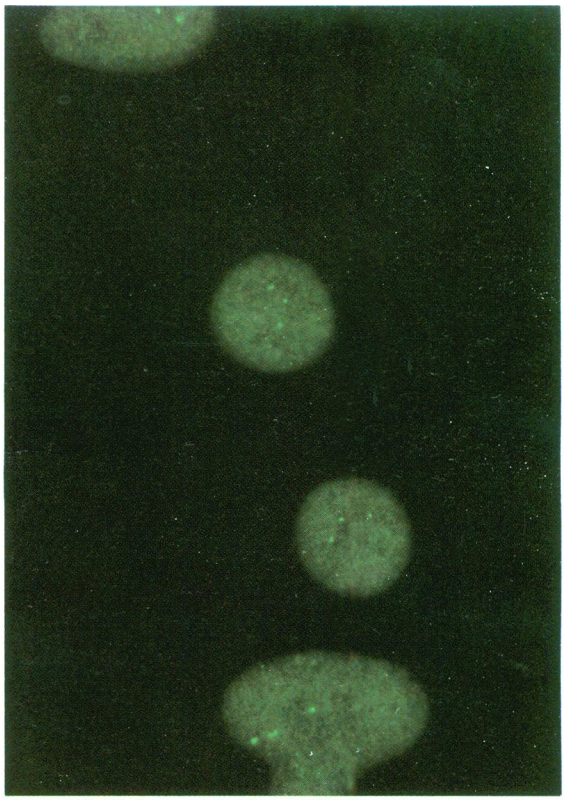


Figure 1 *Facing page,* Ideogramatic representation of human chromosomes 21, 18, 13, X, and Y. For most chromosomes multiple probe sets were constructed with approximately equivalent performance characteristics, as indicated by vertical bars. The heavy bar beside each chromosome denotes the approximate location of the probe set used for that chromosome. Chromosome-in-situ-suppression hybridization of the individual probe sets used in this study to human metaphase chromosomes is shown in the panels on this page. The karyotype of these metaphase spreads is 46,XY. *Top left,* Chromosome 21 probe set. *Top right,* Chromosome 18 probe set. *Middle left,* Chromosome 13 probe set. *Middle right,* Chromosome X probe. *Right,* Chromosome Y probe. Probes were detected by avidin-FITC (yellow), and chromosomes were counterstained with propidium iodide (red).





unequal. The resulting posterior probability that assay value X belongs to classification t is given by $P(t|X) = \{\exp[-0.5D_t^2(X)]\}/\{\Sigma_u \exp[-0.5D_u^2(X)]\}$.

Results

The locus-specific probe sets that we constructed generated bright, easily detected hybridization signals that were spatially well resolved in interphase nuclei. Typical hybridization results obtained from normal and trisomic uncultured amniocytes are shown in figure 2. Hybridization efficiencies were comparable with the results obtained in lymphocytes and cultured cells, since 85%–95% of the nuclei displayed at least one hybridization signal. This result is significantly higher than previous results obtained by ourselves and others using uncultured amniocytes; we attribute this to differences in probe composition and sample processing (Klinger et al. 1990; Yu et al. 1990; Kuo et al. 1991).

The amniotic fluid samples analyzed in this study primarily represented excess fluid remaining after the sample requirements were met for cytogenetic analysis. In all, a total of 526 independent amniotic fluid samples were analyzed. In 117 cases the amniotic fluid was analyzed using all five probe sets. The remaining fluid samples were insufficient for analysis of all five chromosomes. In these cases the sample was usually hybridized to two probe sets, although, depending on sample volume, occasionally one, three, or four chromosomes were analyzed. The choice of probe set(s) for hybridization was random in cases with insufficient volume. Three hundred four samples were hybridized to the chromosome 21 probe; 307 samples were hybridized to the chromosome 18 probe; and 320 samples were hybridized to the chromosome 13 probe. With the X and Y probes, respectively, 239 and 197 samples were analyzed. When the hybridization analysis of the uncultured amniotic fluid samples was complete, data were analyzed in the following manner: First, the percent of total nuclei counted that displayed 0, 1, 2, 3, or 4 signals was calculated as appropriate for each sample. Next, the mean domain distributions were calculated. Samples were categorized as normal or abnormal on the basis of karyotype, and these results were compared with those obtained by interphase cytogenetics.

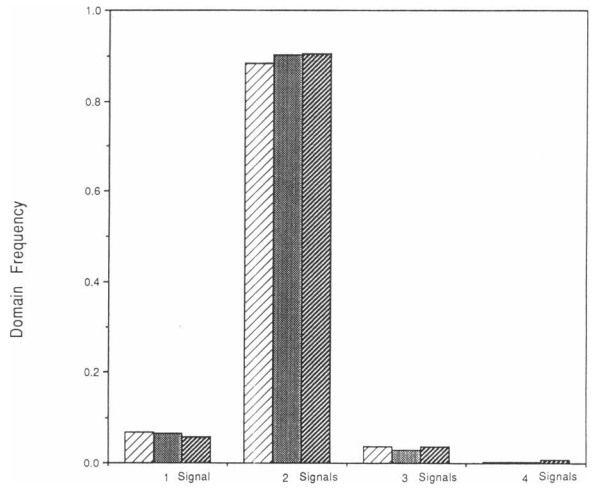


Figure 3 Results of FISH analysis of normal uncultured amniotic fluid cells, using the chromosome 21, 18, and 13 probe sets shown in fig. 2. Domain frequencies represent the mean of 291 analyses for chromosome 21, 306 analyses for chromosome 18, and 318 analyses for chromosome 13. ▨ = Chromosome 21; ■ = Chromosome 18; and ▨ = Chromosome 13.

Figure 3 summarizes the autosomal results obtained from disomic, i.e., "normal," samples. The results indicate that each of the autosomal probe sets has approximately equivalent performance characteristics and that efficient hybridization/detection was achieved. When the results from 915 analyses were pooled, on average, approximately 90% of the hybridized nuclei in any given disomic sample displayed two signals when analyzed using one of the autosomal probe sets. Only 11 samples displayed <70% two-signal nuclei, and in all disomic samples ⩾50% of the hybridized nuclei displayed two signals. Interphase cytogenetics accurately identified all samples as disomic for these autosomes. Few disomic nuclei displayed three hybridization signals ($\bar{x}$ 21 = 4%; $\bar{x}$ 18 = 3%; and $\bar{x}$ 13 = 3%). The distribution of three-signal nu-

Figure 2 Hybridization of the probe sets described in fig. 1 to uncultured amniotic fluid cells. Probes were labeled with biotin-11-dUTP and were detected with avidin-FITC, except for the bottom-right panel, in which the X probe was labeled with biotin-11-dUTP and was detected with avidin–Texas Red and in which the Y probe was labeled with digoxigenin-11-dUTP and was detected with FITC-conjugated sheep-anti digoxigenin Fab fragments. A dual band-pass filter (Omega Optical, Brattleboro, VT) was used to visualize the bottom-right panel. The top-left and middle-left panels were counterstained with DAPI. *Top left,* trisomy 21. *Top right,* Trisomy 21 caused by a Robertsonian translocation. *Middle left,* Trisomy 18. *Middle right,* Trisomy 13. *Bottom left,* Trisomy X. *Bottom right,* Normal male.

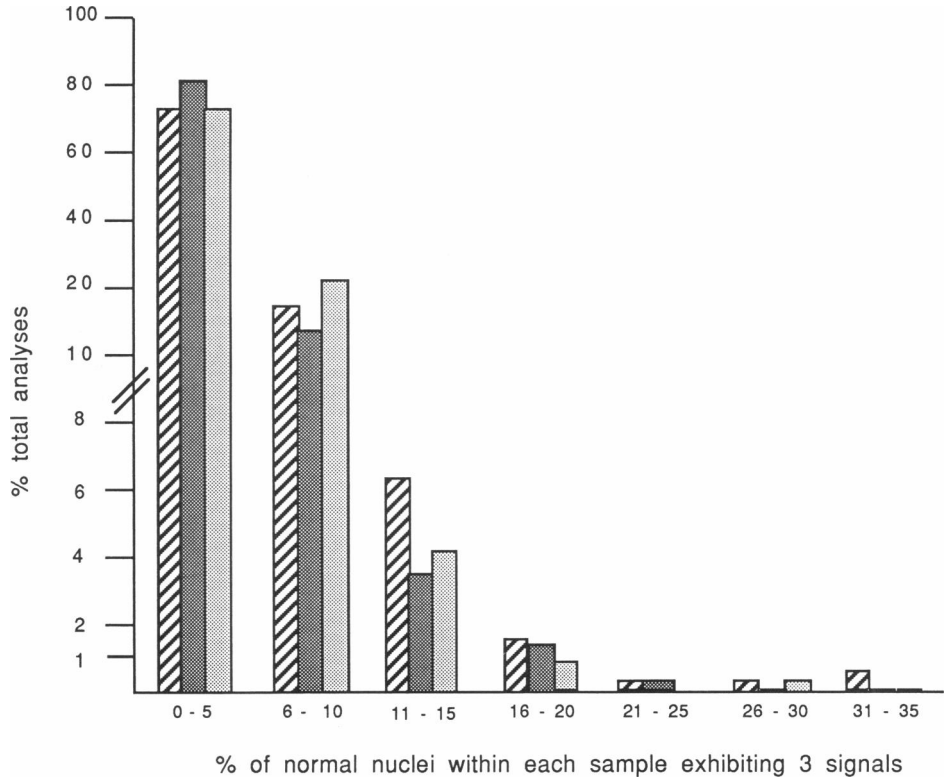


Figure 4 Distribution of three-signal nuclei in normal (i.e., disomic) samples. The frequency was calculated as a function of the percentage of cells with three signals. The majority (i.e., 855/915) of samples displayed <10% three-signal nuclei. Two samples displayed 21%–25%, two displayed 26%–30%, and two displayed 31%–35% three-signal nuclei. ▨ = Chromosome 21; ■ = Chromosome 18; and ▩ = Chromosome 13.

clei in normal samples is shown in figure 4. Similar results were obtained for the sex chromosomes, where XX and XY genotypes were clearly distinguished (fig. 5).

Twenty-one samples of abnormal karyotype were analyzed in the course of this study. Trisomy 21 represented 14 of the 21 samples; there were two cases each of trisomy 18 and 13, and there were three sex-chromosome aneuploidies (one XX/XXX and two XXY). As shown in figure 6, trisomic samples were clearly discriminated from normal samples. The frequency of trisomic cells displaying three hybridization signals was 42%–88%, with the exception of two samples with substantial maternal contamination due to the presence of maternal blood. In this data set, there was no overlap between the percent of three-signal nuclei in normal samples and the percent of three-signal nuclei in trisomic samples. Normal and abnormal genotypes were accurately assigned to all

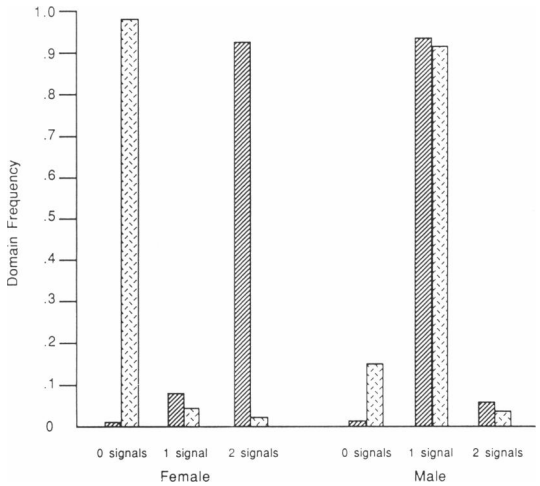


Figure 5 Results of FISH analysis of normal uncultured amniotic fluid cells, using the X and Y probes shown in fig. 2. Domain frequencies represent the mean of 239 female and 197 male analyses. ▨ = X chromosome; ⊠ = Y chromosome.

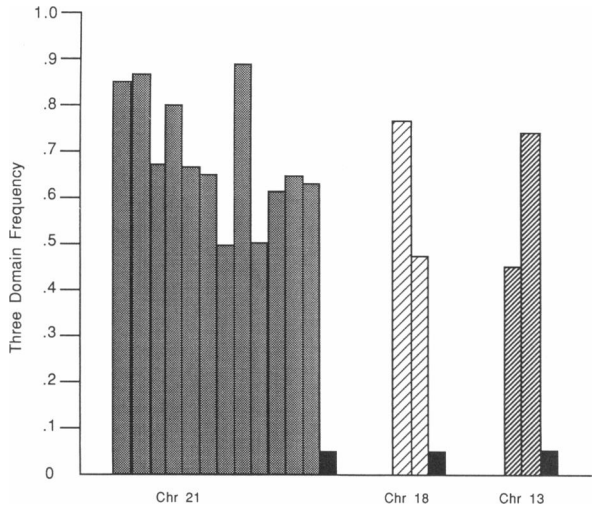


Figure 6 Results of FISH analysis of trisomic uncultured amniotic fluid cells. Each shaded vertical bar represents the three domain frequency results for one sample. Blackened vertical bars represent three domain frequencies for normal amniotic fluid cells, as shown in fig. 3.

samples. This included one case of trisomy 21 caused by a 21:21 Robertsonian translocation, shown in the top-left panel of figure 2 (88% of hybridized nuclei displayed three signals). It should be noted that, while interphase FISH correctly identified this sample as trisomic, diagnosis of a Robertsonian translocation as the cause of the trisomy was made from metaphase spreads. The hybridization efficiency was, on average, somewhat lower in trisomic samples. Although a variety of factors could contribute to decreased efficiency, we believe sample variation was a major contribution. A number of the aneuploid samples were of late gestational age, which, by our methods, have a lower hybridization efficiency.

In this study the hybridization pattern of all trisomic samples was clearly distinct from that seen in normal cells, demonstrating the sensitivity and specificity of interphase cytogenetics. However, reflecting the incidence of chromosomal abnormalities seen in cytogenetics, the number of aneuploid samples present in this series was small (n = 21). Other studies contained even smaller numbers of uncultured (Yu et al. 1990) and cultured (Kuo et al. 1991) samples, demonstrating the difficulty of accumulating significant numbers of abnormal samples. Since most samples in a clinical series will be normal, we felt that it was important to establish assay performance criteria. We therefore addressed the issue of the power of the data set to discriminate between normal and aneuploid samples, i.e., the ability to generalize a protocol from these results in order to make prospective assignments of genotype. Statistical analysis, as described in Material and Methods, showed that the data set supports the assignment of cutoff values such that (*a*) samples in which <23% of the nuclei display three hybridization signals are predicted to be disomic for a given chromosome, (*b*) samples in which ≥42% of the nuclei exhibit three signals are predicted to be trisomic, and (*c*) hybridization patterns that generate 23%–42% three-signal nuclei are held to be indeterminate (<2% of samples in this series). By these criteria, there was no overlap in the confidence intervals predictive of normal or abnormal status at the 99% level. The nonoverlapping distribution of signals obtained suggests that this technique has a direct clinical application as an indicator of the presence of trisomic cells. However, since this is a new and emerging technology, we suggest that more stringent cutoff criteria be used until more-extensive data bases are generated.

Discussion

We have demonstrated that FISH provides efficient and accurate prenatal detection of chromosomal aneuploidies in uncultured cells from amniotic fluid. In this study aneuploidies of all five chromosomes tested were easily identified. In addition, we determined the frequencies with which each hybridization pattern would occur in normal and trisomic samples, which allowed us to establish performance criteria for the assay.

In a given sample, both the percentage of cells that hybridize and the extent to which hybridization reflects the correct genotype are products of probe design and performance, hybridization efficiency, and signal-detection capability. It has been shown elsewhere that subtle variations in sample fixation, cell permeability, and probe size/complexity markedly influence hybridization/detection efficiency (Jordan 1990; Lichter et al. 1990*a;* 1991; McNeil et al. 1991). These parameters may vary with cell type, as demonstrated in earlier studies with tumor tissue (Cremer et al. 1986), and with amniocytes, in which as few as 10% of trisomic nuclei displayed three signals (Klinger et al. 1990; Yu et al. 1990; Kuo et al. 1991; Tkachuk et al. 1991). The hybridization/detection efficiency of the assay has a disproportionate impact on the ability to detect the third signal in trisomic cells and, therefore, to accurately diagnose trisomies. For example, if the aggregate probability of detecting a target chromo-

some is .9, then the probability of detecting two chromosomes in a nucleus is .9 × .9, or .81, and the probability of detecting three chromosomes is .9 × .9 × .9, or .729. It is obvious that high efficiencies must be achieved for in situ hybridization assays to have clinical utility for prenatal diagnosis. The study reported herein shows that high efficiencies can be achieved using uncultured amniocytes as target cells.

The assay would have the following performance characteristics if the diagnostic parameters described in Results (<23% three-signal nuclei designated normal and ⩾42% three-signal nuclei designated trisomic) were applied to the data reported herein: the sensitivity and specificity would be 100%, and the false-positive and false-negative rates would be 0. Two samples were excluded because of excessive maternal cell contamination, and six normal samples would have been indeterminate; that is, the percent of three-signal nuclei would be 23%–42%. Thus the positive and negative predictive values would equal 100%, and <2% of samples would be uninformative.

The most common chromosomal abnormalities in newborns are trisomy 21, with an incidence of 1/800; trisomy 18, with an incidence of 1/8,000; trisomy 13, with an incidence of 1/20,000; monosomy X (Turner syndrome), with an incidence of 1/10,000; and other sex-chromosome aneuploidies, with a combined incidence of 1/1,000 (Thompson and Thompson 1986). Cytogenetic analysis is now routinely offered only to women at increased risk of having a child with a chromosomal abnormality, the most common indications being advanced maternal age, high-risk levels of maternal serum alpha-fetoprotein (MSAFP), or combined levels of MSAFP, βHCG, and estriol, or family history. Thus, approximately 1%–3% of all prenatal karyotypes in a cytogenetics laboratory are abnormal. Together, aneuploidies of the five chromosomes studied in this report can account for up to 95% of liveborn chromosome abnormalities that are accompanied by birth defects in the child (Whiteman and Klinger 1991)—and for 67% of all chromosomal abnormalities, if one includes balanced translocations. Some women routinely referred for amniocentesis are at particularly high risk because of detection of fetal abnormalities by ultrasound, abnormal levels of serum analytes, family history, etc. We believe that rapid analysis of chromosomes 21, 18, 13, X, and Y is particularly valuable in such high-risk situations or in cases of late gestational age. We therefore designed and tested probe sets targeted to these five chromosomes. The same hybridization conditions yield equivalent performance characteristics for all five probe sets, allowing them to be used for multicolor fluorescence analyses (Klinger et al. 1991). It is, of course, possible to design equivalent probe sets for other target chromosomes, broadening the number of abnormalities detected by the assay.

Currently, chromosomal abnormalities are routinely diagnosed by conventional cytogenetic banding of metaphase chromosomes. Cytogenetic assays are accurate and can often detect quite subtle rearrangements. However, even though advances in culture methods and staining techniques have decreased the time required to perform an analysis, under the best circumstances it takes approximately 7 d to complete a test, and turn-around times of >2 wk are common. Methods that allow rapid and accurate detection of the major fetal aneuploidies are valuable, since they will provide prospective parents and medical practitioners with sufficient time to consider the test results thoughtfully and to develop an appropriate course of action. Analysis of interphase chromosomes by using FISH is extremely rapid. The results of the present study support the utilization of this technology to enhance standard cytogenetics, allowing accurate identification of trisomic chromosome constitution in uncultured amniocytes in significantly less time. Subsequent cytogenetic analysis ensures that less common chromosomal abnormalities will also be detected.

Acknowledgments

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