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Case Report

INTERSTITIAL DELETION OF THE LONG ARM OF CHROMOSOME 11 DETERMINED BY FLUORESCENCE *IN SITU* HYBRIDIZATION

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Summary An interstitial deletion, del(11)(q14q22), found in a female infant was examined by fluorescence *in situ* hybridization with cosmid DNA markers mapped on the long arm of chromosome 11. Three cosmids mapped on 11q14.1-11q22.1 region were not hybridized to the del(11) chromosome, while all the other DNA markers mapped on 11cen-11q14.1 and 11q23.1-11qter region gave hybridization signals on the del(11) chromosome. Cytogenetic analysis after R-banding confirmed an apparent deletion of 11q14-q22, but containing a small R-negative band, a part of 11q22.3 and/or 11q14.1, in the middle part of del(11) chromosome. The karyotype thus was determined to be 46, XX, del(11)(q14.1q22.3).

Key Words interstitial deletion, del(11)(q14q22), fluorescence *in situ* hybridization, cosmid clones

INTRODUCTION

Since the first report on a terminal deletion of the long arm of chromosome 11 by Jacobsen *et al.* (1973), 34 cases with deletion of 11q23-qter have been reported (Wardinski *et al.*, 1990) and the characteristic phenotype was remarkably similar when the deletions were involved in a chromosomal region between bands 11q22

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and 11q24.1 (O'Hara *et al.*, 1984; Fryns *et al.*, 1986). On the other hand, *de novo* interstitial deletion of the long arm of chromosome 11 have been documented in only 9 cases including the recent report by Wakazono *et al.* (1992). The interstitial deletion with various size were detected in the regions between bands 11q13 and 11q23, and they exhibit less pronounced, or even absent, clinical signs of the 11q terminal deletion syndrome. The comparison of clinical features associated with the 11q-interstitial deletion did not allow it as a distinct or clinically recognizable syndrome (Wakazono *et al.*, 1992). To evaluate the clinical characteristics associated with 11q-interstitial deletion, it is essential to determine the exact breakpoints involved in the deletion.

In this communication, we examined an interstitial deletion, del(11)(q14q22) in a female infant, by applying fluorescence *in siyu* hydridization with cosmid DNA markers mapped on the long arm of chromosome 11.

MATERIALS AND METHODS

A patient. The patient was a 12-month-old female infant with developmental delay, growth retardation, and dysmorphic features including dolichocephaly, telecanthus, ptosis, flat nasal bridge, anteverted nares, high-arched palate, carp-shaped mouth, microretrognathia, and low-set and posteriorly rotated ears (Wakazono *et al.*, 1992). Chromosome analysis by GTG high-resolution technique on peripheral lymphocytes indicated that the proposita had a karyotype of 46, XX, del(11)(q14q22).

DNA probes. All 10 cosmid cC111 clones used as DNA probes in the present study were isolated by Tokino *et al.* (1991) from a genomic library constructed in the cosmid vector pWEX15 by using DNA from a somatic cell hybrid containing an intact human chromosome 11 as the only human material in Chinese hamster cell background.

Chromosome preparation with R-banding. Preparations of R-banded prometaphase chromosome were carried out by method of Takahashi *et al.* (1990). In brief, PHA-stimulated blood lymphocytes from the patient were cultured in TC199 medium (Nissui) containing 10% FCS for 48 hr. After cell synchronization with excess thymidine (300 μg/ml, Sigma) for 15.5 hr, cells were cultured for 6.5 hr in the presence of 5-bromodeoxyuridine (BrdU, 25 μg/ml, Sigma). Chromosome preparations were made after 30 min-treatment with colcemid and exposed to black light (Takahashi *et al.*, 1991).

Fluorescence in situ hybridization (FISH). The procedure used for FISH with DNA probes containing repetitive DNA sequences was originally described by Hori *et al.* (1990) and had been applied for the construction of high-resolution cytogenetic map of 168 cosmid DNA markers for human chromosome 11 (Hori *et al.*, 1992). To eliminate noise signals from interspersed repetitive DNA sequences such as Alu-sequences, chromosomal *in situ* suppression hybridization

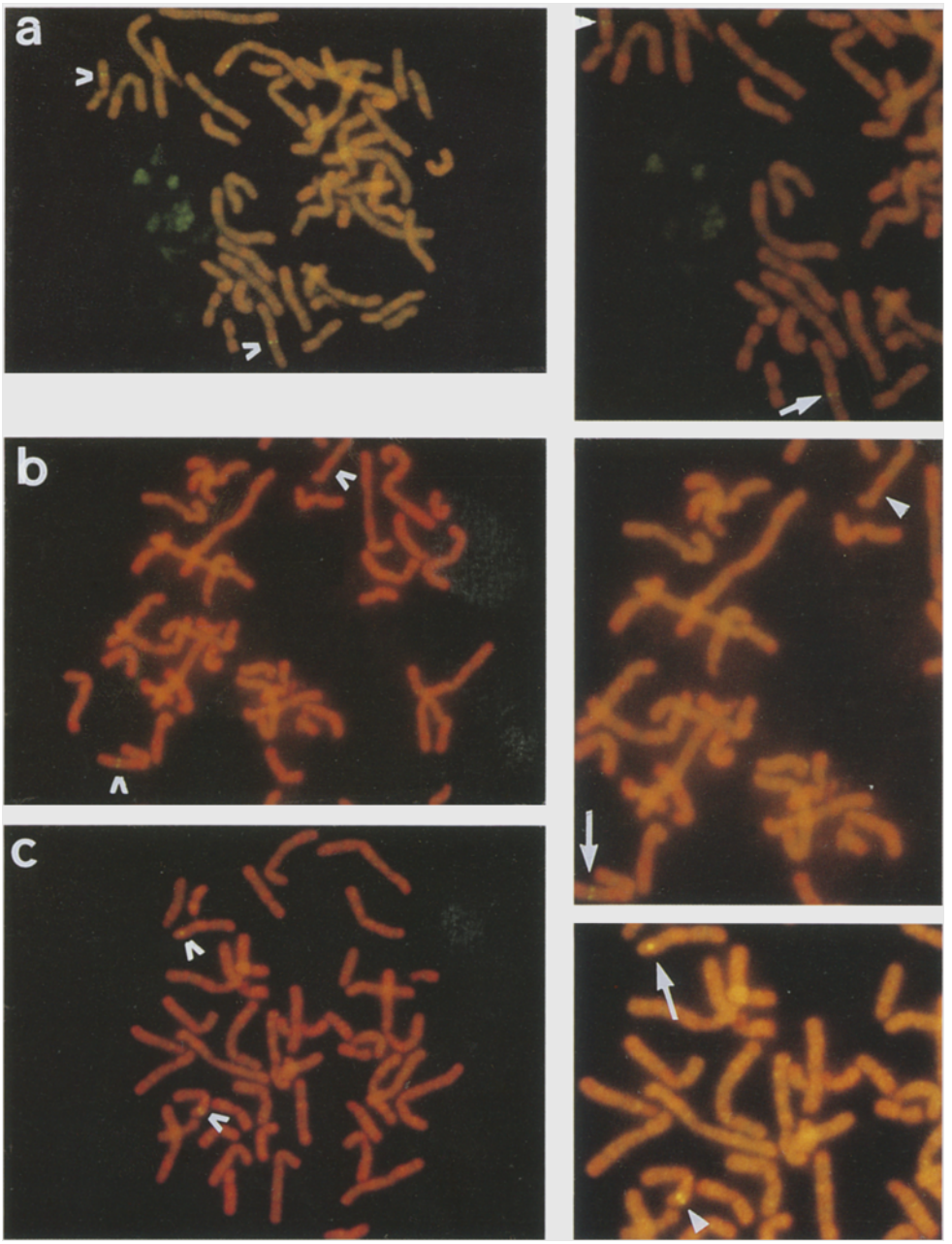

Fig. 1. Fluorescence *in situ* hybridization with biotinylated DNA probes of cosmids cC111-471 (a), -384 (b), and -530 (c) on metaphase (right) and enlarged partial metaphase (left). Hybridization signals on normal chromosome 11 and del(11) chromosome are indicated by arrows and arrowheads, respectively. Note no signal of cC111-384 on the del(11) chromosome.

was carried out, by adding sonicated total human placenta DNA (Sigma) as competitor (5- to 50-fold excess amounts) to the hybridization mixture. The procedures of rpobe labeling with biotin-dUTP, hybridization, rinsing, detection, and microphotography were performed in a routine manner.

RESULTS AND DISCUSSION

In our direct FISH system, which combines fluorescence *in situ* suppression hybridization with replicated prometaphase R-bands, both the fluorescein (FITC)-detected hybridization signal and propidium iodide (PI)-stained chromosomes with R-banding pattern were detected at the same time (Fig. 1). Hybridization signal appeared as twin spots on both chromatids in more than 80% of chromosome 11 examined. The localizations of 10 cosmid clones on normal chromosome 11 are summarized in Fig. 2B. The 6 cosmids, cC111-44, -303, -384, -442, -447, and -471, had been mapped previously by Hori *et al.* (1992) and the present study confirmed their locations. In addition, four cosmid clones, cC111-506, -530, -567, and -607, were newly mapped in this study. As summarized in Fig. 2C, on the other hand, the hybridization signals given by 7 cosmids remained on the chromosome 11 with an interstitial deletion; examples of cC11-471 and -530 are shown in Fig. 1a and c, respectively. However, the signals from 3 cosmids, cC111-384, -506, and -567 mapped to 11q14.1-q22.1 region, could not be detected in the deleted chromosome 11; an example of cC111-384 is shown in Fig. 1b. Thus, it can be deduced from these results that the interstitial deletion included 11q14.1 and 11q22.1, and that both R-positive proximal (11cen-q13.5) and distal parts (11q23.1-qter) are intact.

As can be seen in Fig. 1, a small R-negative band present in the middle part of the del(11) chromosome. This is consistent with the result of the previous cytogenetic analysis with GTG-banding in which a G-positive band remained in the del(11) chromosome. Wakazono *et al.* (1992) have also reported that an immunoblot analysis of her fibroblasts revealed a normal amount of mitochondrial acetoacetyl-coenzyme A thiolase. Since the gene for human mitochondrial acetoacetyl-coenzyme A thiolase has been mapped on chromosome 11q22.3-q23.1 (Masuno *et al.*, 1992), the region around the junction between 11q22.3 and 11q23.1 appears to be intact in the del(11) chromosome. The ascertainment of the breakpoint at 11q14.1 was not clear, because 3 cosmid clones examined were overlapped in this region. It seems thus likely that the R-negative (G-positive) band in the del(11) chromosome might contain a part of 11q22.3 and/or 11q14.1 bands. However, it is difficult to make discrimination between these two bands, because the numbers of DNA markers examined here were limited.

Figure 2A summarizes a review of cytogenetic findings on the patients with interstitial deletion of 11q13-q23 reported by Wakazono *et al.* (1992). According to their review, there is a similarity in both cytogenetic and clinical findings between

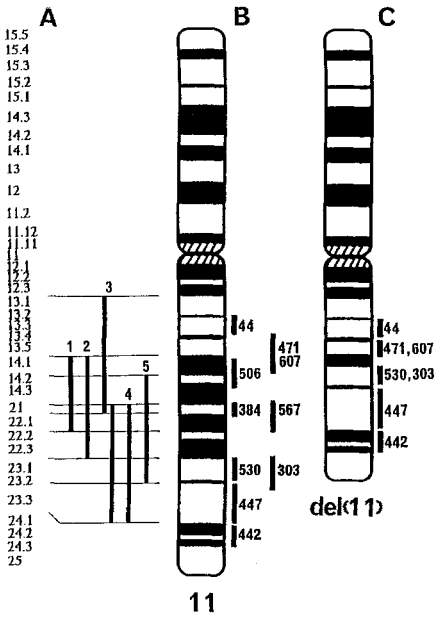

Fig. 2. A: Diagramatic representation of interstitial deletions in 11q13-11q23 regions reported. 1, Taillemite *et al.* (1975); 2, Sorensen *et al.* (1979); Bateman *et al.* (1984), Wakazono *et al.* (1992); 3, McPherson and Meissner (1982), Klep-de Pater *et al.* (1985), Carnevale *et al.* (1987); 4, Taki *et al.* (1983); 5, Okamura *et al.* (1988). B: Localization of 10 cosmid clones on normal chromosome 11. C: Localization of cosmid clones on the del(11) chromosome.

our case and those reported by others (Taillemite *et al.*, 1975; Sorensen *et al.*, 1979; Okamura *et al.*, 1988). The clinical manifestations of mental retardation and dysmorphic features including ptosis, flat nasal bridge, cleft palate and lip, high-arched palate, and low-set and malformed ears have been documented in the cases of del(11)(q14.1q22.1), del(11)(q14q22), and del(11)(q14.2q23.2). To clarify the exact karyotype-phenotype correlation, it is necessary to perform more detailed molecular cytogenetic analysis combined with DNA analysis with polymorphic DNA markers on those patients with varied segments of interstitial deletion of 11q.

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