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Analysis of Reciprocal Translocations by Chromosome Painting: Applications and Limitations of the Technique

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Summary

Fluorescent in situ hybridization with chromosome-specific DNA libraries (chromosome painting) is an important new method for assessing chromosome rearrangements. In the research presented in this paper, two familial reciprocal translocations have been studied in the balanced and unbalanced forms, using both traditional G-banding techniques and chromosome painting. Although for each case two chromosomes were involved in the rearrangement, we found that only one chromosome library was suitable for detecting the translocation. These findings illustrate both the potential and the limitations of chromosome painting as a diagnostic tool in cytogenetics.

Introduction

Fluorescent in situ hybridization has been applied to cytogenetic analysis in both clinical and research situations, with the introduction of centromere-specific repetitive probes. Recently, its use has been expanded with the availability of DNA libraries made from whole chromosomes or from specific regions of chromosomes (Lichter et al. 1988; Pinkel et al. 1988). This approach, called "chromosome painting" (Pinkel et al. 1988), requires suppression of repetitive sequences that are shared with other chromosomes. Painting of chromosomes allows a rapid and striking visualization of chromosomal translocations and represents an important new method for assessing rearrangements (Jauch et al. 1990).

In the present paper, we report two familial translocations, one ascertained after the birth of a child with an unbalanced chromosome translocation and the other referred after abnormal findings in an ultrasound examination. In both cases chromosome-specific DNA libraries were used to visualize the translocation and to confirm the nature of the chromosome rearrangement. In each case, however, one of the libraries we used failed to detect a derivative chromosome.

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Methods and Cases

Cell Culture

Metaphase chromosomes were prepared from samples of both direct and cultured chorionic villi (Simoni et al. 1983; Cheung et al. 1987), amniocyte clones, skin fibroblasts, and peripheral lymphocytes. Chromosome banding was produced by a standard trypsin-Giemsa method (Seabright 1971).

In Situ Hybridization and Immunofluorescence

Probe collections pBS3, pBS13, pBS14, and pBS15 were supplied by J. W. Gray (Lawrence Livermore National Laboratory). For the alphoid sequences of chromosomes 13/21, probe L1.26 was used (Devilee et al. 1986). Probes were biotin labeled by nick-translation (Bethesda Research Laboratories), precipitated in the presence of placental DNA (Sigma), resuspended in 50% formamide, 2 × SSC, 5%–10% dextran sulfate and allowed to preanneal for 1–3 h

after denaturation. The probe mixture was added to denatured metaphase chromosomes and hybridized at 37°C for 25–35 h. After incubation, the slides were washed at 42°C in 50% formamide, 2 × SSC and then in 2 × SSC. Fluorescent detection was accomplished by an incubation in 4 × SSC, 5% nonfat dry milk with fluorescein-avidin (Vector Laboratories), followed by one cycle of amplification. Slides were counterstained with propidium iodide (0.4 μg/ml) in antifade solution (Johnson and Araujo 1981) and were examined using a Leitz Aristoplan microscope equipped with an FITC broad-band-pass filter. For each case, a minimum of 30 metaphase spreads were examined with each library.

Case 1

The patient was a healthy 28-year-old gravida 3 para 1-0-1-0 (one elective abortion) whose first child was a full-term male who had died at 3 mo of age, with multiple major and minor anomalies. These included microcephaly, hydrocephaly, bilateral proptosis and microtia, a flattened nasal bridge, flat philtrum, cleft palate, micrognathia, micropenis, tapering digits with bilateral clinodactyly of the second fingers, and right talipes equinovarus. The infant was small for gestational age, and chromosomal analysis revealed an unbalanced reciprocal translocation. The mother was found to be a balanced translocation carrier. In her next pregnancy, chorionic villus sampling was performed transabdominally at 12 wk gestation.

Case 2

A 27-year-old gravida 5 para 1-0-3-1 was referred for a perinatal genetics consultation at 33½ wk gestation. She had two 8-wk spontaneous abortions and one 20-wk stillbirth. She also had one healthy son. At 31 wk of the current pregnancy, an obstetrical sonogram had shown intrauterine growth retardation. Right-sided displacement of the fetal heart was also present, as was a two-vessel umbilical cord. Amniotic fluid was obtained for cytogenetic analysis. Subsequent sonograms detected a left-sided diaphragmatic hernia, and fetal echocardiography revealed complex congenital heart disease. At 39 wk the patient delivered a 1,280-g female who expired 20 min after birth. Postmortem examination noted, in addition to the prenatal findings, low-set and posteriorly rotated ears, mild micrognathia, fifth fingers with only a single phalangeal crease, and bilateral single transverse palmar creases. Clinodactyly of the second and third toes was present, and nails were hypoplastic on all digits.

Results

Case 1

G-band analysis of the mother's karyotype confirmed a balanced reciprocal translocation: 46,XX, t(13;14)(q12;q24) (fig. 1). The direct chorionic villi chromosome preparation showed an unbalanced reciprocal translocation: 46,XY, − 13, + der(14)t(13; 14)(q12;q24)mat. The fetus was trisomic for most of chromosome 14 and was monosomic for a small region of chromosome 13, and the pregnancy was terminated. The cytogenetic diagnosis was confirmed in both cultured chorionic villi and skin fibroblasts.

In metaphase spreads from the mother, fluorescent in situ hybridization with the chromosome 14 library painted the normal 14, the proximal region of the der(14), and most of the der(13) (fig. 2*a*). In the fetus, two copies of chromosome 14 and one copy of the der(14) were fluorescent (fig. 2*b*). In the mother and the fetus, hybridization of this same material with the chromosome 13 library painted both one copy of chromosome 13 and the distal region of the der(14) (fig. 2*c* and *d*). The der(13), present in the mother, was not painted by this library. Hybridization with a 13/21 alphoid repeat probe to metaphase spreads from the mother showed fluorescence at the centromeric area of one D-group chromosome presumed to be the normal 13, two G-group chromosomes presumed to be the 21's, and one 18-like chromosome presumed to be the der(13) (fig. 2*e*).

Case 2

Cytogenetic analysis of cultured amniocytes had revealed a small deletion of the distal long arm of one fetal chromosome 15. The mother was found to carry the same terminal deletion on chromosome 15, but since she was phenotypically normal her karyotype

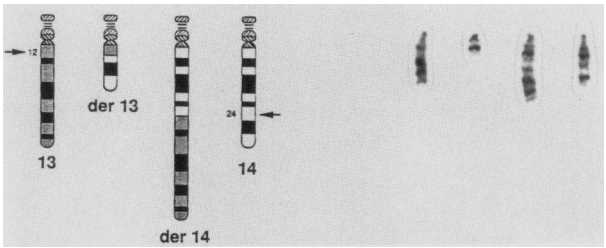
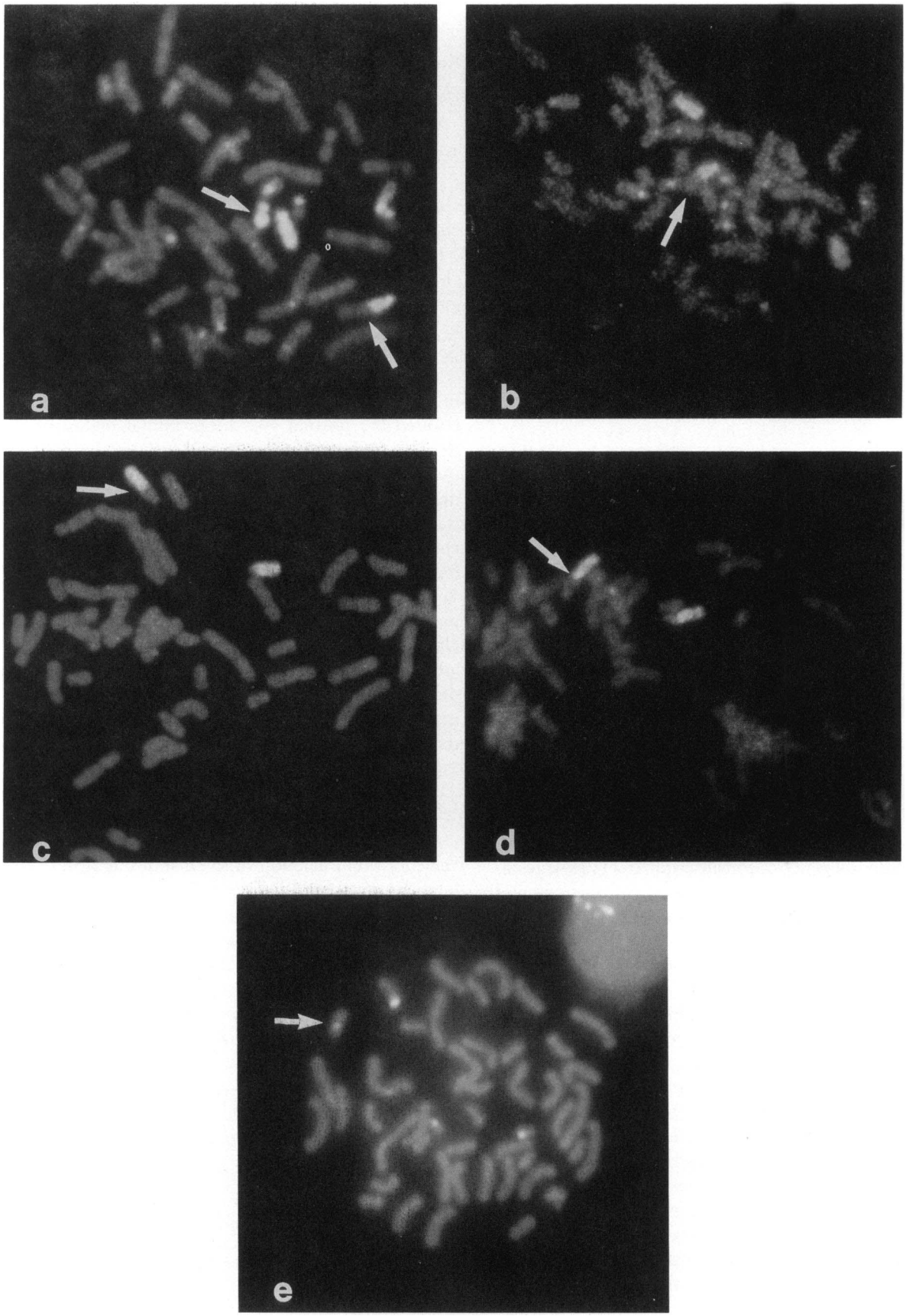


Figure 1 Balanced reciprocal translocation in mother (case 1). t(13;14)(q12;q24) is shown as a schematic diagram (*left*) and as G-banded chromosomes (*right*). Arrows indicate chromosomal breakpoints.


a
b
c
d
e

was assumed to be balanced. An extensive analysis of the mother's chromosomes by both in situ hybridization and G-banding revealed a balanced translocation: 46,XX,t(3;15)(q29;q26.1) (fig. 3), accordingly: 46, XX, − 15, + der(15)t(3;15)(q29;q26.1)mat. These findings were confirmed by studies of cord blood and placental and skin biopsies, all obtained at birth.

Fluorescent in situ hybridization with the library of chromosome 15 painted two chromosomes 15 in metaphase spreads from the mother and from the baby and, in the mother, the distal region of the der(3) (fig. 4*a* and *b*). When metaphase spreads of the mother were analyzed with the chromosome 3 library, both copies of chromosome 3 were painted, but no fluorescence could be detected on the distal end of the der(15), nor could a nonfluorescent region be distinguished on the terminus of the der(3) (fig. 4*c*).

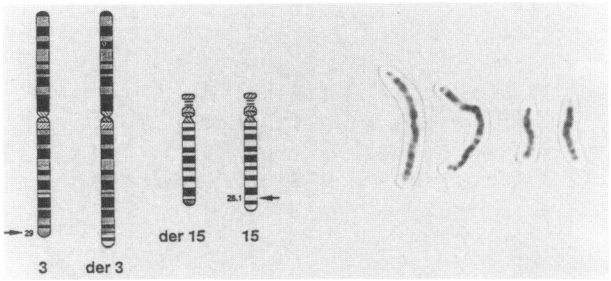


Figure 3 Balanced reciprocal translocation in mother (case 2) t(3;15)(q29;q26.1) shown as schematic diagram (*left*) and as G-banded chromosomes (*right*). Arrows indicate chromosomal breakpoints.

Discussion

The cases presented here demonstrate the potential that DNA libraries and fluorescent in situ hybridization have for enhancing the resolution of prenatal cytogenetic analysis, and they also illustrate the limitations of this technique. In case 1, we were able to characterize the unbalanced karyotype of the fetus by G-banding. Using the chromosome 14 library, we could detect both of the chromosomes involved in the rearrangement—the der(13) and the der(14) (fig. 2*a* and *b*). However, cross-hybridization with most of the acrocentric chromosomes was seen, and the small derivative chromosome was completely fluorescent (fig. 2*a*). The chromosome 13 library failed to detect the der(13) in the mother, where it was known to be present; no centromeres were labeled in this preparation (fig. 2*c*). A high degree of homology among centromeric areas of acrocentric chromosomes is a well-described fact, and suppression of these areas is completely selective. While a more efficient suppression blocks hybridization to all centromeres, a less efficient one allows hybridization to several acrocentric chromosomes. Hybridization with an alphoid repeat probe for chromosomes 13/21 was necessary (fig. 2*e*) to prove that the small derivative in fact carried a 13 centromere—and not just chromosome 14 material, as figure 2*a* and *c* might suggest.

The reciprocal translocation in case 2 was very small, involving only a single band of chromosome material. Because of its size, this translocation was difficult to spot by traditional banding techniques alone. Since this translocation is easily detected with the chromosome 15 library, it will be possible to use chromosome painting to screen family members at risk for carrying the balanced translocation.

For this case, painting with the chromosome 3 library did not show the reciprocal translocation of chromosome 3 material onto the der(15). This rearrangement was apparently below the level of resolution of both G-banding and chromosome painting. Also, the material from chromosome 15, translocated to the der(3), that could easily be visualized with the chromosome 15 library could not be clearly distinguished as a nonfluorescent region when the chromosome 3 library was used. This was not unexpected, since a positive staining detection has a greater sensitivity than a negative one.

Analysis of reciprocal translocations by chromosome painting has some limitations. As documented here, not all libraries have equal specificity and sensitivity in detecting different chromosomal regions. The uneven representation of DNA from different areas of the chromosome may be an artifact of cloning and subsequent amplification of the library but can also result from preferential suppression of some areas, as in case 1 here. The examination of similar translocations with different breakpoints or the use of other libraries can lead to results different from the ones we

Figure 2 Fluorescent in situ hybridization with chromosome 14 library in metaphase chromosomes from balanced carrier (maternal blood) (*a*) and from fetus showing partial trisomy of chromosome 14 (cultured chorionic villi) (*b*); hybridization with chromosome 13 library in metaphase chromosomes from mother (*c*) and fetus (*d*); the der(13) did not fluoresce in (*c*) or (*d*). Hybridization with the probe for 13/21 alphoid repeats in metaphase chromosomes from mother (*e*). Arrows indicate derived chromosomes.

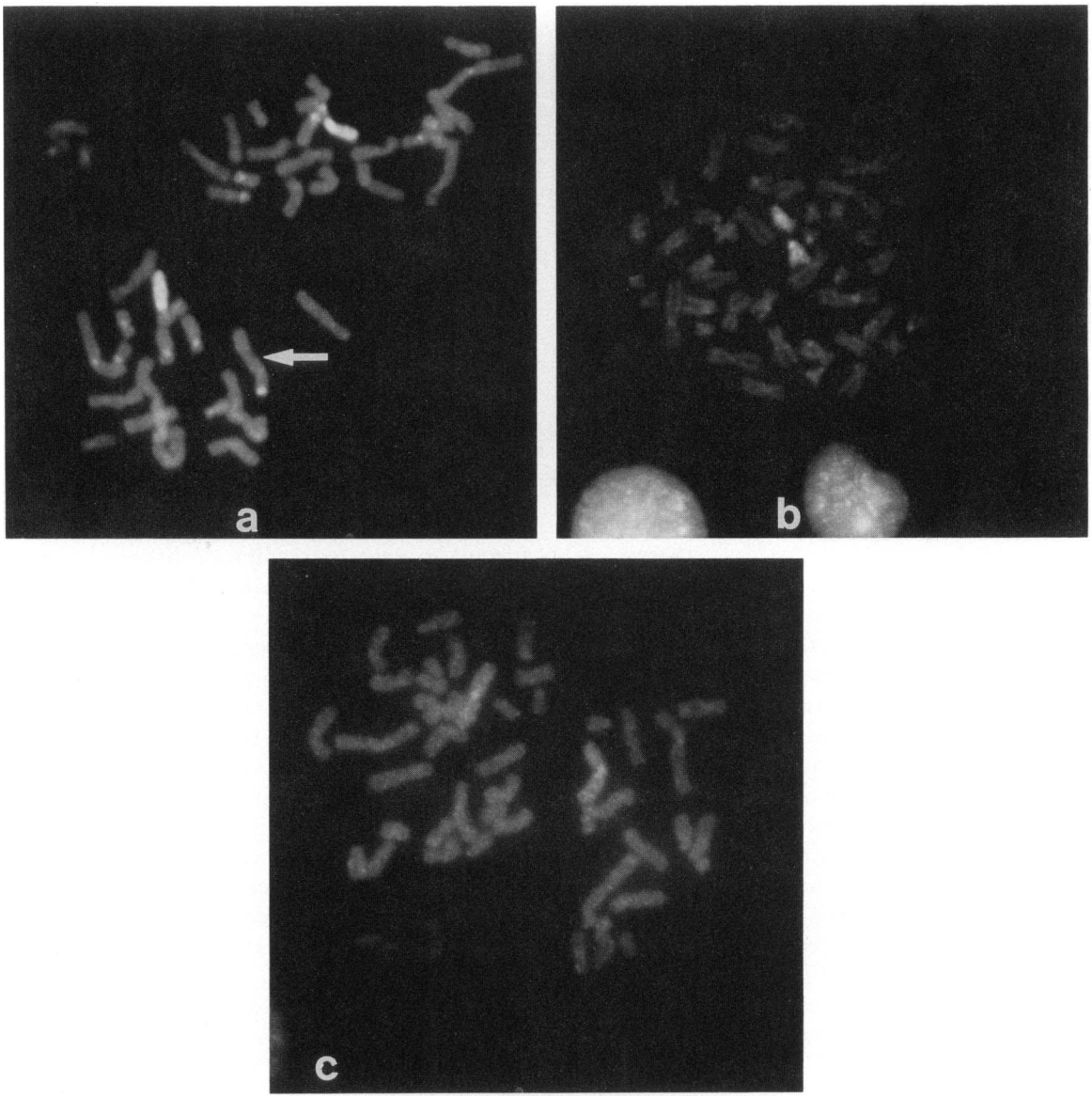


Figure 4 Fluorescent in situ hybridization with chromosome 15 library in metaphase chromosomes from balanced carrier (*a*) and affected child (*b*). The arrow indicates the der(3). Hybridization of chromosome 3 library with metaphase chromosomes from mother is also shown (*c*). Note that the distal tip of the der(15) did not fluoresce.

obtained here. For example, Jauch et al. (1990) have reported detection of a der(13) by using a chromosome 13–specific library. Whenever possible, the balanced-translocation carrier should be studied in order to estimate the utility that a given library has in characterizing the unbalanced translocation. If these limitations are taken into consideration, chromosome painting provides a new and valuable tool for analysis of rearranged chromosomes.

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