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GENETICS

Meiotic segregation and interchromosomal effects in a rare (1:2:10) complex chromosomal rearrangement

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Abstract
Purpose Complex chromosomal rearrangements (CCR) are rare rearrangements involving more than two chromosomes and more than two breakpoints. CCR are associated with male infertility as a result of the disruption of spermatogenesis due to complex meiotic configurations and the production of chromosomally abnormal sperm. We examined a carrier of a t(1:2:10) CCR in order to determine the patterns of segregation and any presence of an interchromosomal effect (ICE).
Methods Centromeric, locus specific and telomeric probes (Vysis, USA) were used for the study. On ~1,000 sperm nuclei from the reciprocal translocation carrier, dual color Fluorescence in situ hybridization (FISH) was performed on each of the involved chromosomes to determine the patterns of segregation. FISH was also performed on chromosome 13, 18, 21, X and Y to determine any ICE.
Results We observed abnormal chromosome complements in 24.3%, 19.5% and 15.8% of sperm for chromosomes 2, 10 and 1, respectively. There was a significantly increased rate of ICEs for chromosomes 13 and 21 when compared with controls.
Conclusions CCR may present a lower risk for producing unbalanced chromosomes than other studies have indicated. CCRs may be at an increased risk for ICE especially among acrocentric chromosomes.

Keywords Complex chromosomal rearrangement · Interchromosomal effect · Meiotic segregation · Sperm aneuploidy · Male infertility · FISH

Capsule Fluorescence in-situ hybridization examination of meiotic segregation and interchromosomal effects in a complex chromosomal rearrangement indicate a frequency of sperm with abnormal chromosome complements comparable with simpler reciprocal translocations.

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Introduction

Complex chromosomal rearrangements (CCR), involving two or more chromosomes and two or more breakpoints are rare, with ~100 being reported in the literature [1]. Carriers of CCR produce a high frequency of chromosomally abnormal sperm due to the aberrant segregation of the rearranged chromosomes during meiosis. Meiotic studies of CCR have shown that these rearrangements adopt structures similar to simpler translocations with several studies finding the rearranged chromosomes in a hexavalent configuration [2–4]. Due to the presence of these structures during meiosis, a variety of patterns of segregation are possible including a normal or balanced chromosome complement or a combination of normal and derivate chromosomes, leading to an unbalanced complement. In addition, the presence of a CCR may interfere with the segregation of other chromosomes, an interchromosomal effect (ICE) [5], furthering the risk of unbalanced sperm which, if involved in a conception, would present a severe risk for miscarriage or mental retardation. *De novo* CCRs, which are most commonly paternally derived, are usually associated with abnormal phenotypes in the carrier, while carriers of familial CCRs, which are more often maternally derived, are often phenotypically normal, but are at risk for reproductive complications [4].

Analysis of chromosome segregation in sperm from translocation carriers has been well investigated. However, in part due to their rarity, few analyses have been carried

out in CCR. Here we report a t(1;2;10) CCR. Fluorescence in situ hybridization (FISH) was used to assess the segregation patterns of each of the involved chromosomes as well as the presence of an ICE in chromosomes 13, 18, 21 X and Y. This study will provide insight into the risk to reproduction posed by carriers of CCR.

Materials and methods

A couple (female, 34 years old; male, 36 years old) presented with a year-long history of primary infertility with no previous attempted cycles of infertility treatment. The female partner displayed no evidence of tubal, ovulatory or pelvic infertility factors. However, the male partner (proband) was found to have left testicular atrophy. Semen analysis revealed severe oligoasthenoteratozoospermia (OAT) from three consecutive semen analyses with a sperm concentration of a few sperm to 0.9 million sperm per ml, 11% motility and 0% normal forms. Serum gonadotropins and testosterone were found to be normal. Cytogenetic analysis showed a male karyotype with a complex chromosomal rearrangement (Fig. 1). Chromosome 1p (breakpoint at p35.1) is translocated onto chromosome 2q. Chromosome 2q (breakpoint at q21.3) is translocated onto chromosome 10q. Chromosome 10q has 3 breakpoints at 10q11.23, 10q24.33 and 10q26.13, with intrachromosomal insertion, paracentric inversion and translocation. The CCR was determined to be a t(1;2;10)(1qter→1p35.1::10q26.13→10qter; 2pter→2q21.3::1p35.1→1pter;10pter→10q11.23::10q24.33→10q26.13::10q24.33→10q11.23::2q21.3→2qter). It is, as yet, unknown if the rearrangement is *de novo* or inherited, though the number of breakpoints and the lack of phenotypic consequence in the carrier would suggest that it is *de novo*. The control spermatozoa used were from an individual of proven fertility. Patient consent was received from the carrier and controls prior to the initiation of experiments. This study was approved by the UBC clinical ethical board prior to the initiation of the experiments.

The methods of sperm preparation, probe hybridization, and FISH analysis were described previously [6]. Probe specificity and the labeling efficiency of each probe were confirmed on karyotyped blood leukocytes prior to analysis. Segregation was analyzed with three separate FISH probe sets, for each of the involved chromosome, on both a patient and control slide. These included p (SpectrumGreen) and q (SpectrumOrange) subtelomere probes for each of chromosomes 1, 2, and 10 (all probes Vysis Inc., Downers Grove, IL, USA). ICE were analyzed as previously reported [7]. Sperm were scored as previously described [6]. Chi-square test was used to compare the aneuploidy rates in the patient with controls. All statistical analyses were performed using GraphPad Prism V5.0 (GraphPad Software, San Diego, CA, USA). $P<0.05$ was considered significant.

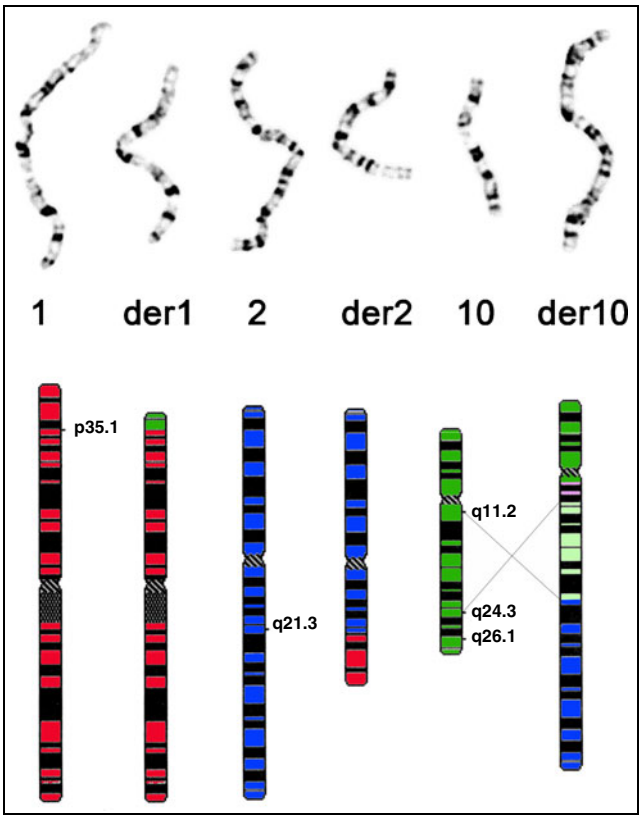

Fig. 1 Karyotype and Ideogram of t(1;2;10) Karyotype for showing the normal and derivative chromosomes from the t(1;2;10) CCR. Below are the corresponding ideograms coloured to display the chromosome of origin with chromosome 1 in *red*, chromosome 2 in *blue* and chromosome 10 in *green*. On the derivative 10 chromosome the area shaded light green corresponds to the inverted region. Lines between chromosome 10 and derivative 10 show the inversion. The area shaded pink corresponds to the inserted region from q24.3–q26.1 on chromosome 10

Results

We examined the frequency of imbalance in each of the involved chromosomes in the CCR individually. The segregation patterns in each chromosome are summarized in Table 1. A total of 3,094 patient sperm and 3,047 control sperm were analyzed, with hybridization efficiencies of 92.7–98.3% and 94.7–97.8% respectively. Chromosome 2 displayed the fewest balanced segregants at 75.8%, while chromosome 1 displayed the highest frequency of normal/balanced segregants with 84.2%. For chromosome 1, the frequency of additional p indicative of an additional derivative 2 chromosome (der 2) and q (der 1) signals were 4.8% ($p<0.05$) and 5.2% ($p<0.05$) respectively, while the frequency of missing p and q signals were 3.3% ($p<0.05$) and 2.2% ($p<0.05$). Analysis of chromosome 2

Table 1 Segregation of chromosomes 1, 2 and 10 in sperm from t(1:2:10)

	1p/1q			2p/2q			10p/10q		
FISH signal	chromosome(s)	t(1:2:10) (1,076) % (#)	Control (1,023) #	chromosome(s)	t(1:2:10) (1,018) % (#)	Control (1,018) #	chromosome(s)	t(1:2:10) (1,000) % (#)	Control (1,006) #
OG	1/der1+der2	84.2[a] (906)	1021	2/der2+der10	75.76[a] (772)	1017	10/der10+der1	80.55[a] (808)	1,006
OOG	1+der1/der1+der2+der1	5.2[b] (56)	0	2+der10/der2+ der10+der10	9.12[b] (93)	1	10+der1/der1+ der10+der1	6.48[b] (65)	1
OGG	1+der2/der1+der2+der2	4.83[b] (52)	0	2+der2/der2+ der10+der2	5.98[b] (61)	2	10+der10/der1+ der10+der10	5.78[b] (58)	2
G	der1	2.23[b] (24)	0	der2	4.71[b] (48)	0	der10	3.38[b] (34)	0
O	der2	3.34[b] (36)	1	der10	2.45[b] (25)	1	der1	2.00[b] (20)	1
OGGG	1+der1+der1	– (0)	0	2+der2+der2	0.39 (4)	0	10+der10+der10	0.3[b] (3)	0
OOOG	1+der2+der2	– (0)	0	2+der10+der10	0.49[b] (5)	0	10+der1+der1	0.4[b] (4)	0
OOGG	1+1/der1+der1+ der2+der2	0.18[b] (2)	0	2+2/der2+der2+ der10+der10	0.98[b] (10)	0	10+10/der1+der1+ der10+der10	0.8[b] (8)	0

[a] $p<0.05$ when compared with the control group (significantly lower)

[b] $p<0,05$ when compared with control group (significantly higher)

revealed additional p (der 2) and q (der 10) signals in 5.9% ($p<0.05$) and 9.1% ($p<0.05$) and missing p and q signals in 2.4% ($p<0.05$) and 4.7% ($p<0.05$). Analyses of chromosome 10 found 80.5% ($p<0.05$) balanced or normal, while additional p (der 10) and q (der 1) signals were 5.8% ($p<0.05$) and 6.5% ($p<0.05$) and missing p and q signals were 2.0% ($p<0.05$) and 3.4% ($p<0.05$). Segregants involving more than one additional p or q signal were found in only 0.88% ($p<0.05$) and 0.7% ($p<0.05$) of spermatozoa from chromosomes 2 and 10. Disomy (or a doubling of the balanced complement) was observed in 0.18% ($p<0.05$), 0.98% ($p<0.05$) and 0.8% ($p<0.05$) of spermatozoa in chromosomes 1, 2 and 10 respectively.

The frequency of unbalanced complements in the involved chromosomes varied between 24.24% ($p<0.05$) in chromosome 2 and 15.8% ($p<0.05$) in chromosome 1. These unbalanced chromosome complements were comprised, almost exclusively, of either normal/balanced complements with the addition of one of the derivative chromosomes, or one of the derivative chromosomes alone, producing partial disomy or nullisomy of one of the rearranged chromosomes.

The results of the ICE analysis were previously reported [7]. When compared with pooled fertile controls, the patient showed a significant increase in disomy for chromosomes 13 (0.32% vs. 0.20% in controls, $P<0.05$) and 21 (0.45% vs. 0.25% in controls, $P<0.001$). While total aneuploidy for the sex chromosomes and chromosome 18 was elevated, this increase was not significant (0.53% vs. 0.49% in controls $P=0.07$).

Discussion

The CCR presented here theoretically adopts a hexivalent structure at meiosis I (Fig. 2). Given the complexity of the rearrangement it is striking that we did not find a higher frequency of unbalanced chromosome complements. CCR have been shown to adopt hexivalent structures during meiosis [2–4], and theoretically, a CCR involving a three-way translocation would have 20 possible 3:3 segregations, and numerous 2:4, 1:5 and 6:0 segregations. Indeed, of the over 100 different possible segregation modes resulting from a three way CCR only two would produce a normal/balanced gamete.

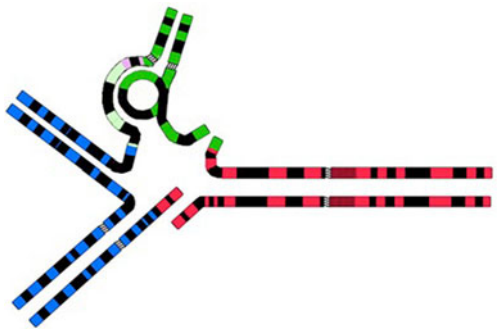

Fig. 2 Hypothetical conformation of t(1;2;10) during meiosis. This figure shows a possible structure formed by the CCR during meiosis. As with Fig. 1, Chromosome 1 is shown in *red*, chromosome 2 in *blue* and chromosome 10 in *green*. Regions on the derivative chromosomes are coloured to denote the chromosome of origin. The *light green* region of derivative 10 corresponds to the inverted region. The *pink* region of derivative 10 corresponds to the inserted region

To date, a few studies have examined segregation in a CCR, a t(2;11;22) [8] , a t(5;13;14) [9], and a t(1;19;13) [10], and these have found much higher frequencies of unbalanced chromosome complements (86.5%, 69.4%, 75.9% respectively). However, a study, similar to this one, that examined chromosome specific frequency of unbalanced complements in a t(2;4;8), found rates lower than those reported here, 3.3% in chromosome 4 and 4.8% chromosomes in chromosome 8 [11].

We hypothesize that a large number of unbalanced chromosome complements are indeed produced during segregation, but selection during spermatogenesis preferentially selects for spermatogonia with balanced/normal chromosome complements. It has been hypothesized that the infertility associated with CCRs is due to spermatogenic arrest that occurs as a result of the complex meiotic configurations that occur during meiosis [12]. Meiotic studies of reciprocal translocations have demonstrated compromised synapsis and silencing in the breakpoints of rearranged chromosomes, likely contributing to spermatogenic arrest [13]. Indeed, spermatogenic arrest at pachytene has been observed in a CCR [14], possibly suggesting that spermatogenesis was disrupted as a result of rearranged chromosomes attempting to adopt these confirmations.

Two checkpoints that operate during meiosis and initiate apoptosis in response to errors in meiosis first ensures proper synapsis between the chromosomes [15] and then ensures that the chromosomes are properly aligned at the metaphase plate prior to anaphase [16]. We suggest that cells in which the CCR septivalent is misaligned, and in which the production of unbalanced sperm is more likely are at greater risk of activating these meiotic checkpoints leading to apoptosis. Such an explanation may explain both the greatly reduced sperm parameters observed in this carrier as well as the relatively low frequency of unbalanced chromosome complements.

Analysis of ICE in this carrier, may suggest that CCR show a preference for disrupting acrocentric chromosomes. While these results may indicate the presence of an ICE, the frequencies of disomy are not significant when compared with OAT men [7], suggesting that the increased level of disomy may be attributable to the patient's OAT status.

We have provided one of the first estimates of chromosome segregation patterns in a carrier of a CCR. Our results confirm that CCRs are at a high risk for producing aneuploid sperm,

though the potential range in the frequency of abnormal chromosome complements may be larger than previously thought. As such CCR carriers may especially benefit from sperm chromosome studies prior to initiation of ART. In addition to abnormal segregation of rearranged chromosomes, our results suggest that CCR carriers are at an increased risk for production of chromosomally abnormal sperm due to mis-segregation of uninvolved chromosomes, a phenomenon that may be due to an ICE, though an increase in disomy associated with OAT status cannot be ruled out. Nevertheless, the results suggest that in this case the patient may be a candidate for ICSI, especially if combined with pre-implantation genetic diagnosis.

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