IDENTIFICATION OF iso(18p) MARKER CHROMOSOME BY FLUORESCENCE *IN SITU* HYBRIDIZATION WITH SINGLE-COPY DNA PROBE

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Summary The patient displayed the clinical features consistent with tetrasomy (18p) syndrome, who had an extra small metacentric iso(18p) chromosome in otherwise normal karyotype. Identification of the marker chromosome used the chromosome 18 band-specific fluorescence *in situ* hybridization strategy.

Key Words fluorescence *in situ* hybridization (FISH), tetrasomy 18p syndrome, iso(18p) chromosome

INTRODUCTION

Many cases of extra marker chromosomes have been reported, including familial and 'de novo' events. In the inherited cases the extra chromosome may not appear to be causally related to the phenotype of the probands. The *de novo* cases present a variety of clinical pictures depending on the different origins of the markers. Small marker chromosomes cannot be easily identified using standard cytogenetic banding techniques because they lack a distinct banding pattern. Marker chromosomes may be associated with a wide range of phenotypes, making it important to identify the chromosomal origin of the markers so that correlations with a clinical phenotype can be evaluated (Froland *et al.*, 1963; Lin *et al.*, 1990; Callen *et al.*, 1990; Mewar *et al.*, 1993).

To develop a method that can differentiate between the iso(18p) and other markers including the 18q- chromosome, cosmid probes were cloned from both arms of chromosome 18 using a somatic cell hybrids, which were then mapped to chromosome 18 by fluorescence *in situ* hybridization (FISH) (Nakashima *et al.*,

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1993). The probe was labeled with biotin and used to identify rearrangements involving the chromosome 18. This approach was used to analyze one patient with the phenotype of tetrasomy(18p).

MATERIALS AND METHODS

Cytogenetic analysis. The patient is a 15-year-old boy. He has microcephaly, epicanthal folds, hypotelorism, hypotonia, a single simian crease, and mildly delayed development. Chromosomes were prepared from peripheral lymphocytes (PBLs) and the karyotype was analyzed by the standard GTG-banding procedure. To induce R-banding stimulated PBLs were synchronized by blocking the cell cycle for 16 h with excess thymidine, and were treated with 5-bromo-2'-deoxyuridine (BrdU) during the final late S-phase before harvesting (Viegus-Pequignot and Dutrillaux, 1978). For R-banding and FISH analysis, chromosome spreads were stained with Hoechst 33258 and were exposed to UV light for 10 min (Takahashi *et al.*, 1991). To induce multiple 4',6-diamino-2-phenylindole (DAPI)-banding, denaturation time was shortened to 30–60 sec as described (Heng and Tsui, 1993), and the step of UV exposure was omitted.

Fluorescence in situ hybridization (FISH). A set of chromosome 18-specific DNA probes were obtained from a cosmid DNA library derived from the cell line MS126, a somatic cell hybrid containing a normal chromosome 18 as its only human component (Nakashima *et al.*, 1993). Cosmid SCW0204F was hybridized specifically to the short arm at 18p11.2, and SCW0207A hybridized to the pericentric region of chromosome 18.

FISH was performed according to the methods described elsewhere (Lichter *et al.*, 1988; Takahashi *et al.*, 1991). Cosmid DNAs were labeled by nick translation using the enzyme kit (Boehringer Mannheim, GmbH) with biotin-11-dUTP (Boehringer Mannheim) and hybridized to chromosome DNA under conditions that suppress signals from repetitive sequences (Lichter *et al.*, 1988). Hybridization signals were localized to a specific chromosome region either by simultaneous R-banding or by DAPI-banding analysis of the same metaphase spread. Hybridized probes were detected by means of fluorescein isothiocyanate (FITC) conjugated to avidin (Vector Laboratory, CA). Fluorescent signals were amplified with biotinylated anti-avidin antibody (Vector Laboratories) (Pinkel *et al.*, 1988). Signals on both metaphase and interphase nuclei were viewed using a Zeiss Axiophot epifluorescence microscope (Carl Zeiss Mikroskop-System, Germany) equipped with a cooled Charge Coupled Device (CCD) camera (Astromed, Cambridge, England).

RESULTS

Chromosome analyses revealed a karyotype, 47,XY,+mar, for nearly all cells examined. The marker chromosome was metacentric and potentially an iso(18p). A probe previously localized on the short arm of chromosome 18 was selected for

Fig. 1. Three partial metaphases showing the specific site of probe hybridization. A: G-banding partial karyotype showing the normal copies of chromosome 18 and a marker chromosome (M). B: Fluorescent signals of a cosmid probe SCW-0207A on the pericentric region, obtained by R-banding and FISH. C: Fluorescent signals of the probe SCW0204F on the short arm at 18p11.2, obtained by DAPI-banding and FISH. Duplicated signals of the probe are observed on both arms of an iso(18p) marker chromosome.

Fig. 2. Small metacentric marker chromosomes from 6 cells of a patient with tetrasomy 18p syndrome. Duplicated fluorescence signals of a cosmid SCW0204F are observed on both arms of an iso(18p) chromosome.

FISH to determine origin of the marker chromosome. Fluorescent signal of the probe SCW0204F DNA was present on the short arm at 18p11.2 of the normal copies of chromosome 18 (Fig. 1). Furthermore, the probe was hybridized to both arms of the metacentric marker chromosome (Figs. 1c and 2). The results clearly indicate that the marker chromosome is the iso(18p). Parental chromosome studies were not carried out.

DISCUSSION

Our initial diagnosis of karyotype in an affected patient was by standard cytogenetic techniques, which was confirmed by FISH. The biotinylated cosmid clones used as a probe were those that hybridized specifically to the pericentric region, to the short or long arm of chromosome 18. The iso(18p) chromosome is difficult to distinguish from chromosome with a large deletion of 18q. Furthermore, translocation products of 18 with another chromosome may appear cytogenetically indistinguishable from the iso(18p). In cases associated with multiple congenital anomaly, clinical features are variable and standard banding techniques allow only a likely diagnosis. Thus, the identification of iso(18p) chromosome should use an 18p probe with attention paid to the distribution of signal on both arms of the marker chromosome as well as on normal copies of chromosome 18 (Callen *et al.*, 1990).

In conclusion, cosmid localization to the specific bands of chromosome 18 will contribute to the physical and genetic mapping of this chromosome in general and may have some potential for their application in clinical cytogenetics as well.

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