

# Female gamete segregation in two carriers of translocations involving 2q and 14q

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FISH, using a combination of whole-chromosome painting and telomeric probes, was used to study the gamete segregation of two female carriers of translocations involving the same chromosome arms, 2q and 14q. Preimplantation genetic diagnosis of the first polar bodies of these oocytes permitted selecting normal embryos for replacement. Copyright © 2000 John Wiley & Sons, Ltd.

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## INTRODUCTION

Chromosome translocations are associated with infertility, high risk of spontaneous abortions and chromosomally unbalanced offspring. They are found in 0.2% of the neonatal population (Maeda *et al.*, 1991; Stern *et al.*, 1999) but this percentage is higher for infertile couples and/or couples with recurrent abortions (Testart *et al.*, 1996; Meschede *et al.*, 1998; Van der Ven *et al.*, 1998; Stern *et al.*, 1999).

For carriers of translocations it is very important to know the chances of conception and the risks of spontaneous abortion, which are both closely linked to the number of normal gametes produced. It is also important to know the chances of carrying a chromosomally unbalanced child, which can be reasonably inferred from prenatal diagnosis and live birth data. Preimplantation genetic diagnosis (PGD) has recently been applied to translocation cases (Munné *et al.*, 1998a,b,c, 2000, Conn *et al.*, 1998) and has achieved a significant reduction of spontaneous abortions (Munné *et al.*, 1998b, in press).

The first studies of segregation modes used post-zygotic material. These results were used to formulate rules to predict unbalanced offspring (Jalbert *et al.*, 1980; Smith and Gaha, 1990; Midro, 1992). However, the specimens used in these studies came from late-stage embryos in which some selective processes would have occurred. Therefore, it is probable that these specimens showed only the most viable segregation types. PGD of first-polar bodies now permits the study of female gamete segregation (Munné *et al.*, 1998a,b); but because of the scarcity of material, little data are yet available. Here we present the segregation data obtained by PGD of two translocation cases involving the same 2q and 14q arms.

## MATERIAL AND METHODS

### Patients

Polar body biopsies were performed at The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center (case B) and at The New England Clinic of Reproductive Medicine (case A). The PGD analysis was performed at The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center. These procedures were performed in accordance with guidelines approved by the Institutional Review Board of Saint Barnabas Medical Center and The New England Clinic of Reproductive Medicine through TUFTS Medical Center, including individual written informed consent. Two couples underwent PGD in which the female partner carried a balanced translocation.

### Case A

A 27-year-old female had a balanced translocation with karyotype 46,XX,t(2;14)(q23;q24). Her husband was found to be karyotypically normal. The couple experienced three spontaneous abortions from natural conception, of which none were genetically analysed.

### Case B

A 33-year-old female had a balanced translocation with karyotype 46,XX,t(2;14)(q31;q24). Her husband was found to be karyotypically normal. The couple obtained three pregnancies from natural conception of which one delivered a normal baby; and two resulted in spontaneous abortions, neither of which was genetically analysed.

### PB biopsy and FISH

Polar body biopsy was performed no later than 6 h after ovarian puncture; the biopsy and fixation were performed as previously described (Munné *et al.*, 1998a). The FISH procedure recommended by Cyto-

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cell (Adderbury, UK) was followed with some modifications. The hybridization solution (10 µl) (Cytocell) was mixed and supplemented with 3 µl of a mix of painting probes, labelled in Spectrum Green (Vysis) and Orange Spectrum (Vysis), for chromosomes 2 and 14 respectively. The final mixture was added on a coverslip supplied by Cytocell, which had telomere probes for the 14q arm labeled with Cy3. The coverslip, with the painting-supplemented hybridization solution, was flipped over the slide containing the fixed cells to be analysed so that the hybridization solution and the coverslip-attached telomere probe faced the cells. The slides were placed for 5 min at 37°C, denatured at 75°C for 5 min, and then placed overnight to hybridize. Next morning, the slides were washed in 1 × SSC for 2 min at 72°C, after which the signals appeared as specified in the Cytocell protocol.

## RESULTS

### Lymphocyte and oocyte controls for painting probes

In each of the translocation carriers, the probes were first tested in fixed lymphocytes to make sure that the painting and telomere probes properly characterized

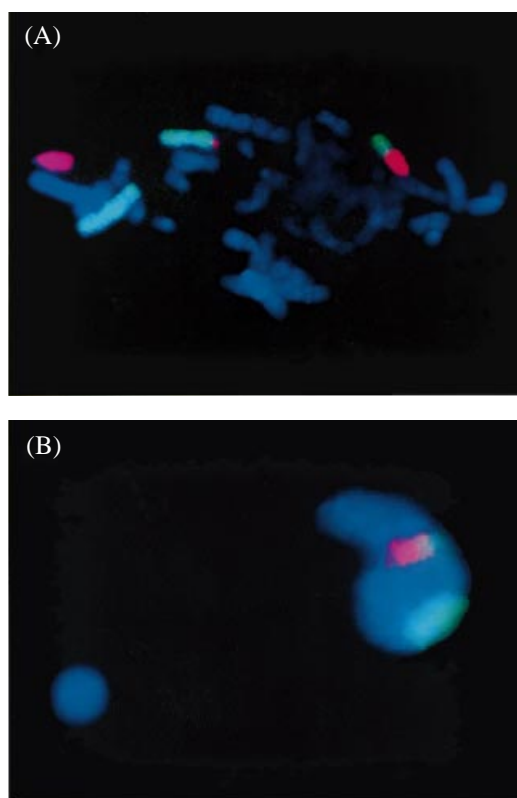


Figure 1—Chromosome painting of a lymphocyte metaphase (A) and an unbalanced 1st PB (B) from case B carrier of a 46,XX,t(2;14)(q31;q24) translocation. The probes used were WCP-2 (green), and WCP-14 (orange), and a telomere probe for 14q also in orange. (A) shows a lymphocyte metaphase with a normal chromosome 2, a normal 14, a derivative 2 and a derivative 14. (B) shows a polar body with a normal chromosome 2 and a derivative 14

the translocation. In all translocations, the lymphocytes at metaphase stage showed one chromosome 2 labelled completely in green, one chromosome derivative 2 labelled in green and red, one chromosome 14 labelled completely in orange and one chromosome derivative 14 labelled in orange and green; thus demonstrating that these probes properly characterized the translocation (Figure 1A). A minimum of 30 lymphocytes at metaphase stage were analysed for each patient and used as controls. The error rate was 4.5% for case A, and 0% for case B, or an average of 2.9% (2/70).

### PGD results

The number of normal or balanced polar bodies detected in these cases was low (Table 1). Overall, only two out of 21 polar bodies (PB) were considered balanced or normal (PB no. 6 of case A was balanced and PB no. 1 of case B was normal). Fifteen were classified as unbalanced, and in five no results were obtained because the polar bodies were inadvertently damaged during biopsy and/or fixation (case A: PB nos 1, 2, 3) or because the probes did not work properly (case A: PB no. 13; case B: PB no. 5). One embryo was replaced in each case, and patient A became pregnant with monozygotic bichorionic twins. Ultrasound analysis did not show any gross abnormalities but the patient rejected prenatal diagnosis. After delivery, the two male twins were karyotyped and found to be normal 46,XY. However, a minor congenital abnormality, hydronephrosis, was found in both of them.

Both translocations are quite similar, so one would expect similarities in the segregation mode; but they were surprisingly different. In case A, the predominant segregation was 3:1 (44.4%), followed by Adjacent-I and -II (22.2% each), and alternate (11.1%). However, in case B the predominant segregation was Adjacent-I (Figure 1B) (57.1%), followed by the other segrega-

Table 1—Polar bodies (PB) that could be analysed

| PB            | Result          | Segregation type | Diagnosis  |
|---------------|-----------------|------------------|------------|
| <i>Case A</i> |                 |                  |            |
| 4             | 2, der14        | Adjacent-I       | Unbalanced |
| 5             | 2, der2         | Adjacent-II      | Unbalanced |
| 6             | der2, der14     | Alternate        | Balanced   |
| 7             | 2               | 3:1              | Unbalanced |
| 8             | der2, 14        | Adjacent-I       | Unbalanced |
| 9             | 2, der2         | Adjacent-II      | Unbalanced |
| 10            | 2               | 3:1              | Unbalanced |
| 11            | der2            | 3:1              | Unbalanced |
| 12            | 2               | 3:1              | Unbalanced |
| <i>Case B</i> |                 |                  |            |
| 1             | 2,14            | Alternate        | Balanced   |
| 2             | 2, der2         | Adjacent-II      | Unbalanced |
| 3             | der2, 14, der14 | 3:1              | Unbalanced |
| 4             | der2, 14        | Adjacent-I       | Unbalanced |
| 6             | der2, 14        | Adjacent-I       | Unbalanced |
| 7             | der2, 14        | Adjacent-I       | Unbalanced |
| 8             | der2, 14        | Adjacent-I       | Unbalanced |

tions (14.3% each). Because of the limited numbers of polar bodies analysed, these differences were not statistically significant. Overall, Adjacent-I (37.5%) and 3:1 (34.3%) were the most common segregations.

## DISCUSSION

By performing polar body biopsy shortly after oocyte retrieval, metaphase stage chromosomes are obtained which can be analysed using chromosome painting probes (Munné *et al.*, 1998a). This approach was followed in the present study, and it has been quite successful in producing chromosomally normal pregnancies after PGD of translocations and obtaining a significant reduction in spontaneous abortions for those cases (Munné *et al.*, 1998b). In the present two cases only 2/21 oocytes were found to be normal or balanced, but even so, one patient achieved a chromosomally normal pregnancy.

This approach also permits the study of gamete segregation in female translocation carriers, including the differentiation of normal from balanced oocytes. We could not find any existing gamete segregation studies involving chromosomes 2 and 14. Translocations involving chromosomes 2 and 14 are uncommon and their frequency has been estimated to be 1% of translocation carriers (Campana *et al.*, 1986; Cohen *et al.*, 1996). In the literature, only two cases of translocation have been reported; one by Stoll *et al.* (1974) and the other by Coco and Penchaszadeh (1977). But studies on spermatozoa of male carriers for translocations other than those involving 2 and 14 showed that the alternate segregation was the most frequent one, with few cases having Adjacent-I as the most frequent segregation (Estop *et al.*, 1995). Although our numbers of PBs analysed are limited, our results differ from that study.

This might be attributed to differences between male and female segregation. Another possibility is the existence of individual differences between carriers. For instance, in a study by Van Assche *et al.* (1999) couples with the exact same translocation showed very different meiotic behaviour. Moreover, in a PGD inversion case performed by Iwarsson *et al.* (1998), they obtained conflicting, different results after two treatments of the same patient. Further studies are required to achieve a reliable pattern of segregation in eggs for these translocations, but at least it can be said that in neither translocation case the alternate segregation is the most frequent.

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