Reproductive history of a healthy woman with mosaic duplication of chromosome 4p

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Objectives Mosaic autosomal duplications are rare and often result in mental retardation and congenital anomalies. Phenotype is not predictable depending on the chromosomal imbalance involved and the percentage and tissues distribution of unbalanced cells. We report on a young woman carrying a mosaic duplication of chromosome 4p, evaluated because of three abortions due to IUGR and fetal malformation.

Methods Mosaic dup(4p) was detected by standard and molecular cytogenetics.

Results Unbalanced cells accounted for about 20 to 30% of nuclei in four examined tissues and did not cause any obvious phenotypic effect.

Conclusion It is likely that mosaic duplications are underascertained because they are not associated with obvious clinical effects in some individuals. Prenatal diagnosis is the method of choice to predict the karyotype in the offspring of subjects carrying mosaic chromosome imbalances. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: abortion; CVS; dup(4p) syndrome; mosaicism; tandem duplication

INTRODUCTION

Mosaic tandem duplications are rare. Thirty cases have been reviewed by Rauen et al. (2001). Although in most individuals mental retardation, dysmorphisms, and/or malformations are found, the phenotypic outcome is not always predictable because of differences in the chromosomes involved, size of genomic imbalance and proportion and tissue distribution of unbalanced cells. About 10% of patients with normal phenotype or manifesting only marginal dysmorphic features have been reported (Tonk et al., 1996; Hocking et al., 1999; Tharapel et al., 1999), suggesting that mosaic tandem duplications could be underascertained.

We report on a phenotypically normal woman who went through three abortions. Her chromosome analysis disclosed a mosaic duplication of the short arm of chromosome 4 in four different tissues examined.

CASE REPORT

A young, Caucasian, non-consanguineous couple had three recorded abortions. The first pregnancy, which was terminated after an ultrasound scan at 24 weeks of gestation, disclosed a congenital heart defect, which was confirmed as an interventricular defect at autopsy. Fetal karyotype disclosed an homogeneous duplication of the short arm of chromosome 4. The father’s karyotype was normal, while the mother had dup(4p) in 30% of the analysed metaphases (46,XX, dup(4p)[30]/46,XX[70]). In the second pregnancy, an ultrasound scan at 10 weeks showed intrauterine growth retardation (IUGR) and cystic hygroma of the neck. This pregnancy resulted in spontaneous abortion at 11 weeks, but no investigations were carried out in the fetus. IUGR was also observed in the third pregnancy, which was terminated following an amniocentesis, which disclosed non-mosaic fetal dup(4p). The couple was evaluated at our institute after the last abortion. An accurate clinical evaluation of the mother was unremarkable. The extent of the 4p duplicated was refined and mosaicism was evaluated in four different tissues. The fourth and the fifth pregnancies were monitored by chorionic villus sampling (CVS), which disclosed 46,XY fetal karyotypes.

MATERIALS AND METHODS

Chromosome analyses of the patients were carried out on cultured peripheral lymphocytes using GTG-banding techniques according to standard procedure. To perform fluorescence in situ hybridization (FISH) on interphase nuclei, buccal mucosa, hair follicle and urinary sediment samples were treated following the protocol described elsewhere (Schwanitz and Schubert, 1999; Davies et al., 1994). Briefly, buccal mucosa cells were smeared on
slides and directly fixed in 100% ethanol for 30 min. Five hair roots were incubated at room temperature for 30 min in 50% acetic acid, vortexed and fixed in 100% methanol. Afterwards, hairs were removed and extracted cells were dropped on cold slides. Immediately before in situ hybridization, slides were treated with a proteinase K solution (20 units per mg) at 37 °C for 20 min and dehydrated in a series of ethanol. Cells from urinary sediment were obtained following the procedure used for uncultured amniotic fluid samples described by Davies et al. (1994) with minor modifications. In brief, sediment was washed once in phosphate buffer saline (PBS) and then treated with a hypotonic solution for 30 min at 37°C. Then cells have been fixed in Carnoy’s solution (methanol: acetic acid = 3 : 1) and dropped on cold slides. FISH painting was carried out using chromosome 4 painting probe (Appligene Oncor SA, Strasbourg, France) according to manufacturer’s instructions. Clones mapping to the distal part of 4p were obtained from YAC Screening Center-DIBIT (Milan, Italy) and from ‘Resources for Molecular Cytogenetics’ (Bari, Italy). Their relevant mapping data were derived from a public database (http://genome.ucsc.edu). Extracted DNA was biotin-16-dUTP-labelled by Nick Translation kit (Invitrogen, Life Technologies, Carlsbad, CA). Hybridisations were carried out having added 15 µl of the denatured probe mixture to each slide, which was then incubated in a moist chamber at 37 °C overnight. After hybridisation, the slides were washed three times in 50% deionized formamide 2X SSC at 42 °C. Specific probe signals were detected by Texas-Red labelled avidin (Appligene Oncor SA, Strasbourg, France), while the control region was detected using FITC-labelled α-satellite probes (Cytocell Ltd, Oxfordshire, UK). A mixture of DAPI/Antifade was used as a counterstain. FISH specimens were viewed using an epifluorescence microscope Nikon Eclipse E1000 (Nikon Instruments, Florence, Italy) and Genikon image analyser system (Nikon Instruments, Florence, Italy).

RESULTS

Cytogenetic analysis in the mother revealed a mosaicism with two cell lines; 70% of metaphases had normal karyotype, whereas 30% of cells showed extra-material on the short arm of one chromosome no. 4 (Figure 1). FISH analysis using a chromosome 4 painting probe proved that the extra-material was derived from chromosome 4. In order to characterise the extension of duplication, FISH analysis was carried out on lymphocytes, using a contig of BAC clones mapping to the distal part of 4p. RP11-1719 and RP11-640B6 probes, mapping to 4p16.1 and 4p14 respectively, showed one signal only on both chromosomes no. 4 (Figure 2A), while RP11-778B12, RP11-654J13 and RP11-121A15 probes, mapping to 4p15 region, showed one signal onto the normal chromosome 4 and two signals onto the rearranged chromosome 4 (Figure 2B). A directly labelled chromosome 4α-satellite probe was co-hybridized as control region. Thus, the proband karyotype was reinterpreted as 46,XX,dup(4)(p15p15)[30]/46,XX[70]. In order to determine the percentage of the unbalanced cell line in different tissues, FISH analysis was carried out on cells obtained from hair bulbs, oral mucosa and urinary sediment. One hundred cells were examined in each experiment. FISH using RP11-778B12 clone as a probe (Figure 3) showed respectively 22%, 25% and 20% of cells bearing dup(4)(p15p15).
A HEALTHY WOMAN WITH MOSAIC DUP(4P)

DISCUSSION

To date, about 30 cases of cytogenetically detectable tandem duplication mosaicism have been reported (Rauen et al., 2001). The majority of patients were ascertained because of their clinical characteristics, but a few cases were discovered only after the birth of an affected offspring carrying a non-mosaic imbalance. Therefore, the frequency of mosaic tandem duplications should be higher than generally considered, based on evidence that a proportion of these individuals have an unremarkable phenotype.

The most likely mechanism leading to mosaic tandem duplication is a post-zygotic unequal sister chromatid exchange at mitosis (Kotzot et al., 2000). Since this event could occur at any time during embryogenesis, and the proportion of abnormal cells could be different and change throughout the time because of selective advantage/disadvantage in different tissues (Youssoufian and Pyeritz, 2002), genotype–phenotype correlation are not easily predictable. The present patient carrying a mosaic dup(4)(p15p15) was a healthy woman with a history of repeated abortions. The first fetus showed IUGR and an interventricular heart defect due to dup(4)(p15p15). The second and third pregnancies resulted in IUGR, dup(4)(p15p15) having been proved in the latter. The last two pregnancies were monitored by CVS and resulted in normal newborns.

Dup(4p) syndrome is a well-characterised chromosome disorder resulting in prenatal and postnatal growth retardation, dysmorphisms, mental retardation and various malformations, including heart defects, coloboma of the iris and retina, cataract, microphthalmia, and cleft lip (Dallapiccola et al., 1977; Gonzales et al., 1977). In the present case, the duplication included only band 4p15, which has been considered critical for development of the dup(4p) phenotype (Curry et al., 1982; Wyant et al., 1993). However, this patient presented a mosaic duplication, with a rather homogeneous distribution of the unbalanced clone, in about 20 to 30% of the cells in four different tissues. The lack of obvious dysmorphisms/defects in this woman could be likely due to the late origin of rearranged cell line or its inadequacy to reach the clinical threshold effect.

The reproductive risk in women carrying mosaic chromosome unbalances cannot be predicted because the proportion of affected oocytes cannot be assessed. In the present case, the presence of gonadal mosaicism was inferred from the two fetuses carrying dup(4)(p15p15). Prenatal analysis of chorionic villi is the method of choice for a precocious prediction of the fetal outcome in individuals carrying constitutional chromosome mosaicism.

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REFERENCES


Figure 3—FISH analysis on interphase nuclei from oral mucosa in the proband, using RP11-778B12 clone (4p15.31). Green signals show the chromosome 4 centromeres. Red signals region 4p15, which is duplicated onto one chromosome (arrow)