Altered bivalent positioning in metaphase I human spermatocytes from Robertsonian translocation carriers

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Abstract

Purpose The study aims to determine whether there is an altered bivalent positioning in metaphase I human spermatocytes from Robertsonian translocation carriers.

Methods Metaphase I human spermatocytes from three 45,XY,der(13;14)(q10;q10) individuals and a 45,XY,der(14;15)(q10;q10) individual were analyzed. Proximity relationships of bivalents were established by analyzing meiotic preparations combining Leishman staining and multiplex-FISH procedures. Poisson regression model was used to determine proximity frequencies between bivalents and to assess associations with chromosome size, gene density, acrocentric morphology, and chromosomes with heterochromatic blocks. The hierarchical cluster Ward method was used to characterize the groups of bivalents with preferred proximities in a cluster analysis. Bivalent groups obtained were individually compared with those obtained in normal karyotype individuals evaluated in a previous study.

Results A total of 1288 bivalents were examined, giving a total of 2289 proximity data. Only four positive significant proximities were detected for each type of Robertsonian translocation. Significant bivalent associations were only observed by small-size chromosomes for MI,22,XY,III(13q14q). These

results were clearly divergent from 46,XY individuals. Moreover, cluster analysis revealed that about 30 % of the bivalents showed changes in their proximity relationships in metaphase I.

Conclusions The territorial organization of bivalents in metaphase I human spermatocytes changes in the presence of a Robertsonian translocation.

Keywords Chromosome territories · Metaphase I · Meiosis · Robertsonian translocation · Spermatocytes

Introduction

Chromosomes occupy specific regions of the nuclear space called chromosome territories (CTs) [1]. Nuclear spatial arrangement of CTs has been related to some chromosomal parameters such as gene density [2–5] or chromosome size [6]. Moreover, the distribution pattern of CTs is distinct, depending on the cell type [7, 8].

The distribution of CTs has functional significance in terms of maintenance and regulation of the genome [9]. It has been suggested that specific nuclear architecture may regulate gene expression [10–12]. Chromosome positioning could promote chromosomal interactions between specific regions of the genome to activate or repress their expression [10, 11]. Actually, alterations in the spatial arrangement of chromosomes have been associated with various diseases. Cremer et al. described different patterns of positioning for chromosomes 18 and 19 between normal and tumor cell lines [13]. A change in the position of chromosome 17 territory has also been described for cells infected with Epstein-Barr virus [14]. Meaburn et al. described changes in territories for chromosomes 13 and 18 in patients with *LMNA* mutations [15].

Capsule The territorial organization of bivalents in metaphase I human spermatocytes changes in the presence of a Robertsonian translocation.

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Little is known about the maintenance and distribution of chromosomes during the cell cycle. Although some authors have proposed that territorial chromosome organization is maintained throughout the cell cycle [16]; others suggested that this organization is re-established in the early G1 phase [17, 18].

Meiotic studies of chromosome positioning have demonstrated a non-random chromosome distribution in spermatogenesis. Some authors have revealed a preferential proximity of the bivalent 15 to the XY pair in human spermatocytes during the pachytene stage [19] and a preferential proximity location of bivalents 15 and 22 to the sex bivalent at metaphase I (MI) [20]. Studies of bivalent positioning at MI have demonstrated a non-random distribution of chromosomes and have observed preferred associations depending on chromosome size, chromosome morphology, and gene density [21].

Several publications have reported a non-random spatial organization of chromosomes in the nuclei of spermatozoa [22–26]. Sperm chromosome position could be crucial in the decondensation and remodeling of chromatin domains and, consequently, achieve an epigenetic control of gene expression in the embryo [26–28].

In humans, Robertsonian translocations are one of the most common structural reorganizations. They are detected in 1/1000 newborns [29], but this percentage is nine times higher in infertile patients [30]. The reduced fertility in Robertsonian translocation carriers is mainly due to the formation of chromosomally abnormal sperm as a result of the regular segregation of the chromosomes involved in the reorganization and interchromosomal effects phenomena (ICE).

Moreover, Robertsonian translocations would be associated with a reduction in gamete production resulting from the activation of meiotic checkpoints triggering apoptosis [31].

Some studies have shown that the presence of chromosomal rearrangements alters the positioning of chromosomes in the sperm nucleus [32–34]. This observation has been related to changes in the expression profile of paternal alleles in the embryo [26–28]. Altogether, this suggests that alterations of sperm chromosomal territoriality could be an additional cause of infertility.

In this work, we have determined the effect of a Robertsonian translocation on the territorial organization of bivalents in MI. To achieve this aim, we compared the relative position of all bivalents in MI in a group of Robertsonian translocation carriers to individuals with normal karyotypes.

Materials and methods

Biological samples

Semen samples (P1, P2) or testicular biopsies (P3, P4) were obtained from four Robertsonian translocation carriers: three

individuals 45,XY,der(13;14)(q10;q10) (P1, P2, P3) and one 45,XY,der(14;15)(q10;q10) (P4). Patients gave their informed consent with regard to the participation in the study, and protocols were approved by our Institutional Ethics Committee.

Seminogram analyses, according to the World Health Organization (WHO) criteria [35], showed oligoasthenoteratozoospermia (P1, P3), oligoasthenozoospermia (P2), and teratozoospermia (P4). Data from testicular tissue samples used as controls have been detailed in a previous study [21].

Semen samples P1 and P2 exhibited high numbers of spermatogenic cells at different meiotic stages, which is quite common in samples from infertile males [36]. Accordingly, these samples were used for downstream analyses. Additionally, testicular tissue samples were obtained for patients P3 and P4.

In any case, samples were incubated in a hypotonic solution (KCl 0.075 M) at 37 °C. Biopsies were mechanically disaggregated to obtain a cell suspension. Semen samples and meiotic cell suspensions were centrifuged, and the pellets were fixed in methanol/acetic acid (3:1). Fixed material was dropped onto dry slides and kept at –20 °C until chromosome analysis.

Bivalent identification

Leishman stain diluted at 20 % in a buffer solution prepared according to Weise was used to stain the chromosome preparations (Fig. 1a). The evaluation was carried out with an Olympus BX60 microscope (Olympus Optical España S.A.) equipped with a capture and image analysis system (CytoVision 2.7, Applied Imaging). MI images were captured, and coordinates were noted. Leishman-stained slides were destained before being processed for multiplex-FISH (M-FISH) (Spectra Vysion Assay, Vysis Inc.) according to a protocol optimized in our laboratory [37]. As a result, bivalents were labeled with a combination of five different fluorochromes, obtaining 24 different color patterns, one for each bivalent (Fig. 1b, c). Thus, M-FISH allowed a karyotype to be created (Fig. 1c), facilitating metaphase proximity analysis by comparing the images captured after the M-FISH protocol with those previously obtained after Leishman staining.

Proximity analysis

All metaphases included in this study were MI,22,XY,III(13q14q) or MI,22,XY,III(14q15q), exhibiting all bivalents, including the XY pair and the chromosomes involved in the reorganization as a trivalent. In each cell, nearby bivalents were determined for each bivalent, including chromosomes involved in the reorganization which were each individually analyzed. We considered those that form the first ring around a specific bivalent to be “nearby.” Proximity was established for the 253 bivalent pair possibilities

Fig. 1 MI in Leishman staining (a) and M-FISH (b) with the corresponding M-FISH karyotype MI,22,XY,III(14q15q) (c). Identification of the nearby bivalents that constitute the first ring around the bivalent 10 (indicated with an arrowhead): 2;4;9;14;15 (a, b)

($C[23, 2] = \frac{1}{2} \times 23 \times 22 = 253$ combinations of two without repetitions). Nearby bivalents were scored as “1” and the absence of proximity as “0.” As a consequence, bivalents involved in the reorganization were always scored as “1.” From these values, tables of proximity for each metaphase were built (Appendix 1). The addition of data from all of the analyzed metaphases allowed the creation of proximity tables for MI,22,XY,III(13q14q) (Appendix 2) and MI,22,XY,III(14q15q) (Appendix 3).

Statistical analysis

In order to determine proximity frequencies between bivalents, a generalized regression model with repeated measures considering a Poisson distribution [38] was performed separately for 45,XY,der(13;14)(q10;q10) and 45,XY,der(14;15)(q10;q10) individuals. Additional regression models were used to assess whether chromosome size, morphology, gene density, or the presence of heterochromatic blocks determined bivalent positions. Wherever possible, the

groups of chromosomes analyzed were set from the five most representative chromosomes of each parameter evaluated (Table 1). Chromosomes involved in the rearrangement were excluded from the acrocentric group to prevent the bias caused by the constant proximity of these chromosomes in a trivalent configuration.

A second analysis was performed with the aim of grouping nearby chromosomes. This was done by a multidimensional scaling (MDS) analysis [39] that summarizes proximities between chromosomes by a set of quantitative variables used in a hierarchical cluster analysis. Results were represented in a tree dendrogram using R-squared distance. Clustering results were compared to those defined in individuals with normal karyotypes and published elsewhere [21]. Briefly, bivalents were distributed in three clusters in normal karyotypes: cluster I constituted larger size chromosomes (1;2;3;4;5;6;7;8;9;10;11;12); cluster II was mainly defined by the presence of acrocentric chromosomes (13;14;15;21;22;XY;18); and cluster III was characterized by rich-gene chromosomes (16;17;19;20). Differences were calculated by taking into account the percentage of bivalents

Table 1 Analysis of associations among preferential bivalent group proximity and chromosome features of MI,22,XY,III(13q14q) and MI,22,XY,III(14q15q)

	Chromosome feature	Bivalents	Percent	Confidence limit		<i>P</i> value	Proximity ^a
				Lower	Upper		
MI,22,XY,III(13q14q)	Large-size chromosomes	(1;2;3;4;5)	12.9	10.1	16.5	0.452	+
	Small-size chromosomes	(18;19;20;21;22)	19.3	17.3	21.5	0.023	
	High-density chromosomes	(11;16;17;19;22)	15.1	13.0	17.6	0.950	
	Low-density chromosomes	(4;5;8;13;18)	16.1	14.8	17.6	0.318	
	Acrocentric chromosomes	(15;21;22)	15.2	14.8	15.6	0.688	
	Chromosomes with heterochromatic blocks	(1;9;16)	17.9	14.7	21.8	0.487	
MI,22,XY,III(14q15q)	Large-size chromosomes	(1;2;3;4;5)	12.0	7.6	19.0	0.080	
	Small-size chromosomes	(18;19;20;21;22)	12.7	8.1	19.8	0.127	
	High-density chromosomes	(11;16;17;19;22)	16.7	11.3	24.7	0.799	
	Low-density chromosomes	(4;5;8;13;18)	19.3	13.4	27.8	0.593	
	Acrocentric chromosomes	(13;21;22)	17.8	11.9	26.5	0.940	
	Chromosomes with heterochromatic blocks	(1;9;16)	24.4	13.6	44.1	0.291	

Note: MI,22,XY,III(13q14q) global mean = 15.2; CI = [14.7–15.7]; MI,22,XY,III(14q15q) global mean = 17.5; CI = [16.2–18.9]

^a Preferential bivalent proximities (+)

remaining in the same cluster and those that changed. The concordance between classifications was calculated by Cohen's Kappa coefficient.

Results

A total of 1288 bivalents were examined, giving a total of 2289 proximity data. Only four positive significant proximities were detected in Robertsonian translocation carriers for

13,14 and five for 14,15 (Table 2). Contrasting these results with proximities described in normal karyotype MI analysis [21], only two of the four bivalent pairs per type of Robertsonian translocation were coincident (Table 2). There were 20 bivalent pairs with significant proximities in normal karyotype MI analyses [21] that did not remain statistically significant for both translocations studied.

Proximity analysis of specific bivalent groups revealed statistically significant nearby locations of small-size chromosomes for MI,22,XY,III(13q14q). Neither of the other bivalent groups showed significant proximities in MI,22,XY,III(13q14q) nor any bivalent group with specific features in MI,22,XY,III(14q15q) (Table 1).

Cluster analysis defined three bivalent groups of preferred proximity in MI,22,XY,III(13q14q) (Fig. 2a) and MI,22,XY,III(14q15q) (Fig. 2b). Comparative analysis between clusters obtained in this study and clusters obtained from normal karyotype individuals showed a difference of 35 % for MI,22,XY,III(13q14q) and 30 % for MI,22,XY,III(14q15q) with a Kappa value of 0.47 (CI = 0.16 to 0.73) and 0.55 (CI = 0.31 to 0.79), respectively.

Discussion

Our study provides evidence that the territorial organization of bivalents in MI changes in the presence of a Robertsonian translocation. We observed an alteration of bivalent pairs with significant proximities compared to those described in normal karyotype individuals, and an alteration of the relationship between chromosome features and bivalent positioning.

Table 2 Significant bivalent pairs associations of MI,22,XY,III(13q14q) (*n* = 41) and MI,22,XY,III(14q15q) (*n* = 15)

	Bivalents	Mean (%)	Confidence limit	
			Lower (%)	Upper (%)
MI,22,XY,III(13q14q)	Chr20;21 ^a	39	24	64
	Chr15;22 ^a	34	20	58
	Chr20;XY	34	20	58
	Chr16;20	29	17	52
MI,22,XY,III(14q15q)	Chr 11;18 ^a	47	22	98
	Chr 22;XY ^a	47	22	98
	Chr 3;XY	47	22	98
	Chr 5;18	47	22	98
	Chr 7;9	47	22	98

Note: global mean = 0.152; 95 % CI = [0.147–0.157]

^a Significant bivalent pairs coinciding with those described in normal karyotypes [21]

Fig. 2 Dendrogram representation of bivalent proximities for MI,22,XY,III(13q14q) (**a**) and MI,22,XY,III(14q15q) (**b**). Different colors indicate groups of chromosomes resulting from clustering of MI

control data [21] Note: MI,22,XY,III(13q14q) Kappa value = 0.47; CI = [0.16–0.73]. MI,22,XY,III(14q15q) Kappa value = 0.55; CI = [0.31–0.79]

There are different non-mutually exclusive possibilities to explain such variations. Studies in Robertsonian translocation carriers have described that the reorganized chromosomes usually show asynaptic regions at pachytene which are often associated with asynaptic regions of other bivalents [40–45]. It has also been described that asynaptic regions suffer heterochromatinization and gene silencing [31, 46]. Moreover, circularized chromosome conformation capture (4C) studies have shown that active and inactive chromatin are found in different nuclear domains [47]. Therefore, heterosynaptic regions at prophase I could alter chromosome positions according to the chromatin state of activation, and this altered position could be maintained at least until the MI stage. Another consequence of heterosynapsis is the alteration of the frequency and distribution of chiasmata in the rearranged chromosomes [48–51], as in other chromosomes [51–54]. In agreement with this, MI included in this study showed a lower mean chiasmata count than MI in the normal karyotype population [55]. This reduction was of six chiasmata in the MI of Robertsonian translocation carriers for 13,14 and of four chiasmata in Robertsonian translocation carriers for 14,15 (excluding chiasmata of reorganized bivalents). Although the number and distribution of chiasmata is crucial to bivalent orientation in the meiotic spindle, it has been demonstrated that preferential proximities between bivalents are not affected by chiasmata count variations [21].

The second possibility could be related to the characteristics of acrocentric chromosomes. They have short arms composed of tandem copies of ribosomal DNA (rDNA) genes

called nucleolar organizing regions (NORs). These regions are specifically associated during interphase to form the nucleolus [56]. Since the genesis of a Robertsonian translocation requires the loss of the short arms, derivative chromosomes may not associate successfully to form the nucleolus. Thus, the relative position between acrocentric chromosomes could be disturbed in interphase, and the same positioning alteration could be still present in MI.

To conclude, our results provide the first evidence of a chromosome territoriality alteration in the presence of a Robertsonian translocation in MI human spermatocytes. Considering the accumulating evidence supporting a functional significance of chromosome territoriality, the characterization of CTs during spermatogenesis could open new insights to determine whether there is a relationship between chromosome positioning, genome regulation, and male infertility.

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Compliance with ethical standards Patients gave their informed consent with regard to the participation in the study, and protocols were approved by our Institutional Ethics Committee.

Appendix

Appendix 1 Count example proximities between the bivalents in MI,22,XY,III(14q15q)

Cr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
1		0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0
2			0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
3				0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
4					0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1
5						0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
6							0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0
7								0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
8									1	0	0	0	0	0	0	1	0	1	1	0	0	0	1
9										1	0	1	0	1	0	0	0	1	0	0	0	0	1
10											0	0	0	1	1	0	0	0	0	0	0	0	0
11												1	1	0	0	0	0	0	0	0	1	0	0
12													0	1	0	0	0	1	0	0	0	0	0
13														0	0	0	1	0	0	0	1	0	0
14															1	0	0	0	0	0	0	0	0
15																0	0	0	0	0	0	0	0
16																	0	1	1	0	0	1	0
17																		0	0	0	1	1	0
18																			0	0	0	1	0
19																				0	0	0	1
20																					0	0	1
21																						0	0
22																							0
XY																							

Appendix 2 Incidence counts of proximities between the bivalents in MI,22,XY,III(13q14q) ($n = 41$)

Cr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
1		4	2	6	8	5	9	9	8	8	7	3	6	6	8	6	7	7	6	6	4	4	7
2			6	1	7	10	4	6	3	4	5	6	7	8	2	5	10	5	6	5	7	3	6
3				11	2	6	5	4	7	4	5	7	6	5	9	9	3	9	4	4	10	4	7
4					6	9	3	7	9	1	7	7	6	2	6	4	7	7	8	0	5	3	9
5						2	9	4	11	8	11	4	6	6	3	7	5	7	7	7	5	9	3
6							7	6	2	3	11	4	3	6	5	5	6	10	5	7	5	4	8
7								7	8	7	7	3	2	3	5	7	11	5	6	3	10	5	7
8									5	11	3	3	8	5	4	8	4	9	3	5	11	7	5
9										10	9	6	4	4	8	8	6	4	8	9	5	4	9
10											3	9	6	7	6	7	5	7	8	11	7	4	10
11												4	5	1	7	7	11	6	2	6	3	4	5
12													5	7	9	5	5	10	6	7	9	9	8
13														41	3	7	5	6	3	3	2	6	10
14															4	4	3	4	8	5	1	8	6
15																8	8	9	8	9	8	14	11
16																	2	6	6	12	7	10	7
17																		3	6	5	10	7	6
18																			9	4	4	10	5
19																				4	8	7	8
20																					16	9	14
21																						8	6
22																							6
XY																							

Appendix 3 Incidence counts of proximities between the bivalents in MI,22,XY,III(14q15q) ($n = 15$)

Cr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
1		1	1	3	1	5	4	3	4	2	5	3	0	2	1	3	6	2	5	2	3	2	2
2			2	4	0	3	5	3	4	5	4	1	2	4	2	3	1	0	1	2	3	0	3
3				3	2	5	3	1	3	2	4	1	0	3	3	3	2	1	1	2	3	4	7
4					1	2	5	2	5	2	2	2	3	1	2	2	3	3	2	2	5	1	3
5						1	4	2	1	2	4	6	2	1	1	5	3	7	2	4	2	2	5
6							2	3	2	1	1	3	3	6	3	3	3	2	2	0	6	5	2
7								5	7	6	2	1	2	0	3	2	4	1	4	2	3	3	4
8									2	4	2	3	4	2	1	1	2	1	5	2	2	1	4
9										6	1	1	1	2	3	4	5	2	2	0	0	4	3
10											0	2	4	4	2	2	3	3	2	3	0	1	1
11												2	5	2	1	3	2	7	1	5	2	2	2
12													4	1	2	4	1	2	1	3	2	2	5
13														1	1	2	4	4	2	3	3	3	4
14															15	3	0	2	2	1	3	2	3
15																3	1	1	3	1	6	2	3
16																	0	4	3	2	1	4	4
17																		5	4	1	6	4	3
18																			2	3	2	3	4
19																				1	1	2	2
20																					1	1	4
21																						3	1
22																							7
XY																							

References

- Cremer T, Cremer M. Chromosome territories. *Cold Spring Harb Perspect Biol.* 2010;2:a003889.
- Cremer M, von Hase J, Volm T, Brero A, Kreth G, Walter J, et al. Non-random radial higher-order chromatin arrangements in nuclei of diploid human cells. *Chromosom Res.* 2001;9:541–67.
- Boyle S, Gilchrist S, Bridger JM, Mahy NL, Ellis JA, Bickmore WA. The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. *Hum Mol Genet.* 2001;10:211–9.
- Küpper K, Kölbl A, Biener D, Dittrich S, von Hase J, Thormeyer T, et al. Radial chromatin positioning is shaped by local gene density, not by gene expression. *Chromosoma.* 2007;116:285–306.
- Hübner MR, Spector DL. Chromatin dynamics. *Annu Rev Biophys.* 2010;39:471–89.
- Sun HB, Shen J, Yokota H. Size-dependent positioning of human chromosomes in interphase nuclei. *Biophys J.* 2000;79:184–90.
- Parada LA, McQueen PG, Misteli T. Tissue-specific spatial organization of genomes. *Genome Biol.* 2004;5:R44.
- Zeitzi MJ, Marella NV, Malyavantham KS, Goetze S, Bode J, Raska I, et al. Organization of the amplified type I interferon gene cluster and associated chromosome regions in the interphase nucleus of human osteosarcoma cells. *Chromosom Res.* 2009;17:305–19.
- Cremer T, Cremer M, Dietzel S, Müller S, Solovei I, Fakan S. Chromosome territories—a functional nuclear landscape. *Curr Opin Cell Biol.* 2006;18:307–16.
- Cavalli G. Chromosome kissing. *Curr Opin Genet Dev.* 2007;17:443–50.
- Hübner MR, Eckersley-Maslin MA, Spector DL. Chromatin organization and transcriptional regulation. *Curr Opin Genet Dev.* 2013;23:89–95.
- Nguyen HQ, Bosco G. Gene positioning effects on expression in eukaryotes. *Annu Rev Genet.* 2015;49:627–46.
- Cremer M, Küpper K, Wagler B, Wizelman L, von Hase J, Weiland Y, et al. Inheritance of gene density-related higher order chromatin arrangements in normal and tumor cell nuclei. *J Cell Biol.* 2003;162:809–20.
- Li C, Shi Z, Zhang L, Huang Y, Liu A, Jin Y, et al. Dynamic changes of territories 17 and 18 during EBV-infection of human lymphocytes. *Mol Biol Rep.* 2010;37:2347–54.
- Meaburn KJ, Cabuy E, Bonne G, Levy N, Morris GE, Novelli G, et al. Primary laminopathy fibroblasts display altered genome organization and apoptosis. *Aging Cell.* 2007;6:139–53.
- Gerlich D, Ellenberg J. Dynamics of chromosome positioning during the cell cycle. *Curr Opin Cell Biol.* 2003;15:664–71.
- Walter J, Schermelleh L, Cremer M, Tashiro S, Cremer T. Chromosome order in HeLa cells changes during mitosis and early

- G1, but is stably maintained during subsequent interphase stages. *J Cell Biol.* 2003;160:685–97.
18. Thomson I, Gilchrist S, Bickmore WA, Chubb JR. The radial positioning of chromatin is not inherited through mitosis but is established de novo in early G1. *Curr Biol.* 2004;14:166–72.
 19. Codina-Pascual M, Navarro J, Oliver-Bonet M, Kraus J, Speicher MR, Arango O, et al. Behaviour of human heterochromatic regions during the synapsis of homologous chromosomes. *Hum Reprod.* 2006;21:1490–7.
 20. Sarrate Z, Blanco J, Vidal F. Acrocentric bivalents positioned preferentially nearby to the XY pair in metaphase I human spermatocytes. *Fertil Steril.* 2012;98:1241–5.
 21. Vergés L, Blanco J, Valero O, Vidal F, Sarrate Z. Chromosome size, morphology, and gene density determine bivalent positioning in metaphase I human spermatocytes. *Fertil Steril.* 2014;101:818–24.
 22. Hazzouri M, Rousseaux S, Mongelard F, Usson Y, Pelletier R, Faure AK, et al. Genome organization in the human sperm nucleus studied by FISH and confocal microscopy. *Mol Reprod Dev.* 2000;55:307–15.
 23. Zalenskaya IA, Zalensky AO. Non-random positioning of chromosomes in human sperm nuclei. *Chromosom Res.* 2004;12:163–73.
 24. Foster HA, Abeydeera LR, Griffin DK, Bridger JM. Non-random chromosome positioning in mammalian sperm nuclei, with migration of the sex chromosomes during late spermatogenesis. *J Cell Sci.* 2005;118:1811–20.
 25. Ioannou D, Meershoek EJ, Christopikou D, Ellis M, Thornhill AR, Griffin DK. Nuclear organisation of sperm remains remarkably unaffected in the presence of defective spermatogenesis. *Chromosom Res.* 2011;19:741–53.
 26. Mudrak OS, Nazarov IB, Jones EL, Zalensky AO. Positioning of chromosomes in human spermatozoa is determined by ordered centromere arrangement. *PLoS One.* 2012;7(12):e52944.
 27. Greaves IK, Rens W, Ferguson-Smith MA, Griffin D, Graves JAM. Conservation of chromosome arrangement and position of the X in mammalian sperm suggests functional significance. *Chromosom Res.* 2003;11:503–12.
 28. Zalensky A, Zalenskaya I. Organization of chromosomes in spermatozoa: an additional layer of epigenetic information? *Biochem Soc Trans.* 2007;35:609–11.
 29. Gardner R, Sutherland G, Shaffer L. Chromosome abnormalities and genetic counseling. New York: Oxford University Press; 2011.
 30. Mau-Holzmann UA. Somatic chromosomal abnormalities in infertile men and women. *Cytogenet Genome Res.* 2005;111:317–36.
 31. Burgoyne PS, Mahadevaiah SK, Turner JMA. The consequences of asynapsis for mammalian meiosis. *Nat Rev Genet.* 2009;10:207–16.
 32. Garagna S, Zuccotti M, Thornhill A, Fernandez-donoso R, Berrios S, Capanna E, et al. Alteration of nuclear architecture in male germ cells of chromosomally derived subfertile mice. 2001;4429–34.
 33. Wiland E, Zegala M, Kurpisz M. Interindividual differences and alterations in the topology of chromosomes in human sperm nuclei of fertile donors and carriers of reciprocal translocations. *Chromosom Res.* 2008;16:291–305.
 34. Acloque H, Bonnet-Garnier A, Mompert F, Pinton A, Yerle-Bouissou M. Sperm nuclear architecture is locally modified in presence of a Robertsonian translocation t(13;17). *PLoS One.* 2013;8:e78005.
 35. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. New York: Cambridge University Press; 1999.
 36. Templado C, Marina S, Coll MD, Egozcue J. Meiotic studies in human semen. Report of 180 cases. *Hum Genet.* 1980;53:335–9.
 37. Sarrate Z, Blanco J, Egozcue S, Vidal F, Egozcue J. Identification of meiotic anomalies with multiplex fluorescence in situ hybridization: Preliminary results. *Fertil Steril.* 2004;82:712–7.
 38. Cameron AC, Trivedi PK. Regression analysis of count data. 1998. Cambridge: University Press; 1998. p. 411.
 39. Cox TF, Cox MAA. Multidimensional scaling, 2nd ed. CRC Press; 2000.
 40. Luciani JM, Guichaoua MR, Mattei A, Morazzani MR. Pachytene analysis of a man with a 13q;14q translocation and infertility. Behavior of the trivalent and nonrandom association with the sex vesicle. *Cytogenet Cell Genet.* 1984;38:14–22.
 41. Guichaoua MR, Quack B, Speed RM, Noel B, Chandley AC, Luciani JM. Infertility in human males with autosomal translocations: meiotic study of a 14;22 Robertsonian translocation. *Hum Genet.* 1990;86:162–6.
 42. Navarro J, Vidal F, Benet J, Templado C, Marina S, Egozcue J. XY-trivalent association and synaptic anomalies in a male carrier of a Robertsonian t(13;14) translocation. *Hum Reprod.* 1991;6:376–81.
 43. Sciurano R, Rahn M, Rey-Valzacchi G, Solari AJ. The asynaptic chromatin in spermatocytes of translocation carriers contains the histone variant gamma-H2AX and associates with the XY body. *Hum Reprod.* 2007;22:142–50.
 44. Sciurano RB, Rahn MI, Rey-Valzacchi G, Coco R, Solari AJ. The role of asynapsis in human spermatocyte failure. *Int J Androl.* 2012;35:541–9.
 45. Kirkpatrick G, Ren H, Liehr T, Chow V, Ma S. Meiotic and sperm aneuploidy studies in three carriers of Robertsonian translocations and small supernumerary marker chromosomes. *Fertil Steril.* 2015;103:1162–9.e7.
 46. Homolka D, Ivanek R, Capkova J, Jansa P, Forejt J. Chromosomal rearrangement interferes with meiotic X chromosome inactivation. *Genome Res.* 2007;17:1431–7.
 47. de Laat W, Grosveld F. Inter-chromosomal gene regulation in the mammalian cell nucleus. *Curr Opin Genet Dev.* 2007;17:456–64.
 48. Armstrong SJ, Goldman AS, Speed RM, Hultén MA. Meiotic studies of a human male carrier of the common translocation, t(11;22), suggests postzygotic selection rather than preferential 3:1 MI segregation as the cause of liveborn offspring with an unbalanced translocation. *Am J Hum Genet.* 2000;67:601–9.
 49. Pigozzi MI, Sciurano RB, Solari AJ. Changes in crossover distribution along a quadrivalent in a man carrier of a reciprocal translocation t(11;14). *Biocell.* 2005;29:195–203.
 50. Ferguson KA, Chow V, Ma S. Silencing of unpaired meiotic chromosomes and altered recombination patterns in an azoospermic carrier of a t(8;13) reciprocal translocation. *Hum Reprod.* 2008;23:988–95.
 51. Leng M, Li G, Zhong L, Hou H, Yu D, Shi Q. Abnormal synapses and recombination in an azoospermic male carrier of a reciprocal translocation t(1;21). *Fertil Steril.* 2009;91:1293. e17–22.
 52. Laurie DA, Palmer RW, Hultén MA. Studies on chiasma frequency and distribution in two fertile men carrying reciprocal translocations; one with a t(9;10) karyotype and one with a t(Y;10) karyotype. *Hum Genet.* 1984;68:235–47.
 53. Goldman AS, Martin RH, Johannisson R, Gould CP, Davison EV, Emslie JE, et al. Meiotic and sperm chromosome analysis in a male carrier of an inverted insertion (3;10)(q13.2;p14p13). *J Med Genet.* 1992;29:460–4.
 54. Oliver-Bonet M, Navarro J, Codina-Pascual M, Abad C, Guitart M, Egozcue J, et al. From spermatocytes to sperm: meiotic behaviour of human male reciprocal translocations. *Hum Reprod.* 2004;19:2515–22.
 55. Sarrate Z, Vidal F, Blanco J. Meiotic abnormalities in metaphase I human spermatocytes from infertile males: frequencies, chromosomes involved, and the relationships with polymorphic karyotype and seminal parameters. *Asian J Androl* 16:838–44.
 56. McStay B, Grummt I. The epigenetics of rRNA genes: from molecular to chromosome biology. *Annu Rev Cell Dev Biol.* 2008;24:131–57.