

Sequential FISH allows the determination of the segregation outcome and the presence of numerical anomalies in spermatozoa from a t(1;8;2)(q42;p21;p15) carrier

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Abstract

Purpose To find out the meiotic segregation behaviour of the t(1;8;2)(q42;p21;p15), to evaluate the occurrence of interchromosomal effects, and to determine whether there is an accumulation of unbalanced products in aneuploid/diploid gametes.

Methods A sequential FISH protocol based on two successive hybridization rounds over the same spermatozoa was performed to determine the segregation outcome of the rearranged chromosomes. The presence of numerical abnormalities for 13, 18, 21, X and Y was also evaluated by sperm FISH. Those aneuploid/diploid gametes were subsequently relocalized and analyzed for their segregation content through additional hybridization rounds.

Results The segregation pattern observed reported a very low production of normal/balanced gametes (11.7 %). Significant increased frequencies of diploidies and disomies for chromosomes X/Y and 18 were detected ($p < 0.001$). Aneuploid and diploid spermatozoa displayed significant increases of 5:1, 6:0 and other unexpected disjunction modes ($p < 0.001$).

Capsule The study reveals a low production of normal/balanced gametes in a t(1;8;2)(q42;p21;p15) carrier and supports previous findings related to the presence of an altered segregation pattern in gametes with numerical abnormalities.

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Conclusions The strategy developed in this study is a reliable new approach to establish the full segregation pattern of complex chromosome rearrangements (CCR). Results corroborate the low number of normal/balanced spermatozoa produced by CCR carriers and support previous findings regarding an altered segregation pattern in gametes with numerical abnormalities. Altogether this confirms the importance of PGD as a tool to prevent the transmission of chromosomal abnormalities to the offspring in CCR patients.

Keywords Complex chromosome rearrangement · Fluorescent in situ hybridization · Interchromosomal effect · Meiotic segregation · Spermatozoa

Introduction

Structural chromosomal rearrangements involving three or more breakpoints are called complex chromosome rearrangements (CCR). The intrinsic complex nature of these rearrangements involves a wide range of categories. They can be classified according to the total number of breaks, the location/distribution of breakpoints or the chromosomal structure of the reorganization (reviewed by Pellestor et al [15]). Based on this last classification criterion, CCR can be divided into three-way rearrangements (when three chromosomes suffer one break each and interchange the distal segments), exceptional CCR (when more than one breakpoint per chromosome is present), and double two-way translocations (involving two or three independent translocations in the same carrier). Despite the lack of surveys referring to a concrete incidence in the general population, the frequency among infertile individuals has been estimated around 0.1 % [14].

Most of the three-way exchange-based rearrangements are balanced, and carriers are phenotypically normal, although

they often have fertility problems. Similar to other structural rearrangements, the reduction in the fertility potential is mainly caused by the formation of either structural or numerical chromosome unbalanced spermatozoa during gametogenesis. In three-way CCR, rearranged chromosomes should form a hexavalent at pachytene in order to achieve synapsis of homologous regions (Fig. 1). The segregation modes of this hexavalent can theoretically be performed by 3:3, 4:2, 5:1 and 6:0 chromosome disjunctions resulting in 64 products. In particular, 3:3 disjunctions include 20 different possibilities, although only the two products derived from the alternate segregation result in a normal or balanced chromosomal content. The disjunctions 4:2, 5:1 and 6:0 can produce 30, 12 and 2 combinations, respectively. Therefore, this meiotic configuration gives rise to a noticeable amount of unbalanced products. Indeed, CCR carriers are at high risk of producing chromosomally abnormal gametes, leading to spontaneous abortions or the transmission of a chromosome abnormality to the offspring (reviewed by Pellestor et al. [15]).

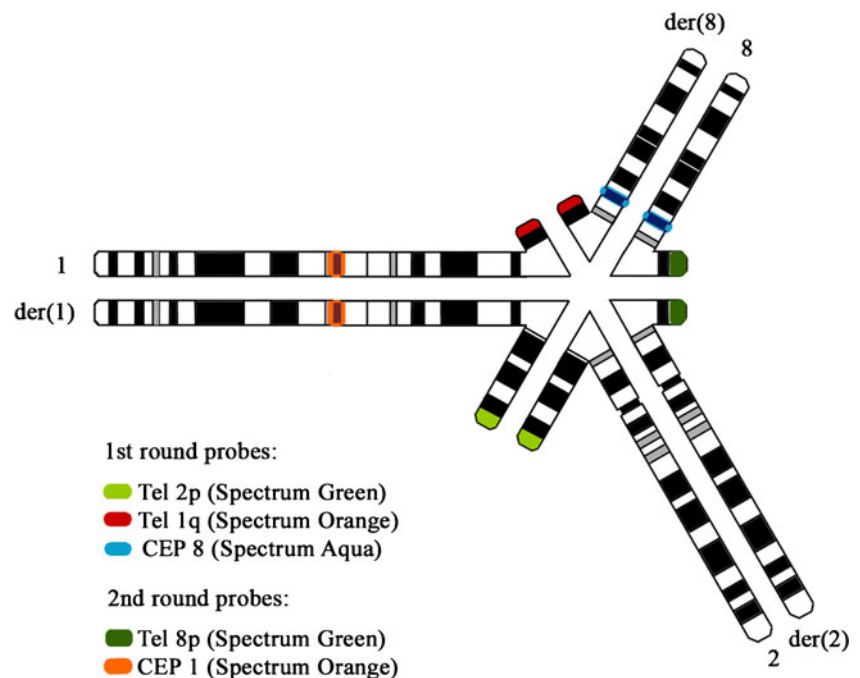
Besides the formation of these imbalances, there is also the possibility that an interchromosomal effect (ICE) occurs increasing the production of chromosomal anomalies. This phenomenon consists of the abnormal pairing and disjunction of chromosomes not involved in the rearrangement because of the meiotic disturbances caused by the rearranged chromosomes [10].

According to all this, male carriers of CCR are likely to present an important increase in the production of chromosomally abnormal spermatozoa. The genetic reproductive risk in two-break reciprocal translocations can be determined by

sperm-fluorescent in situ hybridization (FISH). Segregation analyses require the use of up to three differently labelled DNA probes in order to differentiate among the chromosomes involved in the reorganization [1]. Focussing on three-way CCR, a minimum of five differently labelled DNA probes is required to distinguish the six chromosomes involved in the reorganization. This fact clearly increases the complexity of the analysis, first due to the lack of commercial probes labelled with five different fluorochromes, second because of the difficulty of analysing five probes in the small area of a sperm nucleus. As a consequence, other strategies have been used including sperm karyotyping [3], multi-colour banding [16], primed in situ labelling [12], or aneuploidy screening of the chromosomes involved in the three-way rearrangement [9,13].

Recently, a sequential FISH methodology has been optimized to analyse different probe combinations over the same sperm nuclei [6]. The application of this methodology in spermatozoa from reciprocal translocation carriers has allowed demonstrating an accumulation of unbalanced segregation products of the chromosomes involved in the reorganization, in aneuploid or diploid spermatozoa. In the present study, we have taken advantage of this novel FISH strategy to carry out a comprehensive segregation study in decondensed sperm nuclei from a man heterozygous for the three-way translocation $t(1;8;2)(q42;p21;p15)$. The main objectives of the analysis were to elucidate the segregation pattern of the chromosomes involved in this reorganization and to find out if the accumulation of unbalanced products in aneuploid/diploid gametes also occurs in three-way translocation carriers.

Fig. 1 Hexavalent structure formed by the chromosomes involved in the $t(1;8;2)(q42;p21;p15)$ and location of the probes used in the segregation study. For a better understanding of the figure, Spectrum Green probes (Tel 2p and Tel 8p) and Spectrum Orange probes (Tel 1q and CEP1) have been labelled with different colour tones



Materials and methods

This study was carried out in a semen sample from a 35-year-old infertile patient presenting the karyotype 46,XY,t(1;8;2)(q42;p21;p15). Seminogram analysis revealed asthenozoospermia. The patient gave his informed consent to participate in this study, which was approved by our Institutional ethics Committee.

Segregation analysis

The sample was fixed and processed for FISH as described by Sarrate and Anton [18]. Segregation analysis was carried out using sequential FISH on sperm including two successive hybridization rounds [6]. This allowed the identification of the six chromosomes involved in the three-way translocation (Fig. 1). In the first hybridization round, we used a combination of the telomeric probes Tel 1q (Spectrum Orange, SO) and Tel 2p (Spectrum Green, SG) together with the centromeric CEP 8 probe (Spectrum Aqua, SA) (Abbott Molecular Inc., Des Plaines, IL, USA). After washing-off these probes, a second hybridization round was performed combining the centromeric CEP 1 probe (SO) and the telomeric Tel 8p probe (SG) (Abbott Molecular Inc.) (Fig. 1). All probes were previously tested on a control sperm sample showing a minimum hybridization efficiency of 99.6 %.

The spermatozoa analysed in the first hybridization round were relocalised using the Spot System AX software (Applied Imaging, Newcastle, U.K.) and then were reanalysed for the combination of probes of the second hybridization round. Taking into account the signals observed in both hybridization rounds, we obtained information of the five probes in the same nuclei that allowed the specific identification of each segregation product (see examples in Fig. 2).

ICE analysis versus segregation content

Additionally, segregation analysis was specifically performed on aneuploid and diploid sperm. As the intrinsic limitations of the FISH technique do not allow the analysis of the whole chromosome complement in such small interphase sperm nuclei, chromosomes 13, 18, 21, X and Y were chosen to perform this study. In this case, a previous hybridization round was required in order to first detect spermatozoa with numerical abnormalities for these chromosomes (ICE analysis). In this hybridization round either a combination of centromeric probes for chromosomes 18 (Spectrum Aqua, SA), X (Spectrum Green, SG) and Y (Spectrum Orange, SO), or a combination of locus-specific probes for chromosomes 13 (Spectrum Green, SG) and 21 (Spectrum Orange, SO) (AneuVysion Multicolor DNA Probe Kit, Abbott Molecular Inc.) was used. After the ICE analysis, the same slides were submitted to further successive hybridization rounds for the segregation analysis as described above (see examples in Fig. 3).

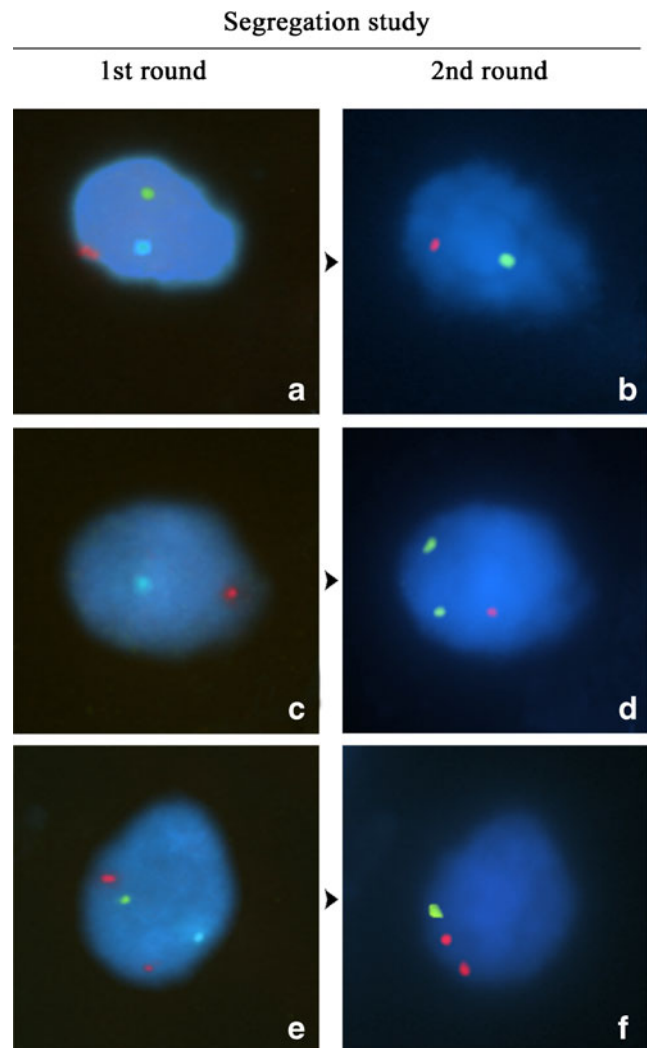
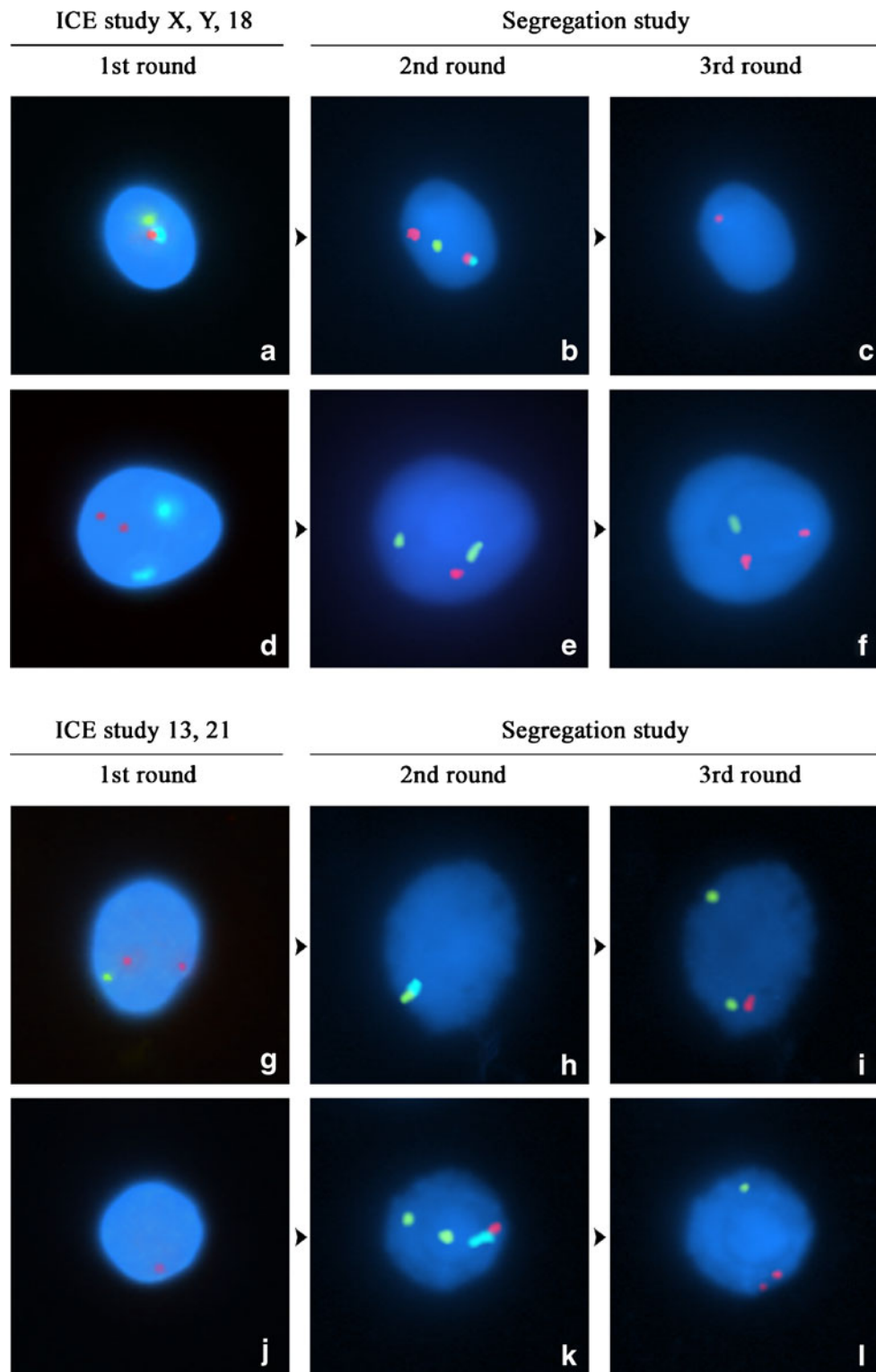


Fig. 2 Sequential FISH images of the segregation analysis. The first column corresponds to the first hybridization round (triple colour FISH) and the second column corresponds to the second hybridization round on the same sperm (dual-colour FISH). **a** Sperm showing one signal for Tel 2q (SG), one signal for Tel 1q (SO) and one signal for CEP 8 (SA). **b** Same sperm as in 2a showing one signal for Tel 8p (SG) and one signal for CEP 1 (SO). The combination of signals displayed in Fig. 2a and b indicates a 3:3 alternate segregation content. **c** Sperm showing one signal for Tel 1q (SO) and one signal for CEP 8 (SA). **d** Same sperm as in 2c showing two signals for Tel 8p (SG) and one signal for CEP 1 (SO). The combination of signals displayed in Fig. 2c and d indicates a 3:3 unbalanced segregation content. **e** Sperm showing one signal for Tel 2q (SG), two signals for Tel 1q (SO) and one signal for CEP 8 (SA). **f** Same sperm as in 2e showing one signal for Tel 8p (SG) and two signals for CEP 1 (SO). The combination of signals displayed in Fig. 2e and f indicates a 4:2 unbalanced segregation content

Microscope evaluation

FISH evaluation was carried out using an Olympus BX-61 microscope (Olympus, Barcelona, Spain) connected to the automatic Spot-Counting scan system (Spot AX software), and equipped with specific filters for DAPI (4',6-diamidino-



2-phenylindole), Aqua, FITC (fluorescein isothiocyanate) and Cy3 (cytochrome 3). Analysis of all nuclei was performed using strict criteria concerning the intensity, the size and the distribution of the signals [2].

Statistical analysis

Data was statistically analysed using a chi-square test, considering significant p -values when $p \leq 0.05$. Aneuploid and diploid

Fig. 3 Sequential FISH images of the ICE and the segregation analysis. The first column corresponds to the first hybridization round combining probes for the ICE analysis of chromosomes X/Y/18 (triple colour FISH) or 13/21 (dual colour FISH). The second column corresponds to the second hybridization round with the first combination of probes for the segregation study (triple colour FISH). The third column corresponds to a third hybridization round with the second combination of probes for the segregation study (dual-colour FISH). **a** Sperm showing one signal for CEP X (SG), one signal for CEP Y (SO) and one signal for CEP 18 (SA). **b** Same sperm as in 3a showing one signal for Tel 2q (SG), two signals for Tel 1q (SO) and one signal for CEP 8 (SA). **c** Same sperm as in 3a and 3b showing one signal for CEP 1 (SO). The combination of signals displayed in Fig. 3a, b and c indicates a 3:3 unbalanced segregation content with a sex chromosomes disomy. **d** Sperm showing two signals for CEP Y (SO) and two signals for CEP 18 (SA). **e** Same sperm as in 3d showing two signals for Tel 2q (SG) and one signal for Tel 1q (SO). **f** Same sperm as in 3d and 3e showing one signal for Tel 8p (SG) and one signal for CEP 1 (SO). The combination of signals displayed in Fig. 3d, e and f indicates a 4:2 unbalanced content and a diploidy. **g** Sperm showing one signal for LSI 13 (SG) and two signals for LSI 21 (SO). **h** Same sperm as in 3g showing one signal for Tel 2q (SG) and one signal for CEP 8 (SA). **i** Same sperm as in 3g and 3h showing two signals for Tel 8p (SG) and one signal for CEP 1 (SO). The combination of signals displayed in Fig. 3g, h and i indicates a 3:3 unbalanced content and a chromosome 21 disomy. **j** Sperm showing one signal for LSI 21 (SO). **k** Same sperm as in 3j showing two signals for Tel 2q (SG), one signal for Tel 1q (SO) and one signal for CEP 8 (SA). **l** Same sperm as in 3j and 3k showing one signal for Tel 8p (SG) and two signals for CEP 1 (SO). The combination of signals displayed in Fig. 3j, k and l indicates a 4:2 unbalanced content and a chromosome 13 nullisomy

frequencies for chromosomes X, Y, 13, 18 and 21 were compared to data from a control population. This population was previously established in our laboratory [19] and was formed by six fertile individuals (ranged in age from 20 to 25 years). Subjects were normozoospermic and showed normal karyotypes. Semen samples were processed as standardized in our laboratory [18]. In the segregation study, frequencies of each segregation mode reported in the non-selected spermatozoa were compared with the frequencies found in spermatozoa carrying numerical abnormalities.

Results

Segregation analysis

The two-round sequential FISH allowed the unequivocal identification of the six chromosomes involved in the CCR and, thus, all the 64 possible segregation products were distinguishable (Table 1). A total of 1,143 nuclei were analysed and 11.7 % showed a normal or balanced content whereas the remaining 88.3 % were unbalanced. Most of these unbalanced products resulted from 3:3 to 4:2 disjunction modes (43.1 and 28.9 %, respectively). The 3:3 unbalanced disjunction modes mainly involved homologous centromere disjunction of all chromosomes (28.9 %), while the rest (14.2 %) included segregation modes with a non-disjunction event in one of the homologous

translocated chromosomes. Segregation 5:1 and 6:0 segregations were very scarce representing 0.7 and 0.0 % of the total gametes analysed. The category named “other”, which included products derived from the occurrence of recombination events at interstitial segments, non-disjunction events at meiosis II, and combinations of signals that could not be ascribed to any of the previous categories, showed a frequency of 15.6 %.

ICE analysis versus segregation content

In the ICE analysis, 10,131 spermatozoa were screened for chromosomes X/Y/18 (Table 2) and 10,204 spermatozoa for chromosomes 13 and 21 (Table 3). Results showed significant increased frequencies of sex chromosomes disomies and chromosome 18 disomies, as well as diploidies when compared to the control population [19] ($p < 0.001$).

Among the 20,335 sperm analysed, 125 were aneuploid for chromosomes X/Y/18 (Table 2) and 38 for chromosomes 13/21 (Table 3). Subsequently, 129 of these 163 spermatozoa were relocated and analysed for their segregation content (sperm population B; Table 1). Results showed that 9.3 % of them were normal or balanced for the reorganized chromosomes, 34.9 % presented 3:3 unbalanced products, and 21.7 % resulted from 4:2 disjunctions. Although these frequencies were not statistically different from those observed among non-selected sperm ($p = 0.4143$, $p = 0.0751$ and $p = 0.7787$, respectively), the non-alternate 3:3 segregation products with homologous centromere disjunction exhibited a significant decrease ($p = 0.0078$). On the contrary, 5:1 and 6:0 disjunction modes (5.4 and 1.6 %, respectively) were statistically increased when compared to non-selected sperm ($p < 0.001$) as well as the category “other” (27.1 %, $p = 0.0019$).

On the other hand, a total of 95 spermatozoa were detected to be diploid either in the ICE analysis of chromosomes X/Y/18 (64) or 13/21 (31) (Tables 2 and 3). These two frequencies appeared to be significantly different ($p < 0.001$). Subsequently, 63 of them were relocated and analysed for their segregation content (sperm population C; Table 1). None of them showed a 3:3 disjunction mode, and the 4:2 products displayed a significantly lower frequency compared to the population of non-selected spermatozoa (14.3 versus 28.9 %, $p = 0.0121$). On the contrary, unbalanced 5:1 and 6:0 disjunction modes, together with category “other” showed statistically significant increases (11.1, 11.1 and 63.5 %, respectively; $p < 0.001$) when compared to the non-selected sperm population.

Discussion

There is few data concerning meiotic segregation of rearranged chromosomes in three-way translocation carriers. In the present study, we designed a strategy based on sequential FISH on sperm, that allowed us to accurately infer the meiotic behaviour

Table 1 Frequencies detected for each segregation mode in the t(1;8;2)(q42;p21;p15) carrier according to the combination of signals in: (A) non-selected spermatozoa, (B) aneuploid spermatozoa and (C) diploid spermatozoa

Segregation mode	Segregation products	N° of signals					Sperm populations <i>n</i> (%)						
		1st round probes			2nd round probes		A	B	C				
		Tel 2p	Tel 1q	CEP8	Tel 8p	CEP1							
3:3 alternate	1,2,8/der(1),der(2),der(8)	1	1	1	1	1	134 (11.7 %)	12 (9.3 %)	0 (0.0 %)*				
Non-alternate 3:3 (homologous centromeres disjunction)	der(1),2,der(8)	2	1	1	0	1	330 (28.9 %)	23 (17.8 %)*	0 (0.0 %)*				
	1,der(2),8	0	1	1	2	1							
	1,2,der(8)	1	2	1	0	1							
	der(1),der(2),8	1	0	1	2	1							
	der(1),2,8	2	0	1	1	1							
	1,der(2),der(8)	0	2	1	1	1							
Non-alternate 3:3 (homologous centromeres non-disjunction)	2,der(2),8	1	0	1	2	0	163 (14.2 %)	22 (17.1 %)	0 (0.0 %)*				
	1,der(1),der(8)	1	2	1	0	2							
	1,2,der(8)	1	2	1	0	1							
	der(1),der(2),8	1	0	1	2	1							
	1,2,der(2)	1	1	0	1	1							
	der(1),8,der(8)	1	1	2	1	1							
	1,2,der(2)	1	1	0	1	1							
	der(1),8,der(8)	1	1	2	1	1							
	der(2),8,der(8)	0	1	2	2	0							
	1,der(1),2	2	1	0	0	2							
	2,der(2),der(8)	1	1	1	1	0							
	1,der(1),8	1	1	1	1	2							
	4:2	1,der(1),2,der(2)	2	1	0	1				2	330 (28.9 %)	28 (21.7 %)	9 (14.3 %)*
		8,der(8)	0	1	2	1				0			
	1,2,der(2),8	1	1	1	2	1							
	der(1),der(8)	1	1	1	0	1							
	1,2,der(2),der(8)	1	2	1	1	1							
	der(1),8	1	0	1	1	1							
	1,2,8,der(8)	1	2	2	1	1							
	der(1),der(2)	1	0	0	1	1							
	1,der(2),8,der(8)	0	2	2	2	1							
	der(1),2	2	0	0	0	1							
	der(1),der(2),8,der(8)	1	1	2	2	1							
	1,2	1	1	0	0	1							
	der(1),2,8,der(8)	2	1	2	1	1							
	1,der(2)	0	1	0	1	1							
	der(1),2,der(2),8	2	0	1	2	1							
	1,der(8)	0	2	1	0	1							
	der(1),2,der(2),der(8)	2	1	1	1	1							
	1,8	0	1	1	1	1							
	1,der(1),8,der(8)	1	2	2	1	2							
	2,der(2)	1	0	0	1	0							
	2,der(2),8,der(8)	1	1	2	2	0							
	1,der(1)	1	1	0	0	2							
	1,der(1),der(2),der(8)	1	2	1	1	2							
	2,8	1	0	1	1	0							
	1,der(1),der(2),8	1	1	1	2	2							
	2,der(8)	1	1	1	0	0							

Table 1 (continued)

Segregation mode	Segregation products	N° of signals					Sperm populations <i>n</i> (%)		
		1st round probes			2nd round probes		A	B	C
		Tel 2p	Tel 1q	CEP8	Tel 8p	CEP1			
5:1	1,der(1),2,der(8)	2	2	1	0	2			
	der(2),8	0	0	1	2	0			
	1,der(1),2,8	2	1	1	1	2			
	der(2),der(8)	0	1	1	1	0			
	der(1),2,der(2),8,der(8)	2	1	2	2	1	8 (0.7 %)	7 (5.4 %)*	7 (11.1 %)*
	1	0	1	0	0	1			
	1,2,der(2),8,der(8)	1	2	2	2	1			
	der(1)	1	0	0	0	1			
	1,der(1),der(2),8,der(8)	1	2	2	2	2			
	2	1	0	0	0	0			
	1,der(1),2,8,der(8)	2	2	2	1	2			
	der(2)	0	0	0	1	0			
	1,der(1),2,der(2),der(8)	2	2	1	1	2			
	8	0	0	1	1	0			
	1,der(1),2,der(2),8	2	1	1	2	2			
der(8)	0	1	1	0	0				
6:0	1,der(1),2,der(2),8,der(8)	2	2	2	2	2	0 (0.0 %)	2 (1.6 %)*	7 (11.1 %)*
	—	0	0	0	0	0			
Other		NS	NS	NS	NS	NS	178 (15.6 %)	35 (27.1 %)*	40 (63.5 %)*
Total							1,143	129	63

*Statistically significant differences ($p < 0.05$) compared to the same segregation mode in the non-selected population of sperm (A)
 NS not shown

of rearranged chromosomes in a carrier of a t(1;8;2)(q42;p21;p15). Although this study is only based in a single case, the remarkable low frequency of normal or balanced products observed confirmed the high risk of CCR carriers of producing unbalanced gametes. Disjunction of the derivative chromosomes and their normal homologues resulted in a high frequency of unbalanced segregation modes, mostly related to 3:3 and 4:2 disjunctions. It is important to mention the considerable number of products classified as “other”, which includes many different signal combinations that can be attributed to the occurrence of several interstitial recombination events and to non-disjunctions at meiosis II. Our data are in good agreement with previous studies carried out in three-way CCR carriers. Those reports found similar segregation patterns with a low production of gametes with normal or balanced chromosomal content, with the non-alternate 3:3 and 4:2 as the most frequent segregation modes,

and with almost no products derived from 5:1 to 6:0 segregations (Table 4).

Regarding the seminal parameters of the carrier, the absence of spermatogenesis’ impairment (sperm count fits within normal values) supports de full pairing of the hexavalent, which leads to a wide range of segregation products. It is important to mention that spermatogenic impairment in CCR carriers is thought to be linked to the complexity of the rearrangement rather than the number or location of breakpoints [8]. In this case, probably because the balanced t(1;8;2) can usually attain the full pairing of chromosomes—either by forming a completely homosynapsed hexavalent or by means of heterosynapsis with other bivalents—the pachytene checkpoint can be overcome.

Regarding the ICE evaluation, the significant increases of aneuploidies for chromosomes X, Y and 18 are consistent with the only two reported studies that evaluated ICE in CCR carriers

Table 2 Frequencies of aneuploid and diploid spermatozoa detected in the interchromosomal effect (ICE) study of chromosomes 18, X and Y

	Sex chromosomes		Chromosome 18		Diploid <i>n</i> (%)	Total sperm (<i>n</i>)	
	Disomy <i>n</i> (%)	Nullisomy <i>n</i> (%)	Disomy <i>n</i> (%)	Nullisomy <i>n</i> (%)			
Statistical significant increases ($p < 0.05$) compared to control data [19]	t(1;8;2)	47 (0.46)	58 (0.57)	19 (0.19)*	1 (0.01)	64 (0.63)*	10,131
	Control	120 (0.19)	346 (0.54)	22 (0.03)	47 (0.07)	123 (0.19)	63,811

Table 3 Frequencies of aneuploid and diploid spermatozoa detected in the interchromosomal effect (ICE) study of chromosomes 13 and 21

	Chromosome 13		Chromosome 21		Diploid <i>n</i> (%)	Total sperm (<i>n</i>)
	Disomy <i>n</i> (%)	Nullisomy <i>n</i> (%)	Disomy <i>n</i> (%)	Nullisomy <i>n</i> (%)		
t(1;8;2)	3 (0.03)	14 (0.14)	8 (0.08)	13 (0.13)	31 (0.30)	10,204
Control	39 (0.06)	159 (0.25)	47 (0.07)	112 (0.18)	159 (0.25)	62,345

[9,16]. This circumstance might be related again with the characteristics of the hexavalent configuration. In fact, some meiotic studies in spermatocytes, besides evidencing the formation of a hexavalent in three-way translocation carriers, also observed asynapsis around chromosome breakpoints [7,17]. Moreover, a study by Coco et al. [4] showed the association of an acrocentric chromosome to the multivalent. As stated above, when hom-synapsis is not fully reached at pachytene, the occurrence of heterosynapsis represents a mechanism that avoids triggering the pachytene checkpoint, and this has been related to higher frequencies of numerical chromosomal abnormalities (ICE).

With reference to the relationship between segregation and the occurrence of ICE, the aneuploid population did not show a significant decrease in alternate segregation as has been observed elsewhere in reciprocal translocation carriers [6]. However, it needs to be considered that in this CCR carrier the alternate segregation mode among non-selected spermatozoa was already very low. Even though unbalanced segregation modes were prevalent both in aneuploid and non-selected spermatozoa, their distribution was different in these two groups: Aneuploid nuclei contained higher frequencies of 5:1, 6:0 and “other” modes and a significant reduction of the non-alternate 3:3 segregation products with homologous disjunction.

Regarding diploid sperm, the fact that they all presented an unbalanced segregation content is consistent with data from reciprocal translocation carriers [6]. Similar to aneuploid gametes, diploid sperm in this CCR also presented a different distribution of the unbalanced segregation modes with significant increases in the modes 5:1, 6:0 and “other” when compared to the non-selected population of sperm. These changes implied that the 5:1, 6:0 and “other” segregation modes became prevalent over the 3:3 and 4:2 disjunctions. On the other hand,

the fact that only 11.1 % of the diploid spermatozoa showed 6:0 segregation suggests that not all spermatozoa of this population had a real diploid chromosome content. Thus, some of these diploidies could actually correspond to double disomies. This data would also explain the differences observed in the frequencies of diploidies from both ICE studies.

Therefore in general, the ICE analysis versus segregation content revealed that in this three-way translocation carrier there is no evidence of a specific accumulation of unbalanced products in numerically abnormal sperm. However, these aneuploid/diploid gametes show an altered segregation pattern that modifies the distribution of the unbalanced segregation modes. The formation of both numerically and structurally unbalanced gametes would probably resemble the condition described in reciprocal translocation carriers [6]. Also in this case, the occurrence of heterosynapsis at pachytene and the consequent formation of a metaphase spindle with misaligned chromosomes, could lead to disjunction failures, resulting in a wrong meiotic outcome involving the rearranged chromosomes and other miss-attached chromosomes.

There is also few data from pre-implantation genetic diagnosis (PGD) procedures carried out in three-way CCR carriers. Escudero et al. [5] reported two normal/balanced embryos out of 22 diagnosed in a three-way translocation carrier. The couple achieved pregnancy and delivery of a child after five PGD cycles. Two more cases were reported by Lim et al. [11], one of them resulted in a normal embryo out of seven diagnosed which did not end up in pregnancy, while the other one did not present any normal/balanced embryo among the 11 diagnosed. Regarding the patient included in the present study, a PGD cycle was performed. A total of eight biopsied embryos could be diagnosed for all the chromosomes involved in the CCR.

Table 4 Segregation patterns described in previously published studies performed in three-way reorganization carriers and in the present study

Author	Rearrangement	Segregation modes						Total sperm (<i>n</i>)
		3:3 alternate (%)	3:3 (%)	4:2 (%)	5:1 (%)	6:0 (%)	Other (%)	
[3]	t(2;22;11)	13.5	64.9	20.7	1.0	0.0	–	208
[12]	t(1;19;13)	14.8	34.1	38.2	3.5	0.05	9.3	1822
[16]	t(5;13;14)	27.4	31.0	35.7	2.7	0.0	3.2	667
Present study	t(1;8;2)	11.7	43.1	28.9	0.7	0.0	15.6	1143

The results showed two normal/balanced embryos (25 %), which were transferred and resulted in pregnancy and delivery of two healthy children. Regarding the content of the unbalanced embryos, one of them corresponded to a 3:3 disjunction (12.5 %) while the remaining five could not be attributed to any specific hexavalent disjunction (62.5 %), thus, corresponding to the “other” category.

Altogether, this work provides new information about the meiotic behaviour of a three-way translocation as well as the presence of structural and numerical anomalies for chromosomes 13, 18, 21, X and Y in the gametes of this CCR carrier. We believe it is important to highlight that these individuals, despite producing a high number of unbalanced gametes, can also present normal sperm counts. This allows them to undergo assisted reproductive techniques and, thus, achieve healthy pregnancies through PGD analysis.

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Conflict of interest The authors declare that there are no conflicts of interest.

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