Characterization of De Novo Duplications in Eight Patients by Using Fluorescence In Situ Hybridization with Chromosome-specific DNA Libraries

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Summary

Fluorescence in situ hybridization (FISH) with chromosome-specific DNA libraries was performed on samples from eight patients with de novo chromosomal duplications. In five cases, the clinical phenotype and/or cytogenetic evaluations suggested a likely origin of the duplicated material. In the remaining three cases, careful examination of the GTG-banding pattern indicated multiple possible origins; hybridization with more than one chromosome-specific library was performed on two of these cases. In all cases, FISH conclusively identified the chromosomal origin of the duplicated material. In addition, the hybridization pattern was useful in quantitatively delineating the duplication in two cases.

Introduction

Fluorescence in situ hybridization (FISH) has rapidly become a highly valued adjunct in clinical diagnostic cytogenetics. One fundamental advantage of FISH over conventional cytogenetic methods is the ability to define the makeup of chromosomal material currently unidentifiable by routine banding or special staining procedures. FISH with pericentric-specific repetitive DNA probes has been used for the identification of small marker chromosomes (Callen et al. 1990, 1991; Schwartz et al. 1990), as well as for the detection of aneuploidy in prenatal and perinatal samples (Klinger et al. 1991; Ward et al. 1991; White and Schwartz 1991) and of various neoplasias (Hopman et al. 1988; Devilee et al. 1989; Nederlof et al. 1989). Although cytogenetic abnormalities not involving a loss, gain, or rearrangement of pericentromeric material cannot be identified using these probes, others have demonstrated the utility of whole-chromosome composite DNA probes ("chromosome painting") in such cases (Pinkel et al. 1988; Jauch et al. 1990). We have employed chromosome painting with chromosome-specific libraries to examine eight cases of de novo chromosomal duplication.

Subjects and Methods

Case Reports

Case 1.—A black male was referred for genetic evaluation at birth, because of features consistent with trisomy 13, including bilateral cleft lip and palate, right iris coloboma, hemangiomata, flattened nasal bridge, and polydactyly. Cytogenetic analysis of peripheral blood lymphocytes revealed a karyotype of 46,XY,18q-.

Case 2.—A 3.5-mo-old white female was karyotyped because of features consistent with trisomy 13, including growth retardation, microcephaly, hypotelorism, absent corpus callosum, hemangiomata, malformed ears, and a high arched palate. The patient was diagnosed cytogenetically as 46,XX/46,XX,10p+. The der(10) was found in 87% of lymphocytes and in 100% of skin fibroblasts (Schwartz et al. 1991).

Case 3.—A 1-d-old female was referred for genetic evaluation, because of features consistent with trisomy 18, including cleft lip, micrognathia, prominent occi-
put, posteriorly rotated ears with malformed helices, cardiac anomalies (VSD, PDA, and aortic arch obstruction), hypoplastic nails, and bilateral overlapping fingers (second digit over third, fifth digit over fourth). Cytogenetic analysis showed a karyotype of 46,XX,13p+.

Case 4.—A 1-d-old white male was referred for genetic evaluation, because of the presence of multiple congenital anomalies, including prominent occiput, hypertelorism, down-slanting palpebral fissures, low-set and posteriorly rotated ears with malformed helices, mild pectus excavatum, undescended testes, and hypotonia. The patient was diagnosed cytogenetically as 46,XY,11q+.

Case 5.—A 10-year-old white female was referred for genetic evaluation, because of learning disabilities, minor dysmorphic facial features, and behavioral problems. Cytogenetic analysis revealed a karyotype of 46,X,Xq+.

Case 6.—Prenatal diagnosis performed for advanced maternal age revealed a 46,XY/46,XY,12p+ fetal karyotype. The pregnancy was continued, and a male infant was delivered at term, with the following phenotypic abnormalities: low-set posteriorly rotated right ear, elfin left ear, slight micrognathia, prominent nasal bridge, and hypotonia. The mosaic karyotype was confirmed by a peripheral blood sample at birth; the der(12) was present in 60% of peripheral blood lymphocytes.

Case 7.—A 3-d-old white male was referred for genetic evaluation, because of the presence of multiple congenital anomalies, including up-slanting palpebral fissures, colobomata, large beaked nose with prominent nasal tip, micrognathia, wide-spaced nipples, syndactyly, hypoplastic nails, and coronal hypospadias. The patient was diagnosed cytogenetically as 46,XY,17q-.

Case 8.—A 9-year-old white female was referred for genetic evaluation, because of facial dysmorphism (thick eyebrows, up-slanting palpebral fissures, prominent nasal bridge, and anteverted nares), seizures, and learning disabilities. Cytogenetic analysis revealed a karyotype of 46,XX/46,XX,22q+. The der(22) was present in 36% of peripheral blood lymphocytes.

In all cases, parental karyotypes were normal.

### Cytogenetic Studies

Metaphase chromosomes were prepared from phytohemagglutinin (PHA)-stimulated cultures (cases 1–8) and from fibroblast cultures (case 2) by using standard procedures. GTG-banding was performed as described by Seabright (1971).

#### DNA Probes

Whole-chromosome composite DNA libraries for chromosomes 13, 17, and 18, prepared in Bluescribe plasmids (Collins et al. 1991), were provided by Dr. J. W. Gray (Lawrence Livermore National Laboratory, Livermore, CA). Library DNA was amplified via plasmid preparation, and purified whole plasmids were biotin-labeled using the BioNick labeling system (Bethesda Research Laboratories, Gaithersburg, MD). Biotin-labeled libraries for chromosomes 3, 4, and 12 were provided by Imagenetics™ (Naperville, IL). A biotin-la-
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Results

Cytogenetic Analyses

A summary of the phenotypic features and karyotypes of the eight cases is given in Table 1. Partial GTG-banded karyotypes depicting the abnormal chromosomes of each case are shown in Figure 1. Cases 2, 6, and 8 were mosaic, each with a normal cell line in addition to the cell line containing the derivative chromosome. In cases 2–5, the large size of the duplication allowed the presumptive identification of its chromosomal origin by GTG-banding. However, in case 5, the breakpoints within the derivative chromosome could not be conclusively determined. The duplications in cases 1 and 6–8 could not be identified by standard GTG-banding alone. In all cases, parental karyotypes were normal, suggesting that the duplicated material was the product of a de novo rearrangement.

FISH

Chromosomal in situ suppression (CIS) hybridization with the Lawrence Livermore libraries was performed according to the method of Lichter et al. (1988), with minor modifications. Both freshly prepared, heat-aged slides and previously GTG-banded slides up to 1 year old were used for hybridization. The probe mixture consisted of 20 μg total library DNA/ml, with 1–2 mg Cot-1 (human competitor) DNA (Bethesda Research Laboratories)/ml, in 55% formamide, 1 × SSC, 10% dextran sulfate, pH 7.0. This mixture was denatured (at 70°C for 5 min), preannealed (at 37°C for 1 h), and then applied (2 μl/cm²) to denatured (at 70°C for 2 min; 70% formamide, 2 × SSC, pH 7.0) slides, which were immediately placed in a moist chamber and incubated at 37°C for 72–120 h. After signal amplification using fluorescein-labeled avidin and biotin-conjugated antivin (both at 5 μg/ml; Vector Laboratories, Burlingame, CA), chromosomes were mounted in an antifade solution (Johnson and de Araujo 1981) containing propidium iodide (2 μg/ml) as a counterstain and were visualized using fluorescence microscopy. Hybridization with the chromosome 3, 4, and 12 libraries was performed according to the manufacturer’s instructions (WCP™ Manual; Imagenetics™), with the following exceptions: 4 × SSC, 0.1% Tween 20 was used in place of PN buffer (0.1 M sodium phosphate, 0.05% Nonidet P-40, pH 8), and detection reagents were made up in 1% BSA, 4 × SSC, 0.1% Tween 20, in place of PNM buffer (PN buffer, 5% nonfat dry milk, 0.02% Na azide).

Figure 1 Partial GTG-banded karyotypes of patients 1–8 (a–h, respectively). The derivative chromosome is indicated by an asterisk in each case. Breakpoints within the derivative chromosome are denoted by arrows on the normal homologue(s), interpreted as follows: a, der(18)t(13;18) (q31;q21); b, der(10)t(10;13) (p15;q14.2); c, der(18)t(13;18) (q11;p11); d, der(11)t(4;11) (q28;q24); e, der(X)t(X;3) (q13;q13); f, dir dup (12) (p13.1p13.3); g, inv dup(17) (q21.3q22); and h, der(22)t(3;22) (q25;q13.3).

beled alpha-satellite probe for the X chromosome was obtained from Oncor (Gaithersburg, MD).
FISH and GTG-banding suggested that the derivative chromosome was a dir dup(12)(p13.1p13.3).

Cases 7 and 8 necessitated multiple hybridizations to identify the chromosomal origin of the duplication. The duplicated material in case 7 was initially thought to be derived from chromosome 1 by GTG-banding (fig. 1g). However, hybridization with pBS-1 demonstrated that the duplication was not derived from chromosome 1. An alternative interpretation of the banding pattern implicated chromosome 17 as a possible source of the extra material, which was confirmed by hybridization with pBS-17 (fig. 2g).

Results of GTG-banding of the duplicated material in case 8 resembled those of GTG-banding of distal 13q (fig. 1h). Hybridization using pBS-13 revealed the presence of two structurally normal chromosome 13's in all cells. A re-examination of the GTG-banded karyotype identified the terminal portion of chromosome 3q as a
likely source. Subsequent hybridization with the chromosome 3 library produced positive results (fig. 2h).

Discussion

The identification and interpretation of de novo chromosomal duplications poses a recurrent diagnostic problem in clinical cytogenetics. Often, a combination of phenotype and banding patterns may suggest a possible source of the extra material. However, further studies are usually necessary to verify the proposed origin, in order to appropriately diagnose and counsel patients.

Dosage studies using biochemical or DNA markers...
are often performed when banding patterns alone are inadequate to characterize a duplicated segment (Schwartz et al. 1984; Lavedan et al. 1989). The duplication in patient 2 was confirmed as material from 13q by using this approach (Schwartz et al. 1991). However, these studies can be very labor intensive, particularly when several different chromosomal regions may be considered as potential sources of the duplication. Often, adequate biochemical or DNA markers are not readily available, and the results of dosage studies may be ambiguous or difficult to interpret for a variety of reasons, including patient mosaicism.

FISH with chromosome-specific DNA libraries provides an advantageous alternative to dosage studies, in characterizing karyotypic abnormalities. Plasmid libraries (Collins et al. 1991) specific for most of the 24 human chromosomes are now available and have been used both to identify chromosomes involved in subtle translocations (Sullivan et al. 1991) and to clarify complex karyotypes in solid tumors (Smit et al. 1991). In the present study of eight de novo duplications, this approach was successfully used to identify the chromosomal origin of the duplication in each case. These cases can be divided into three groups: those with features reminiscent of a chromosomal syndrome (cases 1–3), those whose GTG-banded karyotype allowed presumptive identification of the duplicated material (cases 2–5); and those with nonspecific phenotypes and whose GTG-banded karyotypes were amenable to multiple interpretations (cases 6–8). FISH with a single library confirmed the cytogenetic impression in the first two groups of patients, while multiple hybridizations were necessary to identify the chromosomal source of the duplication in two of three patients in the third group. These latter cases illustrate the primary advantage of FISH over dosage studies: several hybridizations can be performed easily and rapidly, often on the original sample obtained for cytogenetic analysis, thus eliminating the need to obtain additional patient material.

Three patients (cases 2, 6, and 8) were mosaics for the de novo duplicated material, and two of these (cases 6 and 8) presented with nonspecific phenotypes, perhaps reflecting the mosaic nature of their karyotypes. FISH was found to be particularly effectual in these cases, by allowing the rapid screening of a large number of metaphases. Conversely, dosage studies performed on such cases are often difficult to interpret.

FISH with chromosome-specific libraries may also be used to estimate the amount of material that is duplicated. In case 1, the breakpoints in chromosomes 13 and 18 could not be clearly defined by the GTG-banding pattern of the der(18) alone. After FISH, the duplicated material was estimated to represent approximately one-third of 13q, allowing the assignment of the breakpoint at 13q31, rather than more distally. The initial interpretation of the GTG-banding pattern of the der(X) in case 3 placed the breakpoints at Xq23 and 3q21. After hybridization with PBS-3, the duplicated portion of 3q was found to extend almost to the centromere of the X chromosome. The breakpoints within the derivative chromosome were then redefined as der(X)t(X;3) (q13;q13).

In both case 6 and case 7, the duplicated material was derived from the same chromosome in which it was found, although the specific chromosomal region duplicated could not be confirmed using the DNA libraries alone. In a similar case, Speleman et al. (1991) were able to characterize a de novo duplication of 11p as a direct duplication by using FISH with a single-copy probe, in combination with Southern hybridization and dosimetry. Applying FISH with single-copy probes mapping to 12p (case 6) or 17q (case 7) would yield additional information about the precise origin of the duplicated segment in these cases.

These results clearly demonstrate that FISH with chromosome-specific DNA libraries can provide rapid, unequivocal cytogenetic diagnosis for patients with de novo chromosomal duplications. Such definitive identification allows for a better understanding of the long-term prognosis for these patients and enables caregivers to provide appropriate counseling. As the number of these cases grows, karyotype-phenotype correlations can be further defined for subchromosomal portions of the human genome.

Acknowledgments

We gratefully acknowledge Lawrence Livermore National Laboratory and Imagenetics for providing human chromosome libraries. In addition, we would like to thank Rachel Falkler for her expert technical assistance.

References


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