Case report

Meiotic segregation in spermatozoa of a 46,X,t(Y;10)(q11.2;p15.2) fertile translocation carrier

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Abstract

Translocations involving gonosomes are frequent in azoospermic patients and sometimes in oligozoospermic ones, conditions that lead to request for assisted reproduction treatment. This study reports an unexpectedly fertile 49-year-old man bearing a de-novo translocation 46,X,t(Y;10)(q11.2;q15.2) associated with a high chromosomal risk for offspring, and referred for familial investigations after the diagnosis of an unbalanced translocation 46,XX,der(10)t(Y;10)(q11.2;p15.2) in his naturally conceived and mentally retarded daughter. Chromosome molecular investigation confirmed Y long-arm inheritance in the daughter and absence of the Yq deletion in the father. Semen analysis showed a normal sperm count associated with moderate asthenospermia and severe teratospermia. A total of 984 spermatozoa were analysed using fluorescence in-situ hybridization (FISH). Alternate segregation pattern was found in 50.31% of the spermatozoa studied. The frequencies of adjacent I, adjacent II, 3:1 segregation, and diploidy (or 4:0 segregation) were respectively 39.62, 1.63, 7.83, and 0.61%. No interchromosomal effect was observed. This patient is the first fertile man in whom the meiotic segregation pattern of a Y-autosome translocation has been analysed. The imbalance risk was close to those observed for reciprocal translocations, and emphasizes the value of FISH studies in males with a chromosomal translocation in order to provide them a personalized risk evaluation.

Keywords: *FISH, meiotic segregation, sperm FISH analysis, Y-autosome translocation, Yq;10q translocation*

Introduction

Reciprocal translocations occurring between two autosomes are the most frequent structural chromosomal abnormalities in humans, and their frequency was estimated to be 1 in 625 in the general population (Gardner and Sutherland, 1996). From the first large survey of karyotypes in infertile males, it became evident that compared with newborns, infertile males have a higher prevalence of chromosomal abnormalities (Bourrouillou *et al*., 1987). Several chromosome screenings have established the frequencies and types of chromosomal abnormalities in patients either affected by severe oligozoospermia or undergoing IVF/intracytoplasmic sperm injection procedures (Egozcue *et al*., 2000). Reciprocal translocations were estimated to be 6.5 times more frequent among infertile males (De Braekeleer and Dao, 1991), with an excess of chromosome 1 breakpoints in male infertility (Bache *et al*., 2004). Among them, translocations involving gonosomes are rare, but more frequent in azoospermic patients (Van Assche *et al*., 1996) and divided into three groups according to the gonosome involved in the translocation, namely Y-autosome translocations, X-autosome translocations, and X–Y translocations. Due to frequent meiotic

arrest or cryptozoospermia (Hsu, 1994; Ma *et al*., 2003; Brisset *et al*., 2005), meiotic segregations were rarely reported, contrasting with translocation involving two autosomes, mainly retrieved in oligozoospermic males (Van Assche *et al*., 1996) and frequently studied (Benet *et al*., 2005; Roux *et al*., 2005; Ogur *et al*., 2006). Only three cases of Y-autosome translocations were reported with a meiotic segregation pattern and all were infertile (Mennicke *et al*., 1997; Giltay *et al*., 1999; Kekesi *et al*., 2007). There are reports of one case concerning an X-Y translocation (Morel *et al*., 2001) and two concerning X-autosome translocation (Vialard *et al*., 2005; Perrin *et al*., 2008). The first meiotic segregation analysis in a fertile male carrying a Y-autosome translocation is reported here.

Case report

Patient

A 49-year-old male patient was first seen for a family study after the diagnosis of an unbalanced translocation 46,XX,der(10),t(Y;10)(q11.2;p15.2) (**Figure 1**), obtained after venous peripheral blood karyotyping, in his naturally conceived and mentally retarded daughter. Fluorescence in-situ hybridization (FISH) results, using the telomeric probes of chromosome 10 short arm and CBG banding, confirmed the results. Cytogenetic analysis on a peripheral blood sample revealed a 46,X,t(Y;10)(q11.2;p15.2) karyotype (**Figure 1**), also confirmed by FISH and CBG banding. A Y chromosome-sequence tagged sites (STS) study was performed, which confirmed the absence of microdeletion for this patient and the presence of all markers after the KALY region for his daughter (**Figure 2**). Prior to this study, the patient's informed consent had been obtained. Semen analysis showed a normal sperm count: 26 × 10^6/ml, associated with slight asthenospermia: 35% mobility (a + b) and severe teratospermia: 3% of typical form.

Meiotic segregation analysis

Triple FISH was carried out using the specific alphoid probe of chromosome 10 (D10Z1, spectrum aqua, Abbott, Rungis, France), the 10p subtelomere probe (tel 10p, spectrum green, Abbott) and the subtelomere Xq/Yq probe (telXq/Yq, spectrum orange, Abbott). An ideogram showing the translocation, the localization of the probes and the quadrivalent is shown in **Figure 3**.

To assess the interchromosomal effect, triple FISH was also performed with specific alphoid probes for chromosomes 18 (D18Z1, spectrum aqua; Abbott), specific probes for chromosome 21 (D21S55, spectrum orange; Abbott) and for chromosome 13 (RB1, spectrum green; Abbott).

Methods

Detailed procedures for sperm preparation were as follows. Spermatozoa were fixed in a 3:1 methanol/acetic acid solution after two washing steps in water, spread on a slide, air-dried and fixed with methanol for 5 min at room temperature. Sperm decondensation was performed in 3 mol/l NaOH for 1 min. After dehydration, FISH was performed with a specific probes mixture (Vysis-Abbott), at 73°C for 4 min followed by further hybridization at 37°C overnight. Slides were next washed and counterstained with 4,6-diamidino-2-phenylindole solution and spermatozoa were analysed with an Olympus microscope using the PathVysion software. Observation and interpretation criteria were based on the number of spots per probe on the sperm nuclei. Spermatozoa with one green, one orange and one blue signal were scored as normal or balanced, whereas spermatozoa with other signal combinations were scored as unbalanced. For interchromosomal effect evaluation, only spermatozoa with one spot per chromosome were considered as normal, all others being considered abnormal.

Y chromosome STS analysis

STS analysis was as detailed in Charpenel *et al*. (2002), and additional markers were amplified by polymerase chain reaction as detailed in www.ncbi.nlm.nih.gov (accessed 11/09/08). Loci on the Y chromosome and results are described in **Figure 2**.

Results

A total of 984 spermatozoa were analysed using three-colour FISH (**Table 1** and **Figure 4**). The alternate segregation pattern, leading to a normal or balanced chromosomal content, was found in 50.30% of the spermatozoa studied and was the preferential segregation mode. All other spermatozoa were unbalanced (49.70%). Among the unbalanced spermatozoa, the adjacent I segregation mode was the most frequent (39.63% of analysed spermatozoa), followed by the 3:1 mode (7.82%), the adjacent II segregation mode (1.63%) and the 4:0 mode (0.61%). Only the alternate, adjacent I and some 3:1 modes can result in viable fetuses, given that monosomy and trisomy for chromosome 10 are not viable and that more than 90% of Turner syndrome (45,X) girls die before birth. The risk of unbalanced inheritance for offspring was then 45.54%.

Interchromosomal investigation (n = 9676) revealed aneuploidy rates for chromosomes 13, 18 and 21 to be 0.40, 0.11 and 0.22% respectively.

Family study

Because of results obtained, and because of slightly delayed psychomotor development of the patient's son, the genetic status of the family was assessed (**Figure 5**). The translocation was shown to be *de novo* for the proband, but his son had inherited an unbalanced derivative chromosome with a 46,X,der(Y),t(Y;10)(q11.2;p15.2)pat karyotype. The child showed lack of all Y chromosome markers after KALY (**Figure 2**), including the AZFb and AZFc region markers.

The chromosome segregation mode was therefore adjacent I for the two children.

In view of those results, the parents requested karyotyping for their third child, a healthy girl, and this was found to be normal.

Chromosome 10 breakpoint localization

Breakpoint analysis showed that the chromosome 10 breakpoint was located within BAC RP11–298E9 in 10p15.2, about 3 Mb away from 10p telomere (**Figure 6**).

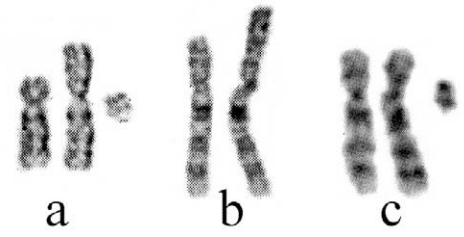

Figure 1. Proband and children's Y and 10 chromosomes. (**a**) Proband's chromosomes 10 and Y; (**b**) daughter's chromosome 10; (**c**) son's chromosomes 10 and Y.

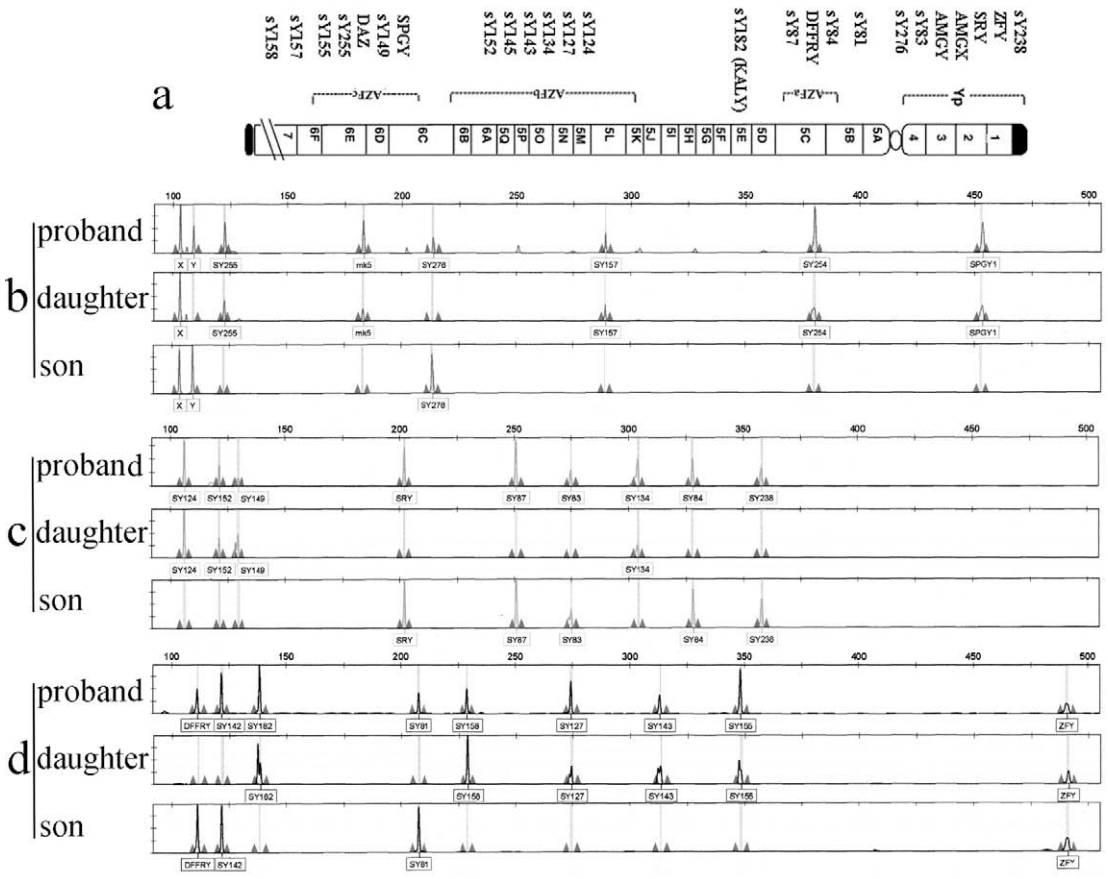

Figure 2. Loci on the Y chromosome. (**a**) Schematic location of the short tandem repeats (STR) analysed for the proband and his children. STR were amplified with the fluorescent-labelled primer pair sequences described in the UniSTS data base (http://www.ncbi.nlm.nih.gov; accessed 23 January 2009). Amplification products were loaded onto an ABI 3100 sequence analyser. (**b**) Electrophoregrams of the proband (top lane), daughter (middle lane) and son (bottom lane) FAM-labelled STR AMG, SY255, mk5, SY276, SY157, SY254, and SPGY1. mk5 is a multilocus marker. (**c**) Electrophoregrams of the proband (top lane), daughter (middle lane) and son (bottom lane) VIC-labelled STR SY124, SY152, SY149, SRY (SY14), SY87, SY83, SY134, and SY238. (**d**) Electrophoregrams of the proband (top lane), daughter (middle lane) and son (bottom lane) NED-labelled STR DFFRY, SY142, SY182, SY81, SY158, SY127, SY143, SY155, and ZFY. There is a ZFY copy on chromosome X.

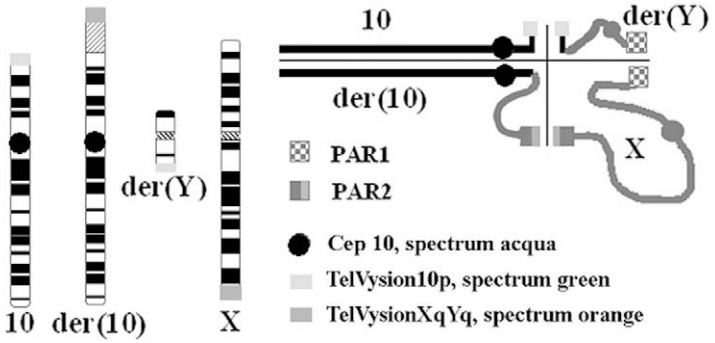

Figure 3. Ideogram showing the 46,X,t(Y;10)(q11.2;p15.2), the localization of probes and the quadrivalent at the pachytene stage.

Table 1. Results of the meiotic segregation in the sample from the 46,X,t(Y;10)(q11.2;p15.2) carrier using D10Z1 (aqua), tel10p (green), and telXqYq (red).

Fluorescent signal	*Segregation mode*	*Chromosomal content*	n *(%) by combination*	*% by mode*	*Viability*	*Viability, % by mode*
AGO	Alternate	X/10 or derY/der10	495 (50.30)	50.30	Yes	54.46%
AGG	Adjacent I	derY/10	181 (18.39)	39.63	Yes	42.90%
AOO		X/der10	209 (21.24)			
AAGO	Adjacent II	10/der10	9 (0.91)	1.63	No	
GO		X/derY	7 (0.71)			
AG	3:1 exchange	10	29 (2.95)	4.47	No	2.64%
AGOO		der10/X/derY	12 (1.22)		Yes	
O		X	1 (0.10)		No	
AAGGO		10/der10/derY	2 (0.20)		No	
AO	3:1 tertiary	der10	19 (1.93)	3.35	No	
AGGO		10/X/derY	12 (1.22)		Yes	
G		derY	1 (0.10)		No	
AAGOO		10/der10/X	1 (0.10)		No	
AAGGOO	4:0 or diploidy		6 (0.61)	0.61	No	0%

A = aqua, G = green, O = red. Total number of spermatozoa analysed was 984.

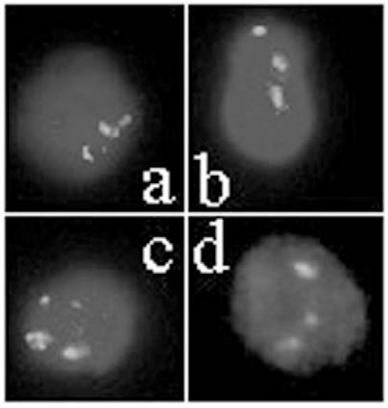

Figure 4. Fluorescence in-situ hybridization on spermatozoa of a 46,X,t(Y;10)(q11.2;p15.2) fertile translocation carrier. (**a**) Balanced spermatozoa; (**b**) unbalanced adjacent 1 configuration with derY and 10 chromosomes; (**c**) unbalanced adjacent 2 configuration with 10 and der10 chromosomes; (**d**) unbalanced adjacent 1 configuration with X and der10 chromosomes.

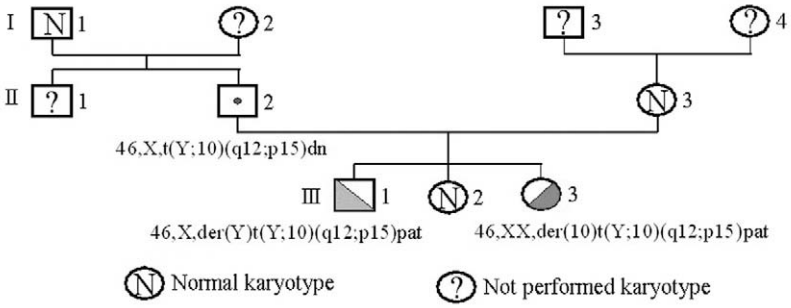

Figure 5. Genealogical tree. II-2 = proband III-1: proband's son; III-2 = proband's healthy daughter III-3: proband's daughter (mentally retarded); I-1 = proband's father.

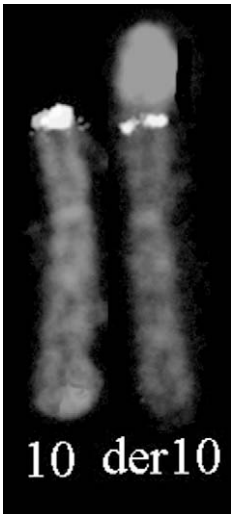

Figure 6. Chromosome 10 breakpoint localization using BAC RP11–298E9 and RP11–631M20. Chromosome 10: RP11–298E9 and RP11–631M20 hybridization. der(10) = asymmetric RP11–298E9 hybridization compared with chromosome 10, RP11–631M20 deletion and Y heterochromatin.

Discussion

Translocations involving gonosomes are rare and are frequently associated with severe male infertility (Van Assche *et al.*, 1996). Due to the spermatogenetic arrest at the pachytene stage frequently observed in histological examinations of biopsied testicular tissue from males carrying a Y-autosome translocation, meiotic segregation cannot usually be analysed (Delobel *et al.*, 1998; Pinho *et al.*, 2005). However, a few germ cells at the spermatid or spermatozoon stage are sometimes found (Brisset *et al.*, 2005). This disruption is mainly related to abnormal sexual vesicle formation, which leads to meiotic disturbance and then to spermatogenetic arrest (Delobel *et al.*, 1998). This patient is of particular interest because of his unexpectedly preserved fertility despite a 46,X,t(Y;10)(q11.2;p15.2) chromosomal complement. In contrast to previous reports, moderate asthenospermia associated with severe teratospermia was the only apparent phenotypic finding.

Hsu (1994) described 130 cases of Y-autosome translocation, with half (60 out 130) bearing the common translocation of the heterochromatic region (Yq12) onto the short arm of an acrocentric chromosome (13, 14, 15, 21 or 22). These translocation cases can be regarded as interesting variant chromosomes, but they are not associated with any clinical consequence (Gardner and Sutherland, 1996).

Brisset *et al.* (2005) also reviewed reciprocal translocations involving the Yq11 region. All 12 men reported with such rearrangements were infertile. In three cases, molecular analysis of the long arm was performed, and no deletion was observed, confirming the leading role of the translocation in failure of spermatogenesis rather than any deletion.

Y-autosome translocation segregations have been previously studied for three infertile men. The first was heterozygous for a t(Y;1)(q12;p34) translocation (Mennicke *et al.*, 1997). Only 36% of the 191 spermatozoa analysed by FISH were normal or balanced. The abnormal segregation pattern found was exclusively the adjacent type (64%). The second showed the heterozygous translocation t(Y;16)(q11.21;q24) (Giltay *et al.*, 1999). Segregation analysis of 500 morphologically normal spermatozoa showed that 51% were normal or balanced, 36% originated from an adjacent I segregation, 12% from a 3:1 segregation and 1% from an aberrant segregation. If morphologically abnormal sperm cells were also included, nearly 90% of all these spermatozoa were unbalanced. Finally, Kekesi *et al.* (2007) described a heterozygous 46,X,t(Y;3)(q12;p21) translocation. Segregation analysis of 450 spermatozoa showed that 29.7% were normal or balanced, 67.3% originated from an adjacent (I or II) segregation, and 3.0% from a 3:1 or 4:0 segregation.

In the present patient, four cases of meiotic segregation analysis could be compared (**Table 2**), showing that the incidence of normal segregation pattern varied from 29.7 to 51.0%, with a mean of 41.8%. This rate is close to those reported (Benet *et al.*, 2005) for autosomal translocations (40.5%). Adjacent II segregation pattern seems to be rare, occurring in close to 1% for the three cases where adjacent segregation was studied. This situation is surprising and has not been described for autosomal translocations (Benet *et al.*, 2005), where the adjacent II frequency was half that of adjacent I. Frequency of other segregation patterns (3:1, 4:0, and aberrant) was 6.1%. This particular meiotic segregation mode, with a prevalence of alternate and adjacent I configuration, has never been described. It seems to favour a segregation mode that permits a mechanistically normal segregation at meiosis, leading to the transfer of one chromosome of each pair into each sperm cell.

The interchromosomal effect of the translocation on chromosomes 13, 18 and 21 was also studied, and rates below 1% were found: 0.40, 0.11 and 0.22% respectively. These rates are similar to the controls, with respective rates of 0.42, 0.11 and 0.32% for chromosomes 13, 18 and 21, and slightly increased compared with normal men in the literature, where aneuploidy rates are respectively 0.10, 0.07 and 0.18% for chromosomes 13, 18 and 21 (Templado *et al.*, 2005; Martin, 2008). When compared with patients heterozygous for a translocation, these figures resemble those reported (Douet-Guilbert *et al.*, 2005), and figures for patients with teratospermia (Templado *et al.*, 2002). An interchromosomal effect does not seem to be present for this patient, and the slight increase observed might be linked to teratospermia.

Table 2. Meiotic segregation in reciprocal translocation carriers involving the Y chromosome: fluorescence in-situ hybridization analysis of sperm nuclei.

Translocation	*Sperm count ($\times 10^6$/ml)*	*No. of sperma-tozoa*	*Normal or balanced*	*Adjacent 1*	*Adjacent 2*	*3:1*	*4:0*	*Others*	*% of human genome haplotype*	*Reference*
t(Y;1)(q12;p34)	10.0	191	36.0	64.0	0.0	0.0	0.0	0.0	1.10	Mennicke *et al.*, 1997
t(Y;16)(q11.1;q24)	1.0	500	51.0	36.0	0.0	12.0	0.0	1.0	0.20	Giltay *et al.*, 1999
t(Y;3)(q12;p21)	0.1	450	29.7	67.3		3.0		0.0	1.45	Kekesi *et al.*, 2007
t(Y;10)(q11.2;p15.2)	26.0	984	50.3	39.6	1.6	7.8	0.6	0.0	0.01	Present study
Mean	–	531	41.8	52.1		6.1			–	–

Values are percentages unless otherwise stated.

By contrast with previously published Y-autosome translocations, the autosome segment involved in this particular translocation is small (3 Mb), corresponding to 0.01% of the human genome haplotype (**Table 2**). It is suggested that at the pachytene stage, a preferential bivalent association for chromosomes 10 and gonosomes occurred. Quadrivalent formation induced sexual vesicle disturbance leading to frequent apoptosis, which may suggest that spermatids completing meiosis had shown predominantly bivalent formation, and that more than 90.0% of spermatozoa analysed were obtained after mechanistically normal meiosis. Adjacent 2, 3:1 and 4:0 segregations derived from quadrivalent association are probably eliminated by apoptosis. Secondly, sperm count may be linked to bivalent/quadrivalent ratio, directly related to the size of the translocated autosome segment. The smaller the segment, the more frequent was bivalent formation, and the higher the sperm count. This hypothesis might explain the sperm segregation pattern of the three previously published infertile cases (**Table 2**), with the great majority of spermatozoa derived from alternate and adjacent I segregation, and spermatogenesis disrupted by frequent quadrivalent formation and apoptosis.

Thus, according to the size of the autosome segment involved in the translocation and apparent chromosome behaviour, the variability of sperm count observed for Y-autosome translocation, and why this patient had a normal sperm count and was fertile, could be explained.

In conclusion, Y-autosome translocations are associated with poor prognosis, with a risk above 50% of inherited unbalanced translocation for offspring. In the case reported here, two out of three children had an unbalanced translocation, confirming the sperm segregation pattern, and the ability of sperm segregation studies to predict translocation segregation even in fertile men.

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