First International Journal of Andrology
ANDROLOGIA

CASE REPORT

Sperm FISH analysis of a 44,X,der(Y),t(Y;15)(q12;q10)pat,rob (13;14)(q10;q10)mat complex chromosome rearrangement

F. Ferfouri[1,2], F. Boitrelle[1,2], P. Clement[3], D. Molina Gomes[1,2], J. Selva[1,2] & F. Vialard[1,2]

1 Department of Reproductive Biology, Cytogenetics, Gynecology and Obstetrics, Poissy Saint Germain Hospital, Poissy, France;
2 EA 2493, University of Versailles Saint-Quentin-en-Yvelines, Versailles, France;
3 Clement Lab, Le Blanc Mesnil, France

Keywords
Complex chromosome rearrangement—inter-chromosomal effect—sperm FISH—sperm nuclear DNA fragmentation

Correspondence
François Vialard, Department of Reproductive Biology, Cytogenetics, Gynecology and Obstetrics, Centre Hospitalier Poissy Saint Germain, 10 rue du Champ-Gaillard, F-78303 Poissy, France.
Tel.: +33 139 274 700;
Fax: +33 139 274 425;
E-mail: fvialard@chi-poissy-st-germain.fr

Accepted: March 25, 2013

doi: 10.1111/and.12112

Summary

Complex chromosome rearrangements (CCR) with two independent chromosome rearrangements are rare. Although CCRs lead to high unbalanced gamete rates, data on meiotic segregation in this context are scarce. A male patient was referred to our clinic as part of a family screening programme prompted by the observation of a 44,X,der(Y),t(Y;15)(q12;q10)pat,rob(13;14)(q10;q10)mat karyotype in his brother. Karyotyping identified the same CCR. Sperm FISH (with locus-specific probes for the segments involved in the translocations and nine chromosomes not involved in both rearrangements) was used to investigate the rearrangements meiotic segregation products and establish whether or not an inter-chromosomal effect was present. Sperm nuclear DNA fragmentation was also evaluated. For rob(13;14) and der(Y), the proportions of unbalanced products were, respectively, 26.4% and 60.6%. Overall, 70.3% of the meiotic segregation products were unbalanced. No evidence of an inter-chromosomal effect was found, and the sperm nuclear DNA fragmentation rate was similar to our laboratory's normal cut-off value. In view of previously published sperm FISH analyses of Robertsonian translocations (and even though the mechanism is still unknown), we hypothesise that cosegregation of der(Y) and rob(13;14) could modify rob(13;14) meiotic segregation.

Introduction

Robertsonian translocations are the most commonly observed structural rearrangements and occur in 0.12% of healthy neonates (Gardner & Sutherland, 1996). These abnormalities result from the centromeric fusion of the long arms of two acrocentric chromosomes (13, 14, 15, 21 and 22) (Nielsen & Wohlert, 1991). Rob (13;14) translocations are the most common and account for 73% of all Robertsonian events of this kind (Gardner & Sutherland, 1996). The unbalanced chromosome segregation induced by these structural aberrations is thought to lead to spontaneous miscarriages, congenital malformations, mental retardations or conditions (as observed in the present case) such as Patau syndrome [46,XY,rob(13;14),+13]. According to the literature (Roux *et al.*, 2005; Ferfouri *et al.*, 2011b), the sperm aneuploidy rate for malsegregation of chromosomes involved in the translocation is about 16% for men heterozygous for the specific rob(13;14) (Ferfouri *et al.*, 2011b). The aneuploidy rate is negatively correlated with the sperm count (Ferfouri *et al.*, 2011b). According to recent data (Ferfouri *et al.*, 2013), sperm chromosome segregation may be affected not only by the sperm count but also by the cosegregation of two independent structural rearrangements. This would modify the proportions of meiotic segregation products found in spermatozoa.

A complex chromosome rearrangement is defined as a balanced or unbalanced structural abnormality that involves at least three breakpoints located on two or more chromosomes, with exchange of genetic material (Pai *et al.*, 1980). According to Kausch *et al.* (1988), these CCRs can be classified into three groups: (i) three-way exchange CCRs; (ii) exceptional CCRs; and (iii) double two-way translocations. Here, we focused on the latter category, which is defined by at least two independent chromosomal rearrangements. There are

literature data on about 255 CCR carriers (Zhang *et al.*, 2009). In about 70% of cases, as the CCR is probably balanced, the male phenotype is normal, without any clinical finding, except frequent infertility (De Gregori *et al.*, 2007). However, it is generally considered that the greater the number of breakpoints, the higher the risk of an abnormal phenotype (Pai *et al.*, 1980). Most *de novo* CCRs (accounting for about 70% of cases) are of paternal origin (Batista *et al.*, 1994). In contrast, familial CCRs (accounting for the remaining 30% of cases) are primarily of maternal origin (Pellestor *et al.*, 2011a). In males, CCR can lead to infertility through failure of spermatogenesis (Joseph & Thomas, 1982; Rodriguez *et al.*, 1985). Only 12 of 130 reported male patients with CCRs were known to be fertile (Grasshoff *et al.*, 2003; Goumy *et al.*, 2006). The meiotic segregation of CCRs frequently results in unbalanced gametes. In the event of fertilisation, partial duplication/deletion causes recurrent spontaneous miscarriage or, in surviving infants, mental retardation and/or congenital abnormalities. Few studies of the meiotic segregation of CCRs have been reported, and all reported high proportions of unbalanced segregation products (ranging from 61.8 to 86.5%) (Pellestor *et al.*, 2011a).

The second chromosomal risk relates to the potential for an inter-chromosomal effect (ICE), as the CCR may disturb the meiotic segregation of chromosomes not involved in the primary recombination event. Although it was initially postulated that the parents of children with Down's syndrome had a greater incidence of translocation (Lindenbaum *et al.*, 1985; Kirkpatrick & Ma, 2012), the significance of ICEs is still subject to debate (Pellestor *et al.*, 2011b).

Here, we report on meiotic segregation in a male with an inherited CCR (44,X,der(Y),t(Y;15)(q12;q10)pat,rob(13;14)(q10;q10)mat) that involved a balanced rob(13;14) rearrangement and an independent, unbalanced t(Y;15) rearrangement. To the best of knowledge, this case corresponds to only the fourth double two-way CCR reported to date and the first with 44 chromosomes.

We sought to evaluate (i) the double two-way unbalanced rates; (ii) the potential impact of one translocation on the other; and (iii) the presence or absence of an ICE on the chromosomes not involved in the CCR (i.e. 7, 9, 11, 12, 16, 17, 18, 20 and 21). We also studied the sperm nuclear DNA fragmentation rate, which is known to be high for single chromosome rearrangements (Brugnon *et al.*, 2006, 2010; Perrin *et al.*, 2009).

Materials and methods

Patient

A 32-year-old man (II-5) was referred to our clinic (during his wife's pregnancy), as part of a family screening programme prompted by the observation of a 44,X,der(Y),t(Y;15)(q12;q10)pat,rob(13;14)(q10;q10)mat karyotype in his brother (II-2) during infertility treatment. The family's pedigree is shown in Fig. 1. The patient had the same CCR 44,X,der(Y),t(Y;15)(q12;q10)pat,rob(13;14)(q10;q10)mat as his brother. After genetic counselling, a prenatal diagnosis was performed and a normal female karyotype was observed.

After the daughter's birth, further genetic counselling was given. The patient provided his informed consent for an evaluation of the sperm chromosome structure and nuclear DNA fragmentation (via sperm FISH and a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay respectively). This case was part of a broader study of genetic aspects of infertility that had been approved by the local investigational review board.

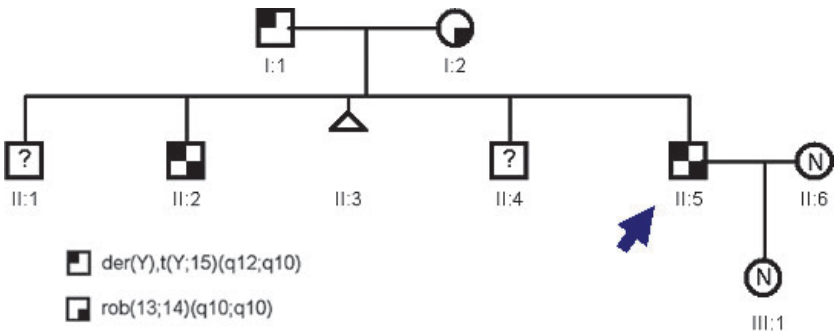

Fig. 1 Family pedigree. Subjects who are heterozygous for der(Y)t(Y;15)(q12;q10) are indicated by a filled top left quadrant. Subjects who are heterozygous for rob(13;14)(q10;q10) are highlighted by a filled bottom right quadrant. The propositus (II:5, indicated by filled top left and bottom right quadrants and an arrow) and his infertile brother (II:2) carry a 44,X,der(Y),t(Y;15)(q12;q10)pat,rob(13;14)(q10;q10)mat CCR.

Methods

A sperm analysis was performed according to the criteria of the World Health Organization (2010). After removal of seminal liquid, the spermatozoa were washed twice with sterile water (300 g for 10 min), fixed with Carnoy's solution (to avoid sperm lysis) to be stored at −20 °C before analysis. Then, sperm fixation was spread on a slide for FISH and DNA fragmentation assays.

Sperm FISH analysis

Slides were prepared as previously described (Vialard *et al.*, 2008; Ferfouri *et al.*, 2011a). Given that chromosome 13-, 14-, 15-, X- and Y-specific probes did not appear to crosshybridise with other chromosomes *in situ*, an accurate analysis of chromosome segregation could be performed.

Different probe mixtures were used to assess the segregation patterns for:

1 The autosome-gonosome translocation der(Y)t(Y;15)(q12;q10), using probes specific for chromosomes X [CEPX (Xp11.1q11.1, DXZ1, SpectrumGreen)], Y [CEPY (Yp11.1q11.1, DYZ3, SpectrumAqua)] and 15 [CEP15 (15p11.2, D15Z1, SpectrumOrange)] (all from Abbott Laboratories, Chicago, IL).

2 The Robertsonian translocation rob(13;14)(q10;q10), using probes specific for chromosomes 13 (LSI13 (13q14, RB1, SpectrumGreen) and 14 (LSI IGH (14q32, Dual colour) (both from Abbott Laboratories).

We also sought to identify an ICE via the use of five mixtures containing specific probes for chromosomes not involved in the CCR (all supplied by Abbott Laboratories).

1 Probes specific for chromosomes 7 [CEP7 (7p11.1q11.1, D7Z1, SpectrumOrange)] and 9 [CEP15 (9p11.1q11.1, D9Z1, SpectrumGreen)].

2 Probes specific for chromosomes 11 [CEP11 (11p11.1q11.1, D11Z1, SpectrumOrange)] and 12 [CEP12 (12p11.1q11.1, D12Z3, SpectrumGreen)].

3 Probes specific for chromosomes 18 (CEP18 (18p11.1q11.1, D18Z1, SpectrumOrange)) and 21 (LSI21 (21q22.13q22.2, D21S342, D21S341 and D21S529, SpectrumOrange)).

4 Probes specific for chromosomes 16 [CEP16 (16p11.2, D16Z3, SpectrumGreen)], 17 [Vysis CEP17 (17p11.1q11.1, D17Z1, SpectrumAqua)] and 20 [Vysis CEP20 (20p11.1q11.1, D20Z1, SpectrumOrange)].

After codenaturation at 73 °C for 4 min, overnight hybridisation was carried out at 37 °C. Slides were washed, counterstained with 4′,6′-diamidino-2-phenylindole (DAPI) and then analysed as previously reported (Vialard *et al.*, 2008) using a Pathvysion Software Smart Capture FISH system, version 1.4 (Digital Scientific, Cambridge, UK).

In line with previous reports [for a review, see (Benet *et al.*, 2005)], we analysed at least 1000 spermatozoa per slide.

For the ICE evaluation, aneuploidy rates were compared with three controls with the following mean (± standard deviation) sperm parameters: sperm count per ejaculate: $191 \pm 21.2 \times 10^6$; progressive motility: 57 ± 13%; percentage of typical normal sperm forms: 39 ± 20%.

When interpreting the FISH results, we considered that one spot corresponded to one chromosome. Two neighbouring spots were considered to be distinct if the distance between them was greater than the spot diameter. In the translocation segregation study, normal, balanced spermatozoa were visualised by the presence of one spot per chromosome. All other combinations corresponded to unbalanced spermatozoa.

In the ICE study, we considered that a spot in a euploid spermatozoon corresponded to a chromosome. All other configurations were considered to be aneuploid cells.

To avoid misinterpretation, only one slide was used per probe mixture and rehybridisation was not performed. The patient's overall proportion of balanced segregation products was calculated by multiplying the proportions observed for each translocation. For the ICE analysis, the total aneuploidy rate was defined as the sum of the results observed for each chromosome.

TUNEL assays

Slides were permeabilised with 0.1% SDS sodium citrate for 15 min. After two washes in phosphate-buffered saline (PBS), slides were incubated with the labelling solution (the fluorescein *in situ* cell death detection kit from Roche Molecular Biochemicals, Rotkreuz, Switzerland) for 2 h at 37 °C. Next, slides were washed three times in PBS and analysed after counterstaining with DAPI. Spermatozoa with DNA fragmentation fluoresced blue and green, as previously described (Frainais *et al.*, 2010). One thousand spermatozoa were counted.

Statistical analyses

Using StatView software (SAS Institute, Cary, NC, USA), a chi-squared test was used to compare patient and control aneuploidy rates and a Wilcoxon test was used to compare sperm DNA fragmentation rates. The threshold for statistical significance was set to $P < 0.05$.

Results

Sperm analysis

According to the WHO (2010) criteria, the propositus II:5 had a slight oligoasthenozoospermia with a semen

volume of 1.5 ml, a sperm count of 7.5 × 10^6/ml, 30% progressive motility and 8% normal sperm forms.

Unbalanced Y-autosome translocation der(Y)t(Y;15)(q12;q10) segregation

For 1017 analysed spermatozoa, the alternate segregation rate was 39.4%, with 21.1% normal (X, 15) and 18.3% balanced [der(Y)] chromosomal spermatozoa (Table 1). All other spermatozoa were unbalanced (60.6%); 53.1% showed adjacent segregation and 7.5% showed 3 : 0 segregation. The pachytene stage is shown in Fig. 2.

Robertsonian translocation rob(13;14)(q10;q10) segregation

For 1016 analysed spermatozoa, alternate segregation was the main segregation mode (at 73.4%) (Table 1). All other segregations patterns were unbalanced, with adjacent segregation (26.3%) and 3 : 0 segregation (0.3%) modes.

Patient's overall euploidy rate

The overall balanced segregation rate for the CCR (i.e. the product of the balanced segregation rates of each reciprocal translocation) was 71.1%.

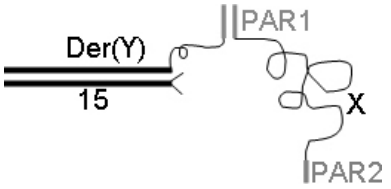

Fig. 2 Diagram of chromosomes 15, der(Y) and X, both of which are involved in the translocation t(Y;15)(q12;q10).

The inter-chromosomal effect

A total of 9087 spermatozoa were counted in the ICE analysis for chromosomes 7, 9, 11, 12, 16, 17, 18, 20 and 21. The chromosome aneuploidy rates varied between 0.2% and 0.5% for the patient and between 0.1% and 0.5% for controls. The carrier's total aneuploidy rate for chromosomes not involved in either of the CCRs did not differ significantly from control values (3.1% versus 2.9% respectively).

Sperm nuclear DNA fragmentation

The nuclear DNA fragmentation rate (n = 1021 spermatozoa analysed) was 12.8%, which is just below our laboratory's normal cut-off value of 13%.

Table 1. Meiotic segregation of the two translocations. A 44,X,der(Y),t(Y;15)(q12;q10)pat,rob(13;14)(q10;q10)mat in the male CCR carrier

	Segregation mode	Genotype	Spot colour	Number of sperm analysed	Proportion (%)
Autosome-gonosome translocation der(Y)t(Y;15)(q12;q10)	Alternate	15,X	GR	215	21.1
		der(Y)	A	186	18.3
		Subtotal		401	39.4
	Adjacent	15,der(Y)	RA	131	12.9
		X	G	142	14.0
		X,der(Y)	GA	128	12.6
		15	R	139	13.7
		Subtotal		540	53.1
	3 : 0	15,X,der(Y)	RGA	76	7.5
	Total			1017	
Robertsonian translocation rob(13;14)(q10;q10)	Alternate	13,14 or der(13;14)	RG	746	73.4
	Adjacent	13, der(13;14)	RGG	65	6.4
		14	R	62	6.1
		14, der(13;14)	RRG	71	7.0
		13	G	69	6.8
		Subtotal		267	26.3
	3 : 0	13,14, der(13;14)	RRGG	3	0.3
	Total			1016	
Overall rate of aneuploidy (both translocations 44,X,der(Y),t(Y;15)(q12;q10)pat,rob(13;14)(q10;q10)mat)					71.1

R, red; A, aqua; G, green.

Discussion

To the best of our knowledge, this study constitutes (i) only the seventh report on sperm meiotic segregation patterns in CCR carriers; and (ii) the fourth report on a double, two-way CCR published to date (Burns *et al.*, 1986; Cifuentes *et al.*, 1998; Loup *et al.*, 2010; Ferfouri *et al.*, 2011a, 2013; Pellestor *et al.*, 2011b); and (iii) the first report on a patient with 44 chromosomes. The CCR studied here results from the dual, independent transmission of a balanced rob (13;14)(q10;q10)mat and an unbalanced der(Y)t(Y;15)(q12;q10)pat origin. This leads to a rare karyotype with 44 chromosomes. In contrast to previously reported CCRs, the present karyotype is unbalanced. Even though the der(Y),t(Y;15)(q12;q10) could be considered as a chromosome variant, loss of chromosome 15′s short arm and the Yq12qter region have no impact on the human phenotype.

The Y chromosome breakpoint on Yq12 (located in the heterochromatic region: Yq12qter) results from deletion of the PAR2 region and leads to pachytene trivalent formation during meiosis (Fig. 2), as is the case for Robertsonian translocations. In contrast to cases of translocations with Yq11.2 breakpoints that are associated with oligozoospermia (Delobel *et al.*, 1998; Alves *et al.*, 2002; Buonadonna *et al.*, 2002) and azoospermia through meiotic arrest (Pinho *et al.*, 2005), patients who are heterozygous for a Yq12 translocation are fertile or subfertile. The Y chromosome breakpoint located on Yq11.2 (with loss of the Y chromosome's distal long arm portion) leads to deletion of the AZF region and results in Sertoli cell-only syndrome, hypospermatogenesis, spermatogenesis arrest and thus azoospermia or oligospermia (Conte *et al.*, 1996; Vogt *et al.*, 1996; Simoni *et al.*, 2004; Patrat *et al.*, 2010).

Furthermore, the der(Y) appears not to disturb pairing, recombination and meiotic segregation. In contrast to previously reported cases (in which autosome-gonosome translocation involving Yq12 breakpoints (Delobel *et al.*, 1998; Pinho *et al.*, 2005) lead to azoospermia), loss of the der(15), paternally inherited, resulted in trivalent formation (as for Robertsonian translocations; Fig. 2) and may explain the absence of azoospermia in the patient and his father. Hence, altered spermatogenesis may have resulted from the combination of two translocations, each of which may predispose to infertility.

Due to frequent azoospermia, few cases of t(Y-autosome) chromosome segregation have been reported to date and all were balanced. Previous published reports featured a patient with oligoasthenoteratozoospermia who was heterozygous for a t(Y;16)(q11.21;q24) (Giltay *et al.*, 1999), a normospermic patient who was heterozygous for a 46,X,t(Y;10)(q11.2;q15.2) (Vialard *et al.*, 2009) and a cryptozoospermic patient who was heterozygous for 46,X,t(Y;3)(q12;p21) (Kekesi *et al.*, 2007). The aneuploidy rates were, respectively, 49.0%, 49.7% and 70.3%. Our patient was normozoospermic and had an aneuploidy rate of 60.6%; even though the translocation was unbalanced, these data agree with previous reports.

In this case, the rob(13;14) sperm aneuploidy rate was 26.6%. This is markedly higher than previously reported values, which averaged 15.9% (n = 65 patients) and ranged from 7% to 42% (Perrin *et al.*, 2008). When considering only oligozoospermic patients who are heterozygous for rob(13;14)(q10;q10), an aneuploidy rate of 18.9% was reported (Ferfouri *et al.*, 2011b).

Even though the segregation pattern for a given translocation might vary from one patient to another, we hypothesise that the presence of both der(Y) and the rob(13;14) modifies the proportion of the various products in mature spermatozoa. This phenomenon might be linked to spatial crowding of the two trivalents and/or differences in germ cell apoptosis. The present findings are in agreement with our previous report on a 46,XY,t(1;16)(q21;p11.2),t(8;9)(q24.3;p24) patient and his 46,XY,t(8;9)(q24.3;p24) brother and cousin (Ferfouri *et al.*, 2013), in whom cosegregation of t(8;9) t(8;9)(q24.3;p24) and t(1;16)(q21;p11.2) modified the proportions of t(8;9) meiotic segregation products found in spermatozoa.

The reciprocal impact of double rearrangements requires further investigation.

Overall rates of aneuploidy for CCRs

We are aware of six reports on meiotic segregation in CCRs: a 46,XY,t(5;11)(p13;q23.2),t(7;14)(ql1;q24.1) (Burns *et al.*, 1986), a 46,XY,t(2;11;22)(q13;q23;q11.2) (Cifuentes *et al.*, 1998), a 46,XY,t(1;19;13)(p31;q13.2;q31)mat (Loup *et al.*, 2010), a 46,XY,t(5;13;14)(q23;q21;q31) (Pellestor *et al.*, 2011b), a 46,XY,t(3;6)(p24;p21.2),inv(8)(p11.2q21.2) (Ferfouri *et al.*, 2011a) and a 46,XY,t(1;16)(q12;p11.2),t(8;9)(q24.3;p24) (Ferfouri *et al.*, 2013). The rates of aneuploidy rates were, respectively, 86.3%, 86.5%, 75.9%, 73.0%, 61.8% and 85.6%. The overall rate of aneuploidy in the present case was 71.1% – a high value that is in agreement with the previous reports. Whereas sperm donation was recommended to the patient's brother on the basis of the karyotype result (and no further analysis), our patient should have been given genetic counselling after the sperm FISH analysis. Paternity is clearly possible in some cases (including the present case), but sperm donation may be the best option for patients confronted with infertility.

Inter-chromosomal effects

We focused on 9 of 19 chromosomes not involved in this CCR (i.e. chromosomes 7, 9, 11, 12, 16, 17, 18, 20 and 21). The aneuploidy rates per chromosome were similar in

patients and controls. Similarly, there was no significant difference in the overall chromosome aneuploidy rate (3.1% in patients and 2.1% in controls). There is unlikely to be an ICE in the present case, despite the fact that an ICE on acrocentric chromosomes was recently suggested in a patient who was heterozygous for rob(13;14) (Ferfouri *et al.*, 2011a). The lack of an ICE could be due to (i) the fact that the propositus' oligoasthenozoospermia was only mild or; (ii) the presence of the second translocation, which might prevent the abnormal meiotic segregation of chromosomes not involved in the two translocations.

Conclusion

Here, we reported on meiotic segregation in a patient with a 44-chromosome karyotype (due to a double, two-way CCR). The rob(13;14) malsegregation rate appears to be slightly greater than those reported in literature for normospermic men heterozygotes for only one robertsonian translocation. The higher rate may be due to the impact of the der(Y)t(Y;15)-derived chromosome on its meiotic segregation. This modifies the proportion of the rob(13;14) meiotic segregation products found in spermatozoa. However, further studies of the reciprocal impact of double rearrangements are required.

Acknowledgement

We thank AMMR-PSG for its financial support.

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