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SHORT COMMUNICATION

Analysis of chromosome abnormalities in sperm and embryos from two 45,XY,t(13;14)(q10;q10) carriers

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Robertsonian translocation t(13q14q) is studied in sperm and embryos of two couples undergoing preimplantation genetic diagnosis (PGD) in which both males are carriers of the translocation. It is already known that the chances of achieving pregnancy for a translocation carrier are directly linked to the number of normal or balanced embryos available for replacement. In our work it was found that the frequency of balanced spermatozoa was almost identical in both patients (74 and 77%), and after PGD, the frequencies of abnormal embryos caused by the translocation were also similar. Sperm chromosome analysis in translocation carriers can provide a reasonable basis for estimating a baseline of chromosome abnormalities to be found in embryos during an assisted reproductive cycle. However, individual factors not linked to the translocation can also produce other chromosome abnormalities (mosaicism, haploidy, polyploidy) and may compromise the chances of achieving a viable pregnancy. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: PGD; FISH; Robertsonian translocation; meiotic segregation; trisomy 13

INTRODUCTION

Robertsonian translocations (RTs) are the most common structural chromosomal abnormalities observed in humans, with an incidence of 1.23 per thousand (Nielsen and Wohlert, 1991). The most frequent RTs are t(13q14q) with an incidence of 0.97 per thousand (Jacobs, 1972). This proportion rises to 1% in infertile men (Guichaoua *et al*., 1990), and has also been associated with infertility (Gosden *et al*., 1978) or oligospermia (Chandley, 1988; Guichaoua *et al*., 1990; De Braekeleer and Dao, 1991).

By using sperm chromatin descondensation techniques (Wyrobeck *et al*., 1990) fluorescence *in situ* hybridization (FISH) can be used to analyse high numbers of sperm to determine more precisely the modes of segregation of a given translocation (Estop *et al*., 1996; Blanco *et al*., 1998). Because the chances of achieving pregnancy for a translocation carrier are directly proportional to the frequency of balanced or normal embryos available for replacement (Munné *et al*., 2000), and because a wide spectrum of unbalanced frequencies has been detected (Estop *et al*., 1996), the frequency of unbalanced gametes could provide a reasonable assessment of the chances for having a normal or balanced embryo. In the present work, the meiotic segregation of chromosomes 13 and 14 was analysed in the embryos of two RT carrier couples undergoing multiple cycles for assisted reproduction, and compared with the abnormalities found in their sperm. If a clear relationship can be found, then sperm chromosome analysis can also provide a reasonable basis for estimating the outcome of assisted reproductive cycles in RT carriers.

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MATERIALS AND METHODS

Patient characteristics

Patient 1 was a 29-year-old G2 P1 SA1 female. Her spouse was a 33-year-old male with a 45,XY,t(13;14)(q10;q10) translocation. They had had five previous IVF cycle attempts. Sperm count was of 2 million/ml with 30% motility and a progression index of 2.

Patient 2 was a 25-year-old G1 P1 SA1 female. Her spouse was a 27-year-old male with a 45,XY,t(13;14)(q10;q10) translocation. Sperm count was in the 5–6 million/ml range with poor motility and morphology. This was their first IVF attempt.

Sperm FISH analysis

Sperm preparation

Samples of the two patients were fixed in methanol : acetic 3 : 1 for FISH analysis. Sperm slides were incubated in 5nM dithiothreitol (DDT) and 1% Triton X-100 solution. Details of sperm fixation, nuclear

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descondensation and FISH processing have been described previously (Vidal *et al.*, 1993).

FISH procedure

FISH was performed using the standard protocol provided by Vysis with slight modifications (Munné *et al.*, 1998) and employing a hybridization mixture containing Locus Specific Indicator (LSI) for chromosome 13 labelled in SpectrumOrange (Vysis, Downess Grove, IL, USA) and Tel 14q in SpectrumGreen (Vysis, Downess Grove, IL, USA).

The slides were observed under a fluorescence scope (Olympus AX70) with the appropriate filters to visualize the fluorochromes and following strict assessment criteria previously described by Blanco *et al.* (1996).

PGD

One cell per embryo at the eight-cell stage was biopsied and fixed on a glass slide as previously described (Munné *et al.*, 1998). Biopsies were performed either at The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center in Livingston, The New England Clinic of Reproductive Medicine of Reading or the IHC Reproductive Clinic of Salt Lake City. Cells were sent to The Institute for Reproductive Medicine and Science of Saint Barnabas for FISH analysis. These procedures were approved by the respective Internal Review Board committees and individual patient consent forms were signed.

FISH was performed as mentioned above. The slides were observed under a fluorescence scope (Olympus AX70) with the appropriate fluorochrome filters. After analysis, the slides were rehybridized with probes for chromosomes 16, 18 and 21, following the above procedure but with a wash of only 30 seconds. This second analysis was performed to differentiate between aneuploidy and chaotic mosaicism. Chaotic mosaics usually have more than one chromosome affected with an abnormal count (Munné *et al.*, 1995), but by analysing five chromosomes, they can be differentiated from aneuploidies, since triple aneuploidies are very rare but chaotic mosaics with three chromosomes and abnormal counts are very common (Munné *et al.*, 1995, Munné and Cohen 1998).

RESULTS

The sperm analysis of both patients produced remarkably similar results, with 74% and 77% normal or balanced gametes, 9% and 8% disomic gametes, 14% and 11% nullisomic gametes, and 3% and 3% of other abnormal forms (Table 1).

Table 1—Sperm analysis

Segregation type	Patient 1	Patient 2
Sperm analysed	1016	1006
Normal or balanced	73.6%	77.4%
Disomics		
Der +13	5.0%	3.7%
Der +14	4.1%	4.2%
Nullisomics		
Only with 13	4.7%	4.4%
Only with 14	9.5%	6.8%
Other types		
No signal	1.5%	0.9%
Two signals of each, with tail	0.5%	0.8%
Two 13, three 14, with tail	0.0%	0.1%
Two 14, three 13, with tail	0.0%	0.2%
Two signals of each, without tail	1.0%	1.2%
Two 13 only, with tail	0.1%	0.0%
Two 14 only, with tail	0.0%	0.1%
Four 13, without tail	0.0%	0.1%
Total normal/balanced	73.6%	77.4%
Total unbalanced	26.4%	22.6%
Total	100.0%	100%

The two patients underwent six cycles of IVF followed by PGD. Of the embryos biopsied in those six cycles, a total of 32 embryos produced nuclei, and of these 32, 30 yielded FISH results. Of those 30, 15 were normal, 6 were aneuploid, 6 were haploid, 1 was tetraploid and 2 were complex abnormal (Table 2). In these last two embryos, three of the five chromosomes analysed gave abnormal numerical results and the embryos were therefore probably mosaics and/or polyploid; but analysis of a single cell cannot be more precise.

The aneuploidies detected for the chromosomes implicated in the RT were three monosomies 13, two monosomies 14 and one trisomy 14, therefore it was estimated that 27% (6/22) of the embryos were abnormal due to this 13q14q RT. This is similar to the rate of abnormalities detected in spermatozoa from both patients (23 and 19%). Patient 1 had less chromosomally normal embryos than patient 2, 43% and 56%, respectively, but the differences were not statistically significant due to the small sample size.

A total of nine embryos were replaced in the five cycles of patient 1; of those nine, two implanted, one in the first cycle resulting in a spontaneous abortion, and one in the third cycle in which was delivered a balanced baby. Patient 2 had three embryos replaced in one cycle, of which one implanted; but the patient miscarried a few weeks later before a fetal heart beat (FHB) could be detected by ultrasonography. No tissue was available for karyotype.

DISCUSSION

FISH sperm analysis accurately describes the proportion of normal sperm in male patients with chromosome abnormalities. This analysis also provides a reasonable basis for predicting the meiotic behaviour

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Table 2—PGD results

Embryos	13	14	16	18	21	PGD diagnosis
Patient 1, cycle 1:						
2	2	2	2	2	2	Normal/balanced (replaced)
3	2	3	2	2	2	Trisomy 14
5	2	2	2	2	2	Normal/balanced (replaced)
6	2	2	2	2	2	Normal/balanced (replaced)
9	1	1	1	1	1	Haploid
Patient 1, cycle 2:						
2	1	2	2	2	2	Monosomy 13
4	1	1	1	1	2	Haploid
5	1	2	2	2	2	Monosomy 13
Patient 1, cycle 3:						
7	2	2	2	2	2	Normal/balanced (replaced)
8	1	1	1	1	1	Haploid
10	1	2	2	2	2	Haploid
Patient 1, cycle 4:						
1	nr	2	2	2	2	NR
3	2	2	2	2	2	Normal/balanced (replaced)
4	2	2	2	2	2	Monosomy 14
8	2	2	2	2	2	Normal/balanced (replaced)
11	2	2	2	2	2	Normal/balanced (replaced)
16	2	2	2	2	2	Normal/balanced
17	2	3	3	4	nr	Complex abnormal
Patient 1, cycle 5:						
1	2	2	2	2	2	Normal/balanced (replaced)
2	2	2	2	2	2	Normal/balanced (replaced)
6	2	2	2	2	2	Monosomy 14
9	3	3	2	2	4	Complex abnormal
16	1	1	1	1	0	Haploid
Patient 2, cycle 1:						
1	nr	2	2	2	2	NR
2	2	2	2	2	2	Normal/balanced (replaced)
3	2	2	2	2	2	Normal/balanced (replaced)
4	0	0	1	1	0	Haploid
5	2	2	2	2	2	Normal/balanced (replaced)
6	2	2	2	2	2	Normal/balanced
8	1	2	2	2	2	Monosomy 13
9	4	4	4	4	4	Tetraploid
10	2	2	2	2	2	Normal/balanced

NR: non result; nr: non result

of such anomalies, and may also serve to compare abnormalities in different patients (Scriven *et al.*, 1998).

Comparison between two reciprocal translocation carries could give different results even if the two translocations imply the same chromosomes (Escudero *et al.*, 2000). Part of this variability could be due to differences in the breakpoints of the translocations. However, the breakpoint positions of RT are the same or vary within a short distance, even in patients that are not blood relations. This similarity between breakpoints in unrelated patients could be helpful in comparative studies of abnormalities present in both spermatozoa and the resulting embryos. This is supported by the findings presented here.

RT 13q14q has breakpoints in the short arms of both chromosomes, which usually generate a dicentric derivative chromosome (Han *et al.*, 1994). Only three previous reports (Pellestor *et al.*, 1987; Martin, 1988; Ogawa *et al.*, 2000), analysed spermatozoa from RT 13q14q carriers to study their meiotic segregation. These three studies showed a high proportion of normal and balanced gametes (74%, 94% and 87% respectively). But their numbers of sperm analysed were low (78, 116 and 45, respectively). Our study counted a much larger number of spermatozoa (Table 1), but also gave the same high proportion of balanced gametes (about 75% in each patient), agreeing with those previous studies, and indicating that the meiotic behaviour of these RT carriers is similar among different male carriers.

A large number of decondensed spermatozoa can be analysed using FISH, which provides better statistical analysis in the unbalanced sperm forms. The proportion of nullisomic sperm found in the present study is higher than disomic sperm (Table 1). Although this difference is not statistically significant, it agrees with other work that shows different proportions among unbalanced forms (Van Hummelen *et al.*, 1997).

PGD analysis shows in both patients a high number of normal or balanced embryos (43% and 56%), but less than the proportion of equilibrated gametes in sperm (74% and 77%). However, the proportion of abnormal forms that could be caused by the translocation in embryos (22% and 11%) was similar to the one found in spermatozoa (23% and 19%).

Other abnormalities present in the embryos (30% and 22%) could be attributed to oocyte factors and/or post-meiotic events, producing haploid and complex abnormal embryos. The frequency of these abnormalities is similar to the 20–25% baseline of chromosome abnormalities usually found in cleavage-stage human embryos (Munné *et al.*, 1995, Harper *et al.*, 1995, Munné and Cohen, 1998). This baseline may vary considerably from couple to couple, and increases with maternal age.

In haploid embryos, RT 13q14q cannot be the cause of the abnormality and may be a result of activated non-fertilized oocytes. Interestingly, a previous report on PGD of RT carriers found a high incidence of chaotic mosaicism in these patients (Conn *et al.*, 1998). This is not the case in our study, and therefore the origin of such mosaicism is unlikely to be caused by the translocation, but by individual female or male differences, or differing embryo culture conditions.

This work has shown that the proportion of abnormal embryos due to the RT 13q14q in embryos is similar to the proportion of abnormal spermatozoa found in the male carriers, plus a patient-specific baseline of other chromosome abnormalities. Thus, sperm analysis with FISH could be a useful tool for predicting the numbers of normal embryos expected to be found in translocation carrier couples undergoing IVF. In certain cases, when a high rate of abnormalities is detected in sperm, it may even be more appropriate to recommend sperm donation, since the chances of producing normal embryos could be unacceptably low.

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