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Identification, characterization and clinical implications of two markers detected at prenatal diagnosis

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Objectives
Marker chromosomes are relatively rare in the general population as its identification at prenatal diagnosis. In this article, we identified and characterized two *de novo* supernumerary marker chromosomes in a mosaic form at prenatal diagnosis.

Methods
The two cases presented were detected during prenatal diagnosis at 17 and 15 weeks of gestation. The analyses were performed due to the advanced maternal age. In both cases, parent's karyotypes were normal. The identification of the marker chromosomes was possible by FISH techniques.

Results
One marker chromosome was derived from chromosome 5 and the other from chromosome 6. Both children are well at the moment.

Conclusion
The two cases described in the present paper, join to the ones already described in the literature. However these results are the first ones without any phenotypical anomalies, at least until the present. Every new characterization of marker chromosomes at prenatal diagnosis should be reported for determining a genotype-phenotype correlation, and thus be used for genetic counselling and risk evaluation.

Key Words: prenatal diagnosis; marker chromosome; mosaicism; chromosome 5; chromosome 6

1. Introduction

Marker chromosomes, supernumerary marker chromosome (SMC) or extra structurally abnormal chromosomes (ESAC) are a class of structurally different chromosomes of the known chromosomes, that generally remain unidentified by conventional cytogenetic analysis due to the presence of a distinct banding pattern (Fergunson-Smith and Yates, 1984; Hook and Cross,1987; Sachs *et al*., 1987; Warburton, 1991; Blennow *et al*., 1994; Hastings *et al*., 1999).

These chromosomes are relatively rare in the general population, being found only in approximately 0.1 – 1.2/1000 live births and more frequently, 3.27/1000, among mentally retarded patients (Buckton *et al*., 1985; Huang *et al*., 1998; Ostroverkhova *et al*., 1999). At prenatal diagnosis, marker chromosome's incidence varies considerably, with a frequency ranging between 0.4/1000 and 1.5/1000. They are maternal-age related and, in many cases, are present in a mosaic form with a normal cell line (Hook *et al*., 1983; Gardner and Sutherland, 1996; Barch *et al*., 1997; Li *et al*., 2000). The clinical significance of a SMC varies widely and the phenotypes associated with the presence of a *de novo* non-familial SMC may vary from normal to severely abnormal (Hengsttschläger *et al*., 2001). The risk of a fetal pathology or pathology in the immediate period of live-born children is estimated in 15% for non-satellited ESACs, and 13% may be associated with congenital abnormalities and mental delay independent of the chromosomal origin (Warburton, 1991; Crolla *et al*., 1992). Several studies have been published and only a few ESACs have a recognized phenotypic outcome (inv dup(15); i(18p); i(12p); inv dup(22)); however, there is consensus on the range of phenotypic expression being related to the size of the marker, its euchromatic content, chromosomal origin and level of mosaicism (Blennow *et al*., 1995; Brondum-Nielsen and Mikkelsen, 1995; Muller-Navia *et al*., 1995; Hsu *et al*., 1996). There is no agreement concerning the real significance of the mosaic; some studies indicate that the prognosis is the same in the case of mosaicism and non-mosaicism, other researchers consider that mosaicism is associated with a less severe prognosis and some even say that it is impossible to accurately predict the phenotype in such cases (Warburton, 1991; Marchina *et al*., 2003).

With the use of conventional cytogenetic and specialized staining techniques, such as C-banding, silver staining of nucleolar organizer regions (NOR) and Dystamicin A/DAPI (DA/DAPI) staining, the determination of the marker chromosomes origin is not always possible. But with the development of fluorescence *in situ* hybridization (FISH) it is now possible to precisely ascertain the chromosomal origin of the markers by performing hybridizations with specific centromeric or whole chromosome painting (WCP) probes.

The authors present two cases with a *de novo* SMC, in a mosaic form found at prenatal diagnosis.

2. Material and methods

Chromosome analyses were performed on cultured cells

from amniotic fluid, peripheral blood, fetal blood and tissue biopsies. Chromosomes were harvested according to standard techniques and analysed by Trypsin – Giemsa method (GTG). C-banding (CBG) and silver staining (NOR) were used to determine the presence or absence of a centromere and satellite stalks, respectively. DA/DAPI was performed to exclude the marker's origin of being from chromosome 15.

FISH analysis was carried out on metaphase chromosomes to characterize the origin of marker chroosomes. Probes used include WCPs (Cambio andChromoprobe Multiprobe Octochrome-Cytocell) and centromere-specific DNA probes (Chromoprobe Multi-probe I System-Cytocell). The chromosomal region origins of both markers were determined by cross-species in situ hybridization. The cross-species colour banding probes were prepared from flow-sorted chromosomes of the Hylobates concolor (HCO) as described elsewhere, following Muller et al. (1998). All these DNA probes were indirectly amplified by DOP-PCR. Briefly, a set of gibbon WCPs homologous to human chromosomes 5 and 6 were indirectly labelled with biotin- dUTP, digoxigenin-dUTP, or a 50 : 50 ratio of biotin-dUTP : digoxigenin-dUTP. Digoxigenin and biotin were detected with rhodamine and FITC, respectively (theyellow colour was produced by the mixture of both fluorochromes in the 50: 50 ratio). Two or three probeswere applied simultaneously in dual- or triple-colour hybridizations. If more than three WCPs were requiredfor any case, sequential painting was carried out on the same metaphase as described by Harrison et al. (2001).

In the analysis of the marker chromosome from case 2 it was also used as a telomeric probe that was labeled indirectly with digoxigenin-dUTP and detected with rhodamine, as described by Ijdo et al. (1991).

3. Case reports

Case 1

Amniocentesis or advanced maternal age was performed in a 39-year-old woman at 17 weeks of gestation. It was a non-consanguineous couple with a normal obstetric history and a phenotypically normal daughter. Cytogenetic analysis in 30 cells from two different cultures revealed a karyotype of 47,XY,+mar/46,XY (60%/40%) (Figure 1(a)). The SMC was CBG positive

(Figure 1(b,c)), DA/DAPI negative (Figure 1(d)) and did not contain nucleolar organizer region positive satellites. Parental blood had normal karyotypes. FISH studies with a centromere-specific DNA probes (Cytocell) revealed the origin of SMC as derived from chromosome 5 and it was confirmed with a WCP probe for chromosome 5 (Cambio) (Figure 1(e)). Accordingly, the fetal karyotype was 47,XY,+mar[30]. ish der(5) (wcp 5+, D5Z2+)/46,XY[20]. Cross-species colour banding with sequential hybridization with two pools of HCO probes disclosed the origin of the marker chromosome at the chromosome region of 5p12-5p15.3 (Figure 1(f) – (i)), since the 6 + 7HCO probe (detected in yellow colour, Figure 1(f,h); or red colour, Figure 1(g,i)) cross-species hybridized to that region on human chromosome 5.

In the ultrasound at the 19th week of pregnancy, no morphological anomalies were detected and, after genetic counselling, the couple decided to continue the pregnancy. A phenotypically normal boy was born at the 40th week of pregnancy. Cord blood and placental tissue were collected for cytogenetic studies. The SMC was seen in 33.7 and 37.8%, of the examined cells. The routine post-natal examination gave normal results; no congenital abnormalities or dysmorphic features were detected.

The child is being observed regularly by a paediatric consultant. Now, at the age of 2, he does not present any morphological alteration and has normal psychomotor development.

Case 2

Amniocentesis was performed at 15 weeks of gestation in a 40-year-old woman. It was a non-consanguineous couple with an obstetric history of four miscarriages. There was no familiar history of mental retardation, congenital malformation, or hereditary disease. GTG analysis revealed a karyotype of 47,XX,+mar/46,XX (73.6%/26,4%) in two different cultures (Figure 2(a)). CBG banding showed positive staining on the marker chromosome (Figure 2(b) – (c)), and did not contain NOR-positive satellites. DA/DAPI was negative (Figure 2(d)). Parent's karyotypes were normal. In an attempt to identify the marker, more chromosome preparations were performed from cultured amniocytes (after 15 days of culture) and we verified that the marker incidence fell to a very small percentage (3%). FISH was performed with a combination of chromosome specific alpha-satellite sequence probes (Cytocell) and we could exclude several chromosomes origin (12; 13; 14; 15; 18; 20; 21; X and Y), but not all of them. It was not possible to identify the small accessory chromosome with FISH analysis since we could not detect it in the new prepared metaphases.

The couple decided to continue the pregnancy since no morphologically ultrasound anomalies were detected at 16 and 20 weeks of gestation. A phenotypically normal girl was born at 37 weeks of gestation. Cord blood and placental tissue were collected for cytogenetic studies. The marker chromosome was seen in 87% of the examined cells (from a total of 150 metaphases analysed). Using FISH with a human painting probe (Cytocell), we identified the chromosomal origin of marker chromosome as derived from chromosome 6 (Figure 2(e)). Accordingly, the fetal karyotype was 47,XX,+mar[38].ish der(6) (wcp 6+, D6Z2+)/46,XX [10]. The precise chromosomal region of the marker chromosome was revealed by cross-species colour banding at the chromosome region 6p11.2-6p12 (Figure 2(f) and 2(h)). Furthermore, it was possible to detect telomere DNA sequences at the marker chromosome (Figure 2(g)), which allowed the exclusion of the traditional ring chromosome syndromes for this case.

Currently, the child is 4 years old and presents normal physical and psychomotor development.

4. Discussion

The identification of marker chromosomes at prenatal diagnosis is always difficult when it is a de novo situation in a mosaic form with a normal cell line. It is difficult to

predict precisely the phenotypic risk of the fetus, since it is dependent on morphological anomalies and pathological development (Schinzel, 1991; Callen et al., 1992; Viersbach et al., 1998). The presence of marker chromosomes at prenatal diagnosis might have no clinical significance but it can also be responsible for mild dysmorphic features as well as severe psychomotor retardation (Crolla, 1998; Va´clav´ık and Kugl´ık, 2001). It is important to note that mental retardation is only possible to exclude at a later age and not at the time of ultrasonography analysis.

In this study, we have characterized two marker chromosomes derived from chromosome 5 (p11-p15.3), and from chromosome 6 (p11.1-p12); both were de novo situations and prenatally detected. In the marker derived from chromosome 6, it was also possible to detect telomere DNA sequences. Several marker chromosomes of these two chromosomes have been reported in the literature, but only a few cases were prenatally diagnosed de novo.

Our first case, as far as we know, is the eighth case of a SMC derived from chromosome 5, and the third at prenatal diagnosis. According to the literature, all the cases presented several anomalies: dysmorphic features (macrocephaly, microretrognathis), psychomotor retardation and recurrent respiratory infections (Avansino et al., 1999; Masuno et al., 1999; Stankiewicz et al., 2000; Sizonenko et al., 2002). In three cases, polyhydramnios was detected during pregnancy. All except one (Avansino et al., 1999) had different levels of mosaicism. Our case had an incidence of 60% in amniotic fluid, 37.8% in chorionic villi and 33.7% in cord blood. The concept of a critical band 5p10 to 5p13.1 was proposed (Lorda-Sanchez et al., 1997; Avansino et al., 1999), and is thought to be associated with the most severe phenotypic effects described. The present report describes a case where the marker-derived chromosome region perfectly overlaps with this ‘critical band’; however, in our case, the patient does not exhibit any phenotypical anomalies, at least, until now.

The second case presents a prenatal diagnosis of a fetus with a marker chromosome in a mosaic form, with an incidence of 73.6%. The couple decided against any risks and proceeded with the pregnancy. Only after birth, could we identify the marker chromosome by FISH techniques; at present the child is well.

The two cases that had a SMC derived from chromosome 6, described by Crolla (1998), are in a mosaic form and are de novo in its origin. The first one had severe mental retardation. The second case described by Crolla (1998) had IUGR, transient neonatal diabetes that resolved spontaneously, and at 3 years, the child developed mild mental delay (Crolla, 1998).

The two cases described in the present article, are similar to the ones already described in the literature; however, these are the first ones without any phenotypical anomalies, at least to date. The identification of a SMC is essential to predict the prognosis. The classical cytogenetic techniques permitted the detection of the markers, but its identification and precise characterization was only possible by FISH techniques. FISH is a complementary technique to more classical cytogenetic methods that proved to be capable of providing important information with respect to chromosome origin and composition of marker chromosomes. The ultrasound investigation and genetic counselling were essential to the pregnancies proceeding in a regular way. Both children developed normally and at present show normal somatic and psychomotor development. However, their psychological development must be followed even during adolescence.

Every new characterization of marker chromosomes at prenatal diagnosis should be reported for determining the genotype – phenotype correlation to be used for genetic counselling and risk evaluation.

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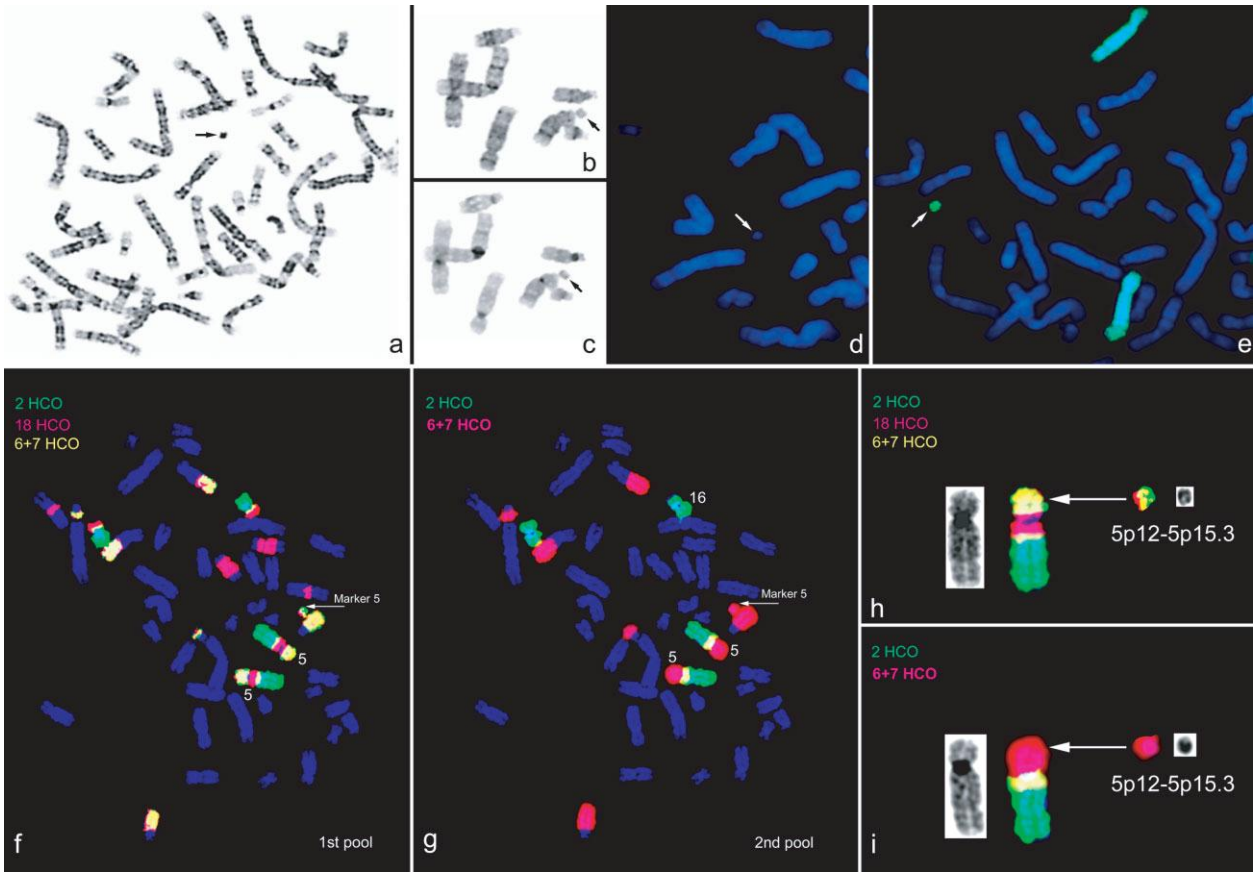

Figure 1 — Amniocyte metaphase spreads from case 1 (marker chromosome is pointed out with small arrows): (a) with GTG-banding; (b) partial metaphase with GTG and (c) after C-banding (marker with euchromatin); (d) DA/DAPI/staining (marker without signal); (e) FISH analysis with an HSA paint probe for chromosome 5 (green signal), shows the presence of green signal on the marker chromosome; (f) and (g) cross-species *in situ* hybridization with two pools of HCO paint probes (indicated in the respective images); (h) and (i) detailed images showing the origin of the marker chromosome based on the cross-species *in situ* hybridization

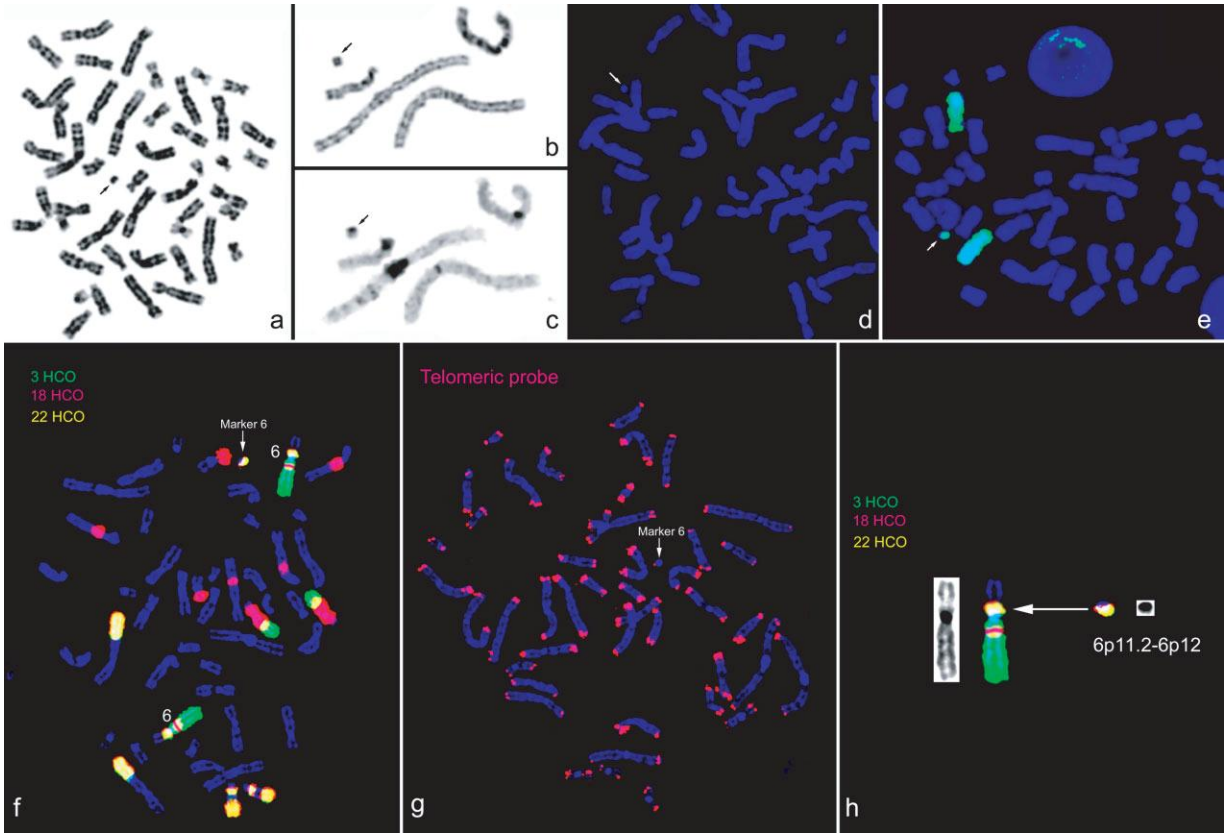

Figure 2 — Amniocyte metaphase spreads from case 2 (marker chromosome is pointed out with small arrows): (a) with GTG-banding; (b) partial metaphase with GTG and (c) after C-banding (marker with euchromatin); (d) DA/DAPI staining (marker without signal); (e) FISH analysis with an HSA paint probe for chromosome 6 (green signal), shows the presence of green signal on the marker chromosome; (f) cross-species *in situ* hybridization with one pool of HCO paint probes (indicated in the image); (g) *in situ* hybridization with a telomeric probe and (h) detailed image showing the origin of the marker chromosome based on the cross-species *in situ* hybridization