Running title: Bivalent position in metaphase I

Title: Acrocentric bivalents positioned preferentially nearby to the XY pair in metaphase I human spermatocytes

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Capsule

Proximity frequencies of autosomic bivalents to the XY pair in metaphase I human spermatocytes were evaluated showing a non-random bivalent distribution and a relative position pattern notably preserved from pachytene to metaphase I.

Abstract

Objective: To analyze if the preferential proximity between acrocentric bivalents and the XY pair described at pachytene was maintained in metaphase I human spermatocytes.

Design: Proximity frequencies of autosomic bivalents to the sex bivalent were evaluated with the analysis of meiotic preparations combining sequentially standard techniques and Multiplex FISH.

Setting: Assisted Reproduction Centers and Universitat Autònoma de Barcelona.

Patient(s): 37 males consulting for fertility problems.

Intervention(s): Unilateral testicular biopsies.

Main Outcome Measure(s): Proximity frequencies analysis to the XY pair, evaluated individually and grouping bivalents was carried out using a logistical regression model with repeated measures.

Result(s): Bivalents 22 and 15 were observed more frequently near to the sex bivalent than the others. Significant interindividual differences were not observed.

Conclusions: Results suggest that bivalents distribution to the metaphase plate is non-random. The maintenance of the acrocentric chromosomes proximity to the sex bivalent from pachytene to metaphase I would indicate that the relative bivalents position would be notably preserved. The observation of non-interindividual variability, in spite of different infertility etiology, proposes that nuclear organization pattern remains largely unaffected even if spermatogenesis is compromised.

Key words: acrocentric chromosomes, chromosome territories, human spermatocytes, metaphase I, XY bivalent.

Introduction

It is generally accepted that chromosomes in the interphase nucleus are organized in distinct domains, called chromosome territories (CTs). This chromosomal location plays an important role in maintaining and regulating the genome functions (1). Several studies suggest that chromosomes are distributed in the nucleus according to size (2), gene density (3, 4), transcriptional activity, early or late replication of sequences of DNA and guanine-cytosine (GC) content (5). The CTs neighbourhood is different depending on tissue origin (6, 7), probably related to its functionality and necessary for maintaining imprints in cells (8).

In the sperm nucleus chromosomes are organized in distinct territories with non-random chromosome positioning (9). For instance, recent studies show evidences that centromeric and sex chromosome loci adopt specific nuclear positions towards the interior of the nucleus (10, 11). These features might be crucial because the chromosomal location could determine the time at which particular chromatin domains are decondensed and remodeled, allowing some epigenetic level of control or influence over subsequent paternal gene expression in the embryo (12-14). It has also been suggested that the topology of chromosomes in the sperm influences the position of chromosomes in the first mitotic division of the zygote (9, 14).

Several published articles including direct and indirect indications suggest that this nuclear organization would be altered in men with severely compromised spermatogenesis (14-17). However, a recent study suggests that this defined pattern of nuclear organization in sperm heads is a remarkably robust process because it remains primarily unaffected even in the presence of defective spermatogenesis (17).

In mammals, a temporal repositioning of CTs during spermatogenesis was proposed (9). Thus, gonosomes CTs are both peripherally located pre-meiosis, separating from one another and each repositioning to the nuclear center by the round spermatid stage. Unfortunately, only few articles analyze some preferentially relative chromosome positions in the early stages of human spermatogenesis. In this sense, two-dimensional studies of synaptonemal complexes spreads showed that the bivalent 15 was more frequently in close proximity to the pair XY than other autosomic bivalents (18, 19).

To go further in this observation, the aim of the present study was to analyze if this preferential proximity between acrocentric chromosomes and the XY pair at pachytene stage was maintained in the next stage of the spermatogenic process. In order to do this, proximity frequencies of autosomal bivalents to the sex bivalent were evaluated in metaphase I human spermatocytes.

Material and methods

Testicular tissue samples were obtained under local anesthesia from 37 males consulting for fertility problems. Samples were kept in an isotonic solution to 4ºC until the moment of its utilization. Protocol was approved by the Institutional Review Board (IRB) of the collaborating centres and the patients gave their informed consent with regard to participation in the study. The somatic karyotype and seminal parameters of these patients are detailed in Table 1.

Biopsied samples were processed according to the method of Evans et al. (21). Cellular spreads were evaluated following a sequential methodology combining Leishman

stained procedures and multiplex fluorescent in situ hybridization protocols (M-FISH) described previously by our group (22).

Briefly, Leishman stained slides were analyzed using an Olympus BX60 microscope (Olympus Optical España S.A., Barcelona, Spain) equipped with a capture and image analysis system CytoVysion 3.6. (Applied Imaging, Newcastle, UK). Metaphase I images from the primary spermatocytes were captured and the coordinates were noted in order to facilitate the location and analysis subsequent to M-FISH protocol. Before the application of the manufacturer's M-FISH protocol (Spectra VysionTM Assay Protocol, Vysis Inc., Downers Grove, IL), the slides were destained in an ethanol solution series in distilled water (70%, 80% and 90%).

Hybridized slide analyses were perform with an Olympus BX60 epifluorescence microscope equipped with a specific filter set to visualize Spectrum Aqua, Spectrum Fred, Spectrum Green, Spectrum Gold, Spectrum Red and DAPI. Capture and image analyses were carried out with a CytoVision system (CytoVysion 3.6., Applied Imaging, Newcastle, UK).

Conjoint analysis of Leishman staining and M-FISH images of the same metaphase I were used to identify to which chromosome each chromosomal unit observed belonged. This information was used to determine which bivalents were nearest to the sex bivalent being part of what we call the "first ring". The "first ring" was conformed by those bivalents located in the first line regarding the XY bivalent regardless of the distance (Figure 1).

For the statistical analysis, and in order to analyze if any bivalent was more frequently near to the sex bivalent than others, an indicative variable was created. Value "1" was assigned when the bivalent was considered near (in the “first ring”) and value "0" when it was not considered near (out of the “first ring”). This proximity analysis was carried out using the following software: SAS v9.1 (SAS Institute Inc., Cary, NC) and SPAD v4.5 (Centre International de Statistiques et d’Informatique Appliquées, Saint Mandé, France). The level of statistical significance was established to 0.05.

The proximity analysis of each bivalent was carried out using a logistical regression model (23) with repeated measures considering the different bivalents as an “explanatory variable”. From the model established the odds ratio was calculated, which represented the risk that a bivalent had to be near to the sex bivalent in relation to the others. In order to check the significance degree of the differences, pair wise comparisons between each bivalent concerning all the others using χ^2 test were done. Moreover, differences of the proximity were analyzed taking in consideration the seven groups of the human karyotype. Accordingly it was necessary to recode the explanatory variable “bivalent” as follow: group A (bivalents 1, 2 and 3), group B (bivalents 4 and 5), group C (bivalents 6, 7, 8, 9, 10, 11 and 12), group D (bivalents 13, 14 and 15), group E (bivalents 16, 17 and 18), group F (bivalents 19 and 20) and group G (bivalents 21 and 22). For each group of bivalents, the percentage of bivalents near to the sex bivalent was calculated. After that, the same statistical model and test used for individual bivalents analysis was applied.

Interindividual variability regarding the proximity between different groups of bivalents and the XY pair were statistically evaluated with a multivariate analysis of multiple correspondences.

Results

A total of 481 metaphases I were evaluated. In 16.2% (6/37) of the individuals, no primary spermatocyte in this stage was observed. All chromosomal units were identified in the 85.7% (412/481) of the cells analyzed, while in the remaining some chromosomal units were not informative (14.3%; 69/481).

From the 412 informative metaphases I, the 67.7% (279/412) showed all chromosomes paired forming bivalents. A percentage of 21.8% (90/412) showed 22 autosomic bivalents plus unpaired XY. The remaining 10.5% include metaphases classified as a hypoploid, tetraploid or metaphases with totally achiasmate bivalents. Therefore, autosomic bivalents nearby to the sex bivalent were identified in the 279 metaphases I where all the chromosomes were paired. The averages of the "0" and "1" values assigned to each bivalent are shown in Table 2.

All autosomic bivalents were observed close to the sex bivalent in any of the metaphases I analyzed. The bivalents 15, 22, 14, 7 and 21 were the bivalents with higher values. The statistical analysis showed significant probabilities for bivalents 22 and 15 ($p = 0.011$ and $p = 0.014$), respectively.

On the other hand, the proximity results obtained from the groups of bivalents established from chromosomes with similar size and morphology were shown in Table 3. Although D and G groups presented the highest values, significant differences were only shown in B and G groups. Group B, which included bivalents 4 and 5, for the absence of proximity to the sex bivalent ($p = 0.038$), and G group, which included

chromosomes 21 and 22, for to be near more frequently ($p = 0.030$). Significant interindividual differences were not observed.

Discussion

The acrocentric bivalents 15 and 22 were observed near the sex bivalent more often than the others, indicating that bivalent distribution in the metaphase plate is non-random. This result is coincident with SC studies describing that bivalent 15 is more frequently nearby to the XY pair than other bivalents (18, 19).

It has been described that the non-centromeric heterochromatin of chromosome 15 presents traces of homology with the non-centromeric Y-chromosome heterochromatin (24). Moreover, some regions of the short arm of chromosome 15 also present homology with Xq/Yq subtelomeric regions (25). Moreover, it has established that material of the short arm of chromosome 15 has been detected in some satellited Y chromosomes (Yqs) (26-28) and also in the short arms of chromosomes 13, 14 and 21 as an inherited polymorphism (26, 29, 30). This homology between the heterochromatin regions of chromosomes 15 and Y, and the fact of part of this material can be also present in other acrocentric chromosomes, could explain the proximity of the bivalents formed by acrocentric chromosomes to the sex bivalent at the pachytene stage (18, 19).

Some indirect data also support the preferential proximity location of bivalents 15 and 22 to the sex bivalent. Revising translocation cases involving the Y chromosome and any autosome, 70% of the cases are produced between the heterocromatin Yq and the short arm of an acrocentric chromosome (31). In such translocations, chromosome 15 is most frequently involved (52%), followed by chromosome 22 (33%), chromosome 21

(7%) and chromosomes 13 and 14 (4% each) (32). These percentages probably reflect the differing degree of homology between satellite DNA sequences of these chromosomes (30); being 15p, 22p and Yq those that share a greater homology (33).

The maintenance of this closeness from pachytene to metaphase I would indicate the preservation of the relative bivalents position, in spite of the formation of the metaphase plate. It is important to remark that there were no significant interindividual differences regarding the bivalents proximity to the XY pair, despite infertility etiology of each patient. This observation is consistent with recent results obtained by Ioannou et al. (11) suggesting that the nuclear organization pattern of centromeric loci in sperm nuclei is a remarkably robust process because it remains largely unaffected even if spermatogenesis is severely compromised. To determine if there is a relative positioning pattern of all chromosomes along meiosis and the establishment of the variables might take part in this organization could be especially interesting to understand chromosome territoriality behavior during spermatogenesis.

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Figure Legends

Table 1. Somatic karyotype and sperm parameters of the individuals studied.

A: Asthenozoospermia, AT: Asthenoteratozoospermia, Az: Azoospermia, N: Normozoospermia, OA: Oligoasthenozoospermia, OAT: Oligoasthenoteratozoospermia, T: Teratozoospermia.

*Classification carried out in accordance with the World Health Organization criteria (20)

Figure 1. Metaphase I from a human testicular biopsy. **(A)** Leishman staining. **(B)** M-FISH image. **(C)** M-FISH karyotype. **A and B:** Identification of bivalents included in the first ring around the sex bivalent (indicated with an arrowhead): 4, 7, 9, 11, 17, 20 and 22.

Table 2. Descriptive statistics of the proximity analysis.

*For the statistical analysis the weighted mean values have been used.

BV: bivalent.

Table 3. Percentage of bivalents belonging to a specific chromosomal group which are located near to the XY pair.

Patient code	Somatic Karyotype	Sperm parameters*	Patient code	Somatic Karyotype	Sperm parameters*
072	46,XY	T	**314**	46,XY	N
118	46,XY	OA	**315**	46,XYqh+,inv9(p12q12)	Az
140	46,XY	AT	**321**	46,XY	N
142	46,XY	AT	**323**	46,XY	A
282	46,XY	AT	**328**	46,XY	OAT
284	46,XY	Az	**331**	46,XY	OAT
285	46,XY,14ps+,15ps+	Az	**360**	46,XY	OAT
287	46,XYqh+	N	**361**	46,XY	OAT
289	46,XY	A	**392**	46,XY	OAT
291	46,XY	AT	**6837**	46,XY	OAT
299	46,XY,inv9(p11q12)	N	**6854**	46,XY	OAT
300	46,XY	A	**6858**	46,XY	OAT
301	46,XY	A	**6859**	46,XY	OAT
302	46,XY	N	**6866**	46,XY	OAT
307	46,XY	A	**6867**	46,XY	OAT
308	46,XY	OAT	**8345**	46,XY	OAT
309	46,XY	OAT	**8362**	46,XY	OAT
310	46,XY	OA	**8514**	46,XY	OAT
312	Non evaluated	Az			

Table 1.

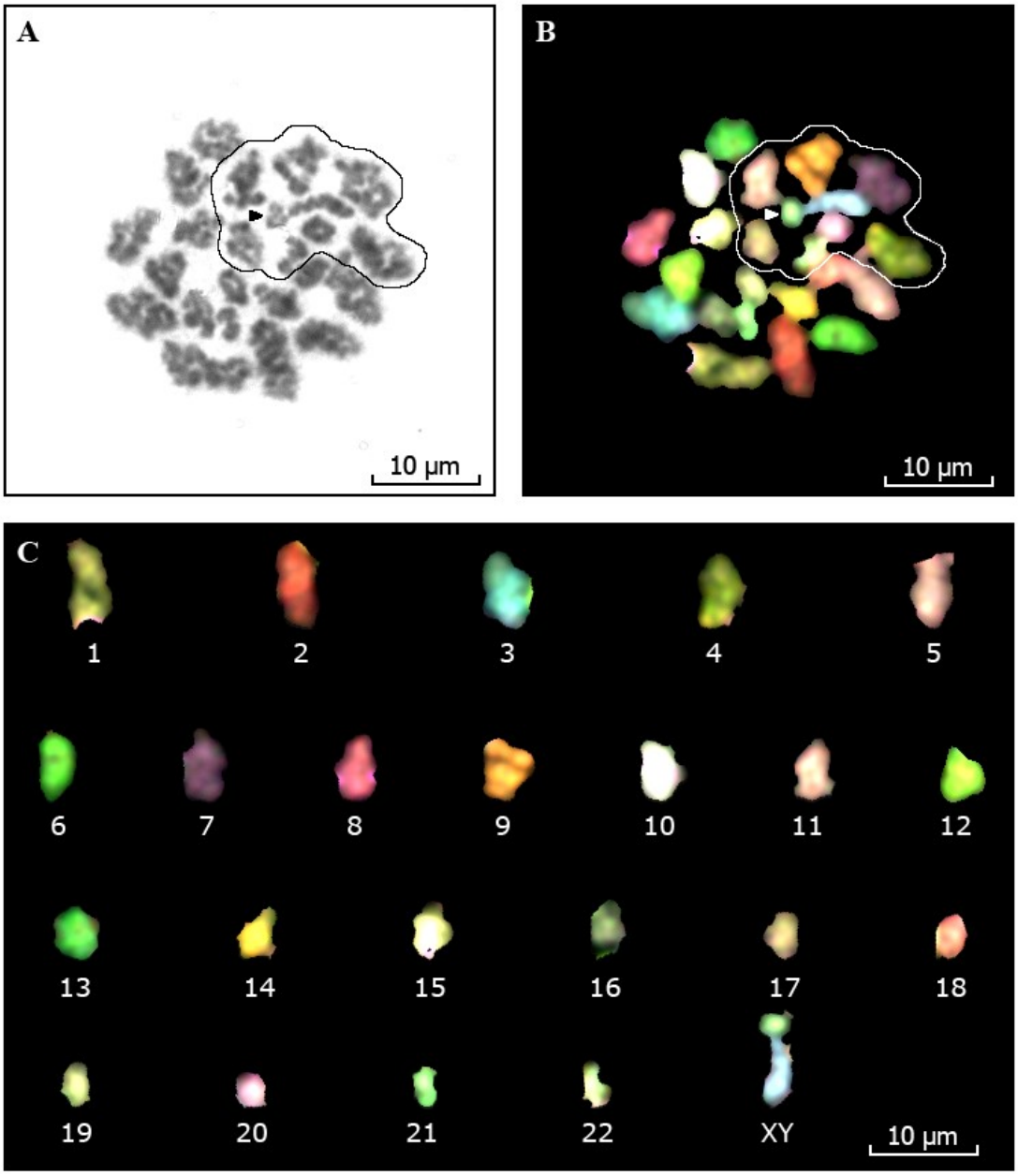
A
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Figure 1.

	BV1	BV2	BV3	BV4	BV5	BV6	BV7	BV8	BV9	BV10	BV11	BV12	BV13	BV14	BV15	BV16	BV17	BV18	BV19	BV20	BV21	BV22
Mean	0.23	0.27	0.20	0.23	0.22	0.31	0.31	0.22	0.24	0.27	0.25	0.26	0.22	0.29	0.32	0.28	0.25	0.23	0.27	0.28	0.28	0.37
Standard deviation	0.18	0.22	0.18	0.20	0.17	0.21	0.20	0.18	0.17	0.17	0.18	0.19	0.18	0.16	0.23	0.19	0.20	0.22	0.23	0.22	0.17	0.25
Maximum	0.55	1.00	0.50	1.00	0.50	1.00	0.67	0.67	0.67	0.67	0.67	0.75	0.50	0.67	1.00	1.00	0.67	1.00	1.00	1.00	0.75	1.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Weighted mean*	0.26	0.26	0.23	0.23	0.24	0.26	0.29	0.22	0.24	0.28	0.28	0.24	0.25	0.30	0.33	0.26	0.26	0.22	0.26	0.25	0.29	0.33

Table 2.

Group	Bivalents	Percentage of bivalents
A	1,2,3	25
B	4,5	24
C	6,7,8,9,10,11,12	26
D	13,14,15	29
E	16,17,18	25
F	19,20	25
G	21,22	31

Table 3.