

# SCE Frequency Measurement Could Be Useful in the Prenatal Diagnosis of Roberts Syndrome

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In a previously published article (Resta et al., 2006) on Robert's syndrome in prenatal diagnosis, a case of a 36-year-old woman and her 36-year-old, nonconsanguineous husband were presented. Our findings suggest the existence of nonsense mediated decay (NMD) variability which could account for the varying severity reported in carriers of identical mutations. Furthermore, fetal cells were used to evaluate the influence of premature centromere separation (PCS) on the sister chromatid exchange (SCE) and micronucleus (MN) frequency. Given the similar variation observed in the SCE frequencies, dependent on tissue/cell type (amniotic fluid sample, chorionic villus sampling) and duration of in vitro cultures (48 hours or 72 hours), the idea was that this new piece of information could be interesting. It seems that the SCE frequency increased proportionally to the cell cycle increasing ( $1^\circ < 2^\circ < 3^\circ \dots n$ ). Obviously, our observations are too scarce to draw conclusions, but further investigation could be useful to corroborate or dispute these results, considering that the two techniques, (MN and SCE), are simple to perform and do not require expensive laboratory equipment.

Roberts syndrome (RS) is rare autosomal recessive defect. In 1919 Roberts described three affected siblings of Italian parents who were first cousins. As reported by Bates (2003), the syndrome was first documented by Bouchard, whose description in 1672 of autoptic findings, or 'Miscellanea curiosa', are consistent with the diagnosis of RS. RS is characterized by pre- and postnatal growth retardation, mental retardation, symmetrical limb defects of variable severity (from hypomelia to tetraphocomelia), and craniofacial abnormalities (hypertelorism, hypoplastic nail alae, cleft lip and palate; Allingham-Hawkins & Tomkins, 1991; Rodriguez et al., 1992). All the patients show a characteristic abnormality of the constitutive heterochromatin, described as 'puffing' or

'repulsion' of the peri- or para-centromeric regions (premature centromere separation [PCS]) together with splaying of the short arm of the acrocentrics and of the distal heterochromatic block of the long arm of the Y chromosome (German, 1979; Louie & German, 1981; Schüle et al., 2005). This cytogenetic phenomenon, called the RS effect, is observed in cells of different tissues and appears to be more evident in chromosomes with large amounts of heterochromatin (Van den Berg & Francke, 1993). The first prenatal detection of the RS effect was reported by Willner et al. (1979). We detected two new cases of RS prenatally by simple cytogenetic analysis in combination with ultrasound evaluation. In both cases the influence of PCS on the micronucleus (MN) and sister chromatid exchange (SCE) frequency was evaluated.

## Case Report

We present a case of a 36-year-old woman, gravida 3, para 1 and her 36-year-old, nonconsanguineous husband. This woman bore a normal child from her first pregnancy. At her next pregnancy, fetal ultrasonography at the 20th week of gestation revealed severe malformations (tetraphocomelia and intrauterine growth retardation), and the mother was referred to us for cytogenetic investigations of amniotic fluid samples (AFS). These investigations performed on amniocyte cultures revealed a male karyotype (46, XY), showing characteristic repulsion of peri- and para-centromeric regions, that is, PCS expression. Genetic counseling was given and the couple decided to terminate the pregnancy at 22 weeks. No further cytogenetic examination was performed on the fetus. Normal karyotypes were present in both parents.

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Nine months later, the mother became pregnant for the third time, but this pregnancy terminated spontaneously at 15 weeks. Cytogenetic investigations of chorionic villus sampling (CVS) showed a female (46, XX) karyotype with the presence of a mild RS effect. Postmortem examination of this aborted fetus showed growth retardation, tetraphocomelia with hypoplastic forearms, three fingers on each hand, microcephaly, micrognathia, hypertelorism, complete cleft lip and palate, nasal hypoplasia, female external genitalia with enlarged clitoris.

## Materials and Methods

Cells from the AFS were cultured using the standard *in situ* technique, while CVS cells collected from the abortion were cultured as already described by Resta et al. (2006). When 80% confluence was reached, subculturing was carried out by treatment with 0.05% trypsin/0.02% EDTA. Fetal cells were used to evaluate the influence of PCS on the SCE and MN frequency. For the SCE analysis, 5-Deoxybromouridine (BrdU) 10 µg/ml (Bukvic et al., 2000; Gebhart, 1981; Lukusa et al., 1988;) was added for the culture period (48 hours and 72 hours). For the final 2 hours, the cells were exposed to Colcemid, at a final concentration of 0.2 µg/ml. After trypsin treatment, slides were made and Fluorescence-plus-Giemsa staining technique was performed. Fifty metaphases were scored for SCE analysis, and at least 200 mitoses were calculated for the proliferative rate index (PRI).

A modified technique Micronucleus Test (MN; Bukvic et al., 2000) described by Fenech (1993) was used. When 80% of confluence was reached, Cytochalasin-B was added at a final concentration of 6 µg/ml. 28 hours after addition of Cytochalasin-B the cells were trypsinized and harvested without Colcemid treatment. Micronuclei were scored only in binucleated cells according to the criteria of Tawn and Holdsworth (1992). At least 2000 binucleated cells were analysed (1000 binucleated cells for every culture preparation — 500 binucleated cells from each of 2 slides from the same preparation).

## Results and Discussion

Once again our cases confirmed that whenever RS is suspected after ultrasonography, cytogenetic investigations are essential to confirm the diagnosis. We evaluated the MN frequency as a bio-indicator of PCS and chromosome lagging in RS cells compared to the normal fetal (female and male) cells. We noted that in the RS fetal cells (AFS and CVS) the MN frequency was significantly increased for the controls (50‰ vs. 11‰ of control–female and male cells [range 7–14‰];  $p < .001$ ). Our data are in accord with those of Benzacken et al. (1996) and earlier observations of Jabs et al. (1991), who demonstrated that during the RS cell anaphase there is an increased incidence of outlying, lagging or premature advancing of the chromosomes. The

lagging chromosomes account for the formation of micronuclei and aneuploidy.

The SCE frequency in CVS cultures harvested after 72 hours of BrdU incorporation was very high in RS fetal cells (17.96%) with respect to the controls (female CVS cells: 8.65%; male CVS cells: 8.80%;  $p < .05$ ). However, the SCE frequency evaluated after 48 hours of BrdU incorporation revealed an absolutely comparable situation between RS fetus and controls (SCE RS fetus 5% vs. 5.33% of controls range 4% to 6%;  $p > .05$ ). In the 72 hour culture experiment the PRI for fetal and control cells was assessed. To exactly calculate this parameter it is necessary to observe and distinguish cells in first, second and third division. Such possibility does not exist for the 48-hour cell cultures. Significantly ( $p < .05$ ) lower PRI was observed for RS fetal cells (2.28) with respect to the controls (female CVS culture: 2.85; male CVS culture: 2.89). Our results, in accord with those of Pavlopoulos et al. (1998), confirm that fetal RS cells show a decreased cell proliferation rate.

We did not find significant differences of SCE frequency between RS amniocytes and normal controls (7.49% vs. 7.8%). However, these results are inconclusive, as only one culture was used for one experiment, and the quality of the chromosomal preparation was poor.

In conclusion, whenever Roberts syndrome is suspected, cytogenetic (QFQ, GBG, and CBG banding procedures) investigations are essential to confirm the diagnosis. First trimester ultrasound controls and early diagnosis by CVS should be suggested in familial cases.

Interestingly, different cell types expressed different levels of SCE frequency (amniocytes and CVS stromal cells). Furthermore, the SCE frequency increased proportionally to the cell cycle increasing ( $1^{\circ} < 2^{\circ} < 3^{\circ} \dots n$ ; differences observed between 48-hour and 72-hour cultures–CVS). Obviously, our observations are too scarce to draw conclusions, but if confirmed, could be useful for future investigations, especially considering that the two techniques (MN and SCE) are simple to perform and do not require expensive laboratory equipment. Furthermore, in the same case of RS that we previously published (Resta et al., 2006), our results suggested the existence of intertissue and interindividual NMD variability, which could account for the varying severity reported in carriers of identical mutations.

It is well known that SCEs are produced during DNA replication and reflect a DNA repair process. SCE may be the result of recombination repair occurring at the stalled replication fork due to obstacles on the template DNA (Sonoda, 1999). The significantly increased rate of SCE in Roberts CVS stromal cells might reflect a greater proneness to DNA replication errors of these cells with respect to amniocytes. The increased SCE frequency in CVS cells could be independent of ESCO2 gene expression or, alternatively, it could be a consequence of the absence of wild type

ESCO2 in the cells, in which we have observed (Resta et al., 2006) a higher efficiency of NMD and a lower expression of ESCO2 gene at the mRNA and protein levels.

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