

FISH analysis of the chromosomal status of spermatozoa from three men with 45,XY,der(13;14)(q10;q10) karyotype

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Meiotic segregation of chromosomes 13 and 14 was studied in the ejaculated spermatozoa of three men carrying a translocation der(13;14)(q10;q10). The spermatozoa of these patients and of a donor with a normal 46,XY karyotype (control) were analysed by two-colour fluorescent in-situ hybridization (FISH) with specific chromosomal painting of chromosomes 13 and 14, by two-colour FISH detecting chromosomes 18 and 21 and by triple-colour FISH for chromosomes X, Y and 8. For patients 1, 2 and 3, respectively, 81.34, 82.60 and 88.90% of the analysed nuclei showed normal or balanced chromosomal status, resulting from the alternate segregation of the translocation. The rates of spermatozoa with an unbalanced status (disomy and nullisomy, 13 or 14) resulting from the adjacent mode of segregation were estimated respectively at 18.06, 16.32 and 10.80 (for patients 1, 2 and 3). Additional colour FISH analysis with probes specific for chromosomes X, Y, 8, 18 and 21 showed a significant increase in some disomy frequencies (8, 18, 21, X and Y for patient 1, only 18 for patient 2) in comparison with the control. These results would seem to indicate an interchromosomal effect.

Key words: fluorescence in-situ hybridization/interchromosomal effect/meiotic segregation/spermatozoa/13;14 translocation

Introduction

Robertsonian translocations are common in man with an approximate incidence of 1 in 1000 births (Evans *et al.*, 1978), and translocation (13;14) is one of the most frequent. This translocation can occur *de novo* or be transmitted by one of the parents. In the latter case, the risk of free 21 trisomy by interchromosomal effect is 5%. Men carrying this translocation have a normal phenotype, but can have problems of infertility associated with more or less severe oligozoospermia. Nevertheless, when these men have offspring, the children generally have a chromosomal status which is either normal (46,XX or 46,XY) or balanced [45,XX,der(13;14)(q10;q10) or 45,XY,der(13;14)(q10;q10)] (Friedrich and Nielsen, 1974). During pre-natal diagnosis in 230 pregnancies in which one of the parents was a carrier of this translocation, no unbalanced offspring were found (Boué and Gallano, 1984). This suggests that there is very early loss of potentially unbalanced fetuses.

The studies evaluating the risks of imbalances in the chromosomal status of spermatozoa of 45,XY,der(13;14)(q10;q10) men with disorders of spermatogenesis were initially studies of meiotic cytogenetics on testicular biopsies (Vidal *et al.*, 1982; Luciani *et al.*, 1984). An association of trivalent and sex vesicle in the majority of the nuclei at the pachytene stage in a sterile man carrying a translocation t(13;14) has been shown (Luciani *et al.*, 1984). According to two studies

(Johannisson *et al.*, 1993; Gabriel-Robez and Rumpler, 1996), this association could lead to gametogenic arrest for Robertsonian translocation carriers. Luciani *et al.* observed that the trivalent was always in the *cis* configuration and suggested that this *cis* configuration of the 13;14 trivalent promotes an alternate segregation which should produce an equal frequency of normal and balanced spermatocytes (Luciani *et al.*, 1984). These data obtained by the analysis of spermatocytes seem to be in agreement with the results provided by a family study of carriers of t(13;14) (Dutrillaux and Lejeune, 1970) showing a similar number of normal offspring and carriers of the balanced translocation. On the other hand, some studies have indicated an excess of offspring carrying the balanced translocation (Hamerton, 1970; Boué and Gallano, 1984).

The results of the meiotic segregation of these translocations have also been analysed by karyotyping spermatozoa after penetration of zona-free hamster oocytes (Pellestor *et al.*, 1987; Martin, 1988) or by injection of human spermatozoa into mouse oocytes (Ogawa *et al.*, 2000), although these data are limited.

Although fluorescent in-situ hybridization (FISH) can be used to analyse a large number of spermatozoa (Downie *et al.*, 1997; Guttenbach *et al.*, 1997; Morel *et al.*, 1997), for purely technical reasons due to the fact that centromeric probes 13cen,

Table I. Semen parameters of the three 45,XY,der(13;14)(q10;q10) patients

| | Age (years) | Volume (ml) | Sperm concentration ($\times 10^6/\text{ml}$) | Motility (%) | Abnormal morphology (%) | Necrozoospermia (%) |
|-----------|----------------|----------------|---|-----------------|-------------------------------|------------------------|
| Patient 1 | 28 | 7 | 40.64 | 5–10 | 64 | 45–50 |
| Patient 2 | 34 | 5 | 2.7 | 35–40 | 94 | 40–45 |
| Patient 3 | 41 | 5 | 0.5 | 8 | 77 | — ^a |

^aNot performed.

21cen or 14cen, 22cen indistinctly detect the centromere of chromosomes 13 and 21 or 14 and 22, there are still very few studies of this kind (Rousseaux *et al.*, 1995; Honda *et al.*, 2000). The studies (Rousseaux *et al.*, 1995; Honda *et al.*, 2000) carried out with specific probes for subtelomeric regions of chromosomes could not differentiate normal or balanced spermatozoa. To avoid the above problems, we have used specific chromosomal painting of chromosomes 13 and 14 to differentiate the normal spermatozoa (two signals of different colour separated by at least one diameter) or balanced spermatozoa (two signals of different colour coupled one with the other) (Figure 1).

This study presents the results obtained on the ejaculated spermatozoa of three men with 45,XY,der(13;14)(q10;q10) karyotype.

Materials and methods

Cytogenetic analysis

Robertsonian translocations were found for patient 1 when his child was born with a mosaic 8 trisomy syndrome and with a 45,XY,der(13;14)(q10;q10)/46,XY,+8,der(13;14)(q10;q10) karyotype, and for patients 2 and 3, when they sought advice for infertility.

Translocation carriers and sperm characteristics

The characteristics of the three analysed ejaculates were different: asthenonecroteratozoospermia for patient 1, severe oligoteratozoospermia and necrozoospermia for patient 2, and very severe oligoasthenozoospermia and teratozoospermia for patient 3 (Table I). A fertile man with a normal 46,XY karyotype and normal sperm characteristics (World Health Organization, 1999) was used as the control. Prior to this study, patients were informed of the investigations and gave their consent. This study was reviewed by the ethics committee of Besançon University Hospital.

Analysis of aneuploidy

Sperm preparation

The procedure has been described elsewhere (Morel *et al.*, 1997). Each semen sample was washed twice in phosphate-buffered saline, and 100 μl of sperm suspension was dropped and fixed onto the slide. Sperm nuclei were lightly decondensed for 1 min with a solution of 1 mol/l NaOH.

Molecular DNA probes

The sperm status of these patients and of a donor with a 46,XY normal karyotype (control) was analysed firstly by two-colour FISH with specific chromosomal painting of chromosomes 13 (P5613-RW; Appligene Oncor, Illkirch, France) and 14 (P5614-GW; Appligene Oncor), secondly by two-colour FISH with painting probes (P5618-RW and P5621-GW; Appligene Oncor) for chromosomes 18 and 21 (respectively) and thirdly by triple-colour FISH using DNA probes

for the centromeric regions of chromosome X (DXZ1; M.Morris, Geneva, Switzerland) and of chromosome 8 (pJM128; American Type Culture Collection, Rockville, MA, USA) and for the heterochromatin region of chromosome Y (pHY2.1; J.H.Cooke, Edinburgh) (Cooke *et al.*, 1982).

Hybridization procedure and analysis

Hybridization procedure and analysis have been described previously (Mercier *et al.*, 1996, 1998; Morel *et al.*, 1999, 2000). Slides were included for scoring if the efficiency of hybridization was $>95\%$. If the efficiency was lower, the results of FISH analysis would not be reliable. Nuclei without a fluorescent signal, either nullisomic nuclei for the analysed chromosomes or non-hybridized nuclei resulting from technical failure, were not included in our study. In two-colour FISH 13–14, spermatozoa with two signals of different colours (red for chromosome 13 and green for chromosome 14), separated by at least one diameter were considered as normal, with one chromosome 13 and one chromosome 14. Spermatozoa with two signals of different colours coupled one with the other, associated with a central yellow signal (visualized by the association of the two fluorescence signals), were considered as balanced with der(13;14). Subsequent image acquisition using a CCD camera with digital image enhancement was performed (Cytovision; Applied Imaging).

Statistical analysis

An independent χ^2 -test was used to compare the results obtained for spermatozoa from the three 45,XY,der(13;14)(q10;q10) patients and those observed in the control spermatozoa.

Results

Using two-colour FISH 13–14, 2984 (patient 1), 1109 (patient 2), 1009 (patient 3) and 10 019 (control) spermatozoa were analysed. For patients 1, 2 and 3 respectively, 81.34, 82.60 and 88.90% of the analysed nuclei showed normal or balanced chromosomal status, resulting from the alternate segregation of the translocation (Table II). The proportion of spermatozoa with unbalanced status (disomy and nullisomy for chromosomes 13 or 14) resulting from the adjacent mode of segregation were estimated at 18.06, 16.32 and 10.80% for patients 1, 2 and 3 respectively.

When only FISH probes for chromosomes 13 and 14 are used, it is not possible to differentiate between 3:0 or diploid cells. Thus the analysed cells resulting from either segregation 3:0 or diploid cells accounted for 0.6, 1.08 and 0.30% of spermatozoa for patients 1, 2 and 3 respectively (Table II). The percentages of 13 and 14 disomies, for control spermatozoa, were estimated at 0.25 and 0.16% respectively and the diploidies were estimated at 0.12%.

Using two-colour FISH 18–21 (Table III), there was an

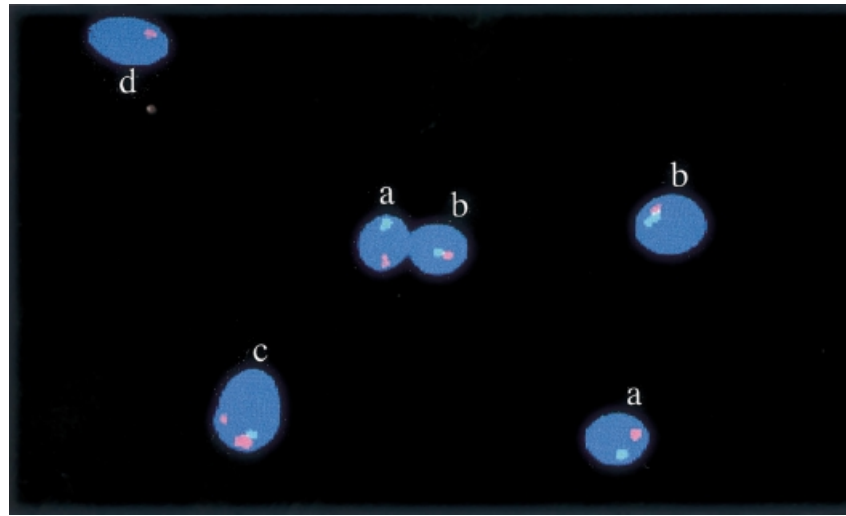


Figure 1. Spermatozoa from a t(13;14) carrier, hybridized with P5613-RW (chromosome 13, red) and P5614-GW (chromosome 14, green). (a) Nucleus with normal chromosome status (alternate segregation). (b) Nucleus with balanced chromosome status (alternate segregation). (c and d) Nuclei with abnormal chromosome status (adjacent segregation).

Table II. Results from two-colour fluorescent in-situ hybridization 13–14 for the analysis of the chromosomal status in spermatozoa of the three 45,XY,der(13;14)(q10;q10) patients and the control

| Combinations | Meiotic segregation | Patient 1 (%) | Sum | Patient 2 (%) | Sum | Patient 3 (%) | Sum | Control |
|----------------------|---------------------|---------------|-------|---------------|-------|---------------|-------|----------------|
| 13q/14q | Alternate | 38.21 | | 40.76 | | 44 | | |
| der(13q;14q) | Alternate | 43.13 | | 41.84 | | 44.9 | | |
| Sum | | | 81.34 | | 82.60 | | 88.90 | |
| 13q/der(13q;14q) | Adjacent | 3.08 | | 1.89 | | 2.67 | | Disomy 13 0.25 |
| 14q | Adjacent | 6.1 | | 4.51 | | 3.67 | | |
| 14q/der(13q;14q) | Adjacent | 3.82 | | 4.15 | | 1.68 | | Disomy 14 0.16 |
| 13q | Adjacent | 5.06 | | 5.77 | | 2.78 | | |
| Sum | | | 18.06 | | 16.32 | | 10.80 | |
| 13q/14q/der(13q;14q) | 3:0 or diploidies | 0.60 | | 1.08 | | 0.30 | | 0.12 |
| Sum | | | 0.60 | | 1.08 | | 0.30 | |
| No. of spermatozoa | | 2984 | | 1109 | | 1009 | | 10 019 |

Table III. Results from two-colour fluorescent in-situ hybridization 18–21 for the analysis of the chromosomal status in spermatozoa of the control and of the three 45,XY,der(13;14)(q10;q10) patients

| | Disomy 18 (%) | Disomy 21 (%) | Diploidy (%) | No. of spermatozoa |
|-----------|-------------------|-------------------|-------------------|--------------------|
| Patient 1 | 0.77 ^a | 0.80 ^a | 0.83 ^a | 3001 |
| Patient 2 | 0.78 ^a | 0.49 | 0.88 ^a | 1025 |
| Patient 3 | 0.30 | 0.40 | 0.10 | 1007 |
| Control | 0.19 | 0.33 | 0.09 | 10 082 |

^a $P < 0.05$ versus control.

increased incidence of chromosome 18 and 21 disomies (patient 1) and 18 disomies (patient 2) when compared with those estimated in the control. Triple-colour FISH X-Y-8 (Table IV) showed a significant increase in XX, YY and chromosome 8 disomy frequencies (patient 1) compared to the control.

Discussion

To our knowledge, few data are available on the chromosomal status in spermatozoa of men carrying Robertsonian transloca-

tions (Table V). By heterospecific fertilization, six studies (Balkan and Martin, 1983; Pellestor *et al.*, 1987; Martin, 1988; Pellestor, 1990; Martin *et al.*, 1992; Syme and Martin, 1992), and only one study by sperm injection into oocytes (Ogawa *et al.*, 2000), have been reported. For the different Robertsonian translocations (13–14, 13–15, 14–21, 15–22 or 21–22), the results show a prevalence of the alternate mode of segregation from 73.5% (out of 117 analysed karyotypes) (Martin, 1988) to 96.6% (out of 149 karyotypes) (Syme and Martin, 1992). The rate of unbalanced gametes varies for the seven studied patients from 2.7 to 26.5%, and only one spermatozoa resulting from the segregation mode 3:0 has been observed (Syme and Martin, 1992).

None of the studies showed an increase in the rates of numerical and structural chromosomal abnormalities in these patients carrying a Robertsonian translocation, when compared to the rates observed in the control patients with normal 46,XY karyotype. Therefore these results would seem to indicate that there is not an interchromosomal effect.

Only three studies have used FISH to analyse the sperm nuclei of Robertsonian carriers: two for t(14q;21q) men (Rousseaux *et al.*, 1995; Honda *et al.*, 2000) and one for t(21q;

Table IV. Results from three-colour fluorescent in-situ hybridization X-Y-8 for the analysis of the chromosomal status in spermatozoa of the control and of the three 45,XY,der(13;14)(q10;q10) patients

| Presumed karyotype | % 23, | | % 24, | | | % 46, | | | % 24, | No. of spermatozoa |
|--------------------|-------|-------|-------------------|-------------------|------|-------------------|-------------------|-------------------|-------------------|--------------------|
| | X | Y | XX | YY | XY | XX | YY | XY | X/Y+8 | |
| Patient 1 | 48.02 | 48.67 | 0.20 ^a | 0.60 ^a | 0.23 | 0.13 ^a | 0.26 ^a | 0.69 ^a | 1.21 ^a | 3055 |
| Patient 2 | 50.24 | 48.36 | 0.10 | 0.10 | 0.20 | — | — | 0.50 ^a | 0.50 | 1005 |
| Patient 3 | 49.12 | 50.57 | — | — | 0.1 | — | — | — | 0.21 | 969 |
| Control | 50.20 | 49.24 | 0.04 | 0.06 | 0.13 | — | — | 0.10 | 0.23 | 10 021 |

^a*P* < 0.05 versus control.**Table V.** Recapitulation of molecular studies of the chromosomal status in the spermatozoa of Robertsonian translocation carriers by heterospecific fertilization or fluorescent in-situ hybridization (FISH)

| Translocation | Age (years) | No. of spermatozoa studied | Segregation mode | | Adjacent | Diploid cells 3:0 | Ambiguous and/or non-hybridization | References |
|--------------------------------|-------------|----------------------------|------------------|----------|----------|-------------------|------------------------------------|--------------------------------|
| | | | Alternate | Balanced | | | | |
| | | | Normal | Balanced | | | | |
| t(14q;21) ^a | | 24 | 70.8 | 16.7 | 12.5 | 0 | | Balkan and Martin (1983) |
| t(13;14)(p11;q11) ^a | 41 | 78 | 50 | 42.3 | 7.7 | 0 | | Pellestor <i>et al.</i> (1987) |
| t(13q;14q) ^a | 23 | 117 | 35.9 | 37.6 | 26.5 | 0 | | Martin (1988) |
| t(13;15)(p11;q11) ^a | 40 | 67 | 46.3 | 43.3 | 10.4 | 0 | | Pellestor (1990) |
| t(15q;22q) ^a | 35 | 115 | 42.6 | 47 | 10.4 | 0 | | Martin <i>et al.</i> (1992) |
| t(21q;22q) ^a | 52 | 149 | 49.6 | 47 | 2.7 | 0.7 | | Syme and Martin (1992) |
| t(13q;14q) ^b | 32 | 45 | 37.77 | 53.33 | 8.9 | 0 | | Ogawa <i>et al.</i> (2000) |
| t(14q;21q) ^c | 34 | 1116 | | 72.22 | 18.01 | 0.8 | 8.97 | Rousseaux <i>et al.</i> (1995) |
| t(21q;22q) ^c | 54 | 350 | 38 | 22 | 36 | | 4 | Mennicke <i>et al.</i> (1997) |
| t(14q;21q) ^c | 32 | 16 578 | | 88.42 | 11.25 | 0.18 | 0.15 | Honda <i>et al.</i> (2000) |

^aStudies by heterospecific fertilization.^bStudy by sperm injection into mouse oocyte.^cStudies by FISH.

22q) men (Mennicke *et al.*, 1997). These studies found a majority (60–88.42%) of sperm cells with normal or balanced chromosomal status (Table V).

For our three 45,XY,der(13;14)(q10;q10) patients, the majority of spermatozoa analysed (from 81.34 to 88.90%) showed a chromosomal status resulting from an alternate mode of segregation. The frequencies of normal or balanced spermatozoa were similar for patients 2 and 3, but were different for patient 1, with a lower level of normal spermatozoa and a relative excess of balanced spermatozoa. As has been the case for most research teams, it is considered that the chromosomal status of spermatozoa does not interfere with their fertilizing capacity. The difference as observed in patient 1 could explain the discrepancy between the results provided by family studies of carriers of t(13;14); some of them have shown a similar number of normal offspring and offspring carrying balanced translocations (Dutrillaux and Lejeune, 1970) and others revealed an excess of carriers of the balanced translocation (Hamerton, 1970; Boué and Gallano, 1984).

Our results are in agreement with previous studies with regard to the chromosome analysis of human spermatozoa from 13;14 Robertsonian translocation carriers performed by heterospecific fertilization (Pellestor *et al.*, 1987; Martin, 1988) or by using sperm injection into oocytes (Ogawa *et al.*, 2000). All these studies have indicated a prevalence of the alternate mode of segregation, 73.5% out of 117 spermatozoa (Martin, 1988) and 92.3% out of 78 karyotypes (Pellestor *et al.*, 1987).

This prevalence of the alternate mode can be explained by meiotic cytogenetic studies which indicate that the trivalent at pachytene are always in *cis* configuration (Luciani *et al.*, 1984).

The different frequencies of unbalanced spermatozoa, resulting from the adjacent modes of segregation (estimated at between 10.8 and 18.06%) point to an interindividual or an intraindividual variation concerning these 45,XY,der(13;14)(q10;q10) men. These variations have also been found from studying heterospecific fertilization or sperm injection into oocytes (Pellestor *et al.*, 1987; Martin, 1988; Ogawa *et al.*, 2000). Moreover, such variabilities have been demonstrated for different Robertsonian translocations, including some with the same chromosomal pairs [translocation 14–21 (Balkan and Martin, 1983; Rousseaux *et al.*, 1995; Honda *et al.*, 2000), translocation 21–22 (Syme and Martin, 1992; Mennicke *et al.*, 1997)]

For our three patients, the percentages of chromosome 13 or 14 nullisomic spermatozoa were higher than those of chromosome 13 or 14 disomic gametes (Table II). However, in Robertsonian translocations, the adjacent modes of segregation should theoretically involve the same rate of spermatozoa carrying a 13 or 14 supernumerary chromosome as that of nullisomic spermatozoa for these same chromosomes.

As previously suggested (Rousseaux *et al.*, 1995), the observed results could be due to an artefactual overestimation of the nullisomies, resulting from unequal hybridization efficiencies of both probes. Moreover, in the two studies on

the 13–14 Robertsonian translocations using heterospecific fertilization, the percentages of 13 or 14 nullisomic spermatozoa were similar to those of disomic gametes.

If it is assumed that the rates of nullisomies must be identical to those of disomies, the estimations of unbalanced spermatozoa resulting from the adjacent mode of segregation could be corrected to 13.8% (patient 1), 12.08% (patient 2) and 8.7% (patient 3).

A small percentage of analysed spermatozoa resulted from segregation 3:0 or are diploid cells. With two-colour FISH 13–14, it is not possible to differentiate between these two types of cells. Only triple-colour FISH with another autosomal probe could make this distinction, but it would involve superpositions of fluorescent signals. However, for our three patients, the rates of diploidies were evaluated in two-colour FISH 18–21 and triple-colour FISH X–Y–8 at respectively 0.83 and 1.08% (patient 1), 0.88 and 0.50% (patient 2), 0.10 and 0% (patient 3). These results tend to suggest that the majority of the cells with 13q/14q/der(13q;14q) status, estimated by two-colour FISH 13–14, are diploid cells (Bernardini *et al.*, 1997; Pellestor *et al.*, 1997; Blanco *et al.*, 1998; Morel *et al.*, 1998).

In triple-colour FISH X–Y–8, there was a high frequency of chromosome 8 disomy in the spermatozoa of patient 1 (1.21%). This frequency could possibly be explained by the fact that this man, whose son has a mosaic chromosome 8 trisomy, is also carrying this mosaicism, although unfortunately this hypothesis could not be verified. Alternatively it could be explained by the presence of an abnormally high rate of non-segregation due to an interchromosomal effect. This latter hypothesis is supported by the fact that there was also a notable increase in chromosome 18, 21, XX, and YY disomies in the spermatozoa of this patient and by the fact that there was an increased rate of chromosome 18 disomies in the spermatozoa of patient 2.

These observations in favour of an interchromosomal effect confirm those of other studies (Rousseaux *et al.*, 1995; Mercier *et al.*, 1998), but refute the data obtained by heterospecific fertilization. However, weak increases in disomy frequencies are not always highlighted using the heterospecific fertilization technique (Martin *et al.*, 1999).

The increase of chromosome 21 disomies for patient 1 should perhaps be considered with the old hypothesis (Lejeune, 1965) which suggests that there is a predisposition for patients carrying a t(D;D) Robertsonian translocation to have offspring with 21 trisomy (5% risk) (de Grouchy and Turleau, 1982). This hypothesis could be explained by a preferential association between the 13–14 trivalent and 21 bivalent observed in the nuclei in the pachytene stage (Luciani *et al.*, 1984). However, another study (Harris *et al.*, 1979) found no trisomy in the offspring of 86 carriers of t(13;14). In fact, few cases of 21 trisomy associated with a translocation are sporadic (Lindenbaum *et al.*, 1985; Couzin *et al.*, 1987).

For patient 3, who had the highest frequency of normal or balanced spermatozoa, there was no significant increase in the disomy frequencies of the other studied chromosomes. This suggests a possible interindividual variation in the interchromosomal effect for the 45,XY,der(13;14)(q10;q10) men.

Moreover, it seems that the higher the frequency of normal or balanced spermatozoa, the less significant the interchromosomal effect.

In conclusion, although this study was based on only three cases, it suggests that, in men with 45,XY,der(13;14)(q10;q10) karyotype, the majority of spermatozoa result from alternate segregation. Nevertheless, a relatively large proportion may have an unbalanced chromosomal status. This raises the question of the chromosomal risk for the offspring of 45,XY,der(13;14)(q10;q10) males and the importance of genetic counselling prior to ICSI or IVF treatment for couples where the male carries a Robertsonian translocation.

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