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SHORT COMMUNICATION

Prenatal Diagnosis of a Mosaic Extra Structurally Abnormal Chromosome by Spectral Karyotyping

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A *de novo* mosaic extra structurally abnormal chromosome (ESAC) was detected in 33 per cent of cultured amniotic fluid cells from a pregnant woman. Neither Q-banding nor fluorescence *in situ* hybridization (FISH) employing a DNA probe for nucleolar organizer region demonstrated the presence of satellites on the ESAC. Spectral karyotyping (SKY) was performed in this prenatal case and led to a quick and accurate determination of the ESAC as chromosome 14 in origin. The SKY finding was confirmed by conventional FISH analysis using a chromosome 14 specific painting probe. Subsequent hybridizations with a centromeric probe and a 14q subtelomeric probe were also performed to further characterize the ESAC. Absence of (TTAGGG)n sequence on the ESAC, determined postnatally, suggested it is a ring chromosome 14. Genetic counselling concerning these findings was provided to the parents who chose to continue the pregnancy. The male infant had no apparent abnormal phenotype at birth. Copyright © 1999 John Wiley & Sons, Ltd.

INTRODUCTION

Extra structurally abnormal chromosomes (ESACs) occur with a frequency of 0.14–0.72 per 1000 births (Gardner and Sutherland, 1996; Warburton, 1991). FISH with alphoid (centromeric) probes and whole chromosome painting probes have enhanced our ability to identify the origins of ESACs, but the process is limited to one or a few chromosomes at a time. A novel technology, termed spectral karyotyping (SKY), has been developed to allow simultaneous hybridization of 24 chromosome painting probes and rapid identification of all chromosomes in 24 colours (Schröck *et al*., 1996, 1997). We report here, what to our knowledge, is the first use of SKY in the prospective prenatal diagnosis of a mosaic ESAC in cultured amniotic fluid cells.

CASE REPORT AND CYTOGENETIC STUDIES

A 36-year-old gravida 2, para 1 female was referred for prenatal cytogenetics analysis with the indication of increased maternal age. A second-trimester ultrasound and amniocentesis were performed and cells were cultured *in situ*. The amniotic fluid alpha-fetoprotein level was within the normative range. GTG-banded chromosome analysis showed that 7 out of 21 colonies from 3 different *in situ* cultures had a small ESAC of unknown origin (Fig. 1, top panel). The ESAC was Q-banding negative, and did not contain a nucleolus organizer region. GTG-banding with ethidium bromide extension allowed an analysis at the 600 band level that showed the ESAC appeared to be linear in some cells and circular in others. There was no evidence of a double ring structure in any of the metaphases analysed. Analysis of parental blood chromosomes showed no evidence of the ESAC.

In order to identify the chromosome origin of the ESAC, we employed the newly developed spectral karyotyping (SKY) technique. This technique permits the simultaneous analysis of all 24 human chromosomes by FISH and spectral imaging. It led to a rapid identification of the ESAC as chromosome 14 in origin (Fig. 1, middle panel). A chromosome 14 specific painting probe was subsequently used to confirm the SKY result. Further FISH analysis with a 14/22 centromeric probe (D14Z1/D22Z1, Oncor) and a 14q subtelomeric probe (Ning *et al*., 1996) demonstrated that the marker is positive for the homologous sequence in the centromere of chromosomes 14 and 22, but negative for the 14q subtelomeric sequence (Fig. 1, bottom panel). These results suggest, but do not confirm that the ESAC is comprised of proximal 14q chromosomal material. Callen *et al*. (1991) illustrated the often complex formation of ring chromosomes.

Genetic counselling was provided to the parents. The empirical risk of approximately 15 per cent for an abnormal phenotype associated with *de novo* non-satellited ESAC (Warburton, 1991) as well as reported outcomes of other cases with ESACs which were chromosome 14 in origin was discussed. The parents elected to continue the pregnancy without additional

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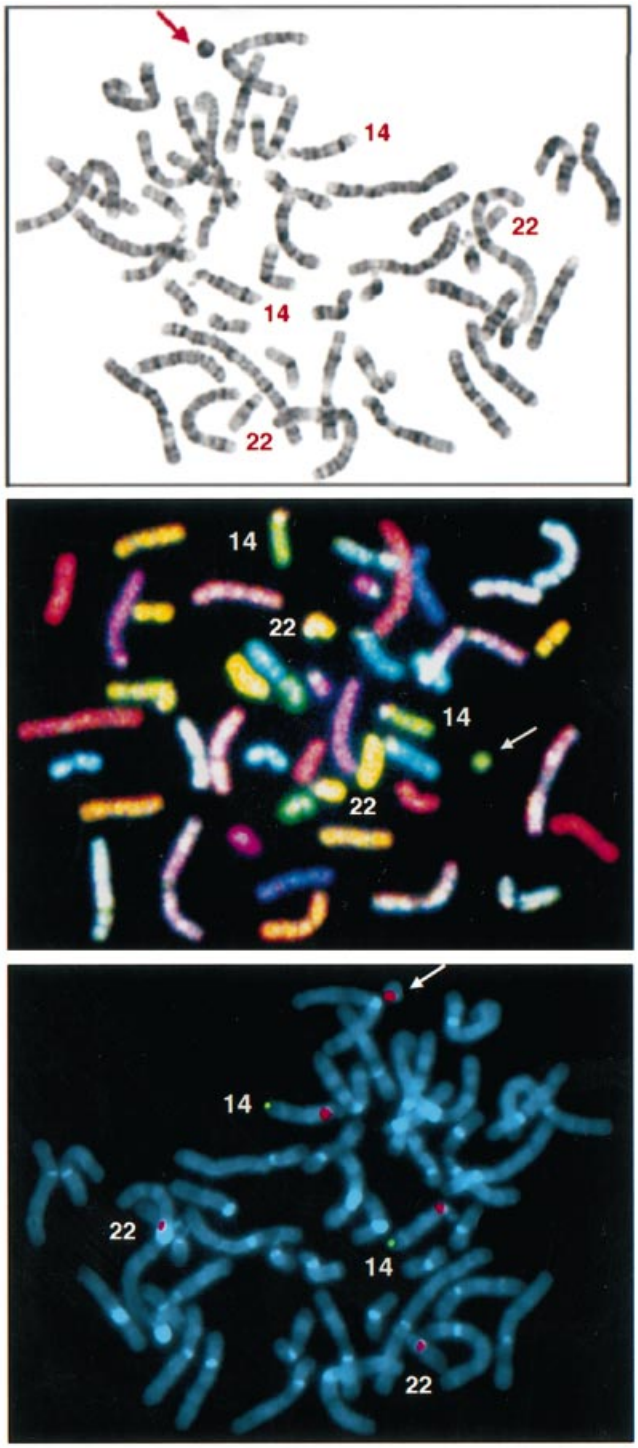
cytogenetic or ultrasound testing. The infant was born at term. Postnatal dysmorphic and neurological evaluations at birth and at six months of age did not show dysmorphic features or developmental delay. However, at six months of age, the infant developed infantile spasms, which resolved after treatment. Postnatal blood chromosome analysis performed in another institution by GTG-banding and FISH with a chromosome 14 painting probe showed 6 of 20 cells (30 per cent) have the same ESAC chromosome 14, which confirmed the prenatal findings. An all human telomere probe (Oncor) did not hybridize to the ESAC, suggesting it is a ring chromosome.



DISCUSSION

Extra structurally abnormal chromosomes pose a unique dilemma in prenatal diagnosis. The phenotypic consequences of a *de novo* abnormal chromosome are difficult to foresee. The first step towards understanding the phenotypic effects due to an ESAC is to precisely identify its chromosomal origin. Five patients with small ESAC chromosomes originating from chromosome 14 have been reported to be phenotyically normal at ages ranging from newborn to 21 years old (Stetten *et al.*, 1992; Gravholt and Friedrich, 1995). However, the ESAC was *de novo* in only two of these patients. The two *de novo* cases were reported from a cytogenetic study of a large unselected group of children. The empirical risk for an abnormal phenotype associated with *de novo* non-satellited ESAC is about 15 per cent (Warburton, 1991). Warburton's study also demonstrated that there was no evidence of a different risk of abnormalities for mosaic versus non-mosaic cases. The pregnancy outcome of our case is summarized earlier in this report.

The advent of FISH techniques has augmented the identification of chromosomal origins of small ESACs. However, sequentially testing a variety of probes can be problematic with the limited specimen and time frame available for prenatal diagnosis. In contrast to conventional FISH procedures, SKY permits the simultaneous analysis of all 24 human chromosomes in a single hybridization experiment. The analysis of a large series of cases with ESACs would help to establish correlations between the chromosomal origin of the ESAC and the clinical outcome for the patient, which in turn would provide more complete information for genetic counselling.

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Fig. 1—Top panel: G-banded metaphase spread shows the presence of a small ESAC (arrow). Middle panel: SKY analysis revealed that the ESAC (arrow) originated from chromosome 14. The metaphase spread is shown in display colours by assigning red, green and blue colours to specific spectral ranges to convert the emission spectra of the painting probes for visualization. Bottom panel: a mirror image of the same G-banded metaphase as shown in the top panel. FISH with a centromeric probe D14Z1/D22Z1 demonstrated the presence of such a sequence (red signal) on the ESAC. No 14q subtelomeric sequence (green signal) was present on the ESAC

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