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LETTER TO THE EDITOR

Reproduction

Chromosome heteromorphisms: do they entail a reproductive risk for male carriers?

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Dear Editor,

Chromosome heteromorphisms are described as variations in size and morphology at specific regions that can be detected through classical banding methods. They are mitotically stable variants usually present in a heterozygous state (only one of the homologous chromosomes is heteromorphic). In humans, the most commonly detected heteromorphisms involve the heterochromatic regions of chromosomes 1, 9, 16, and Y (designated as 1qh, 9qh, 16qh, and Yqh, respectively), and the short-arms, satellites, or stalks of the acrocentric chromosomes 13, 14, 15, 21, and 22 (*e.g.*, for chromosome 13 designated as 13p, 13ps, and 13pstk, respectively). Pericentric inversions involving the heterochromatic region of chromosomes 1, 9, and Y are also frequently observed.[1]

Heteromorphisms are not associated with any phenotypic alteration.[2] Nevertheless, several pieces of data suggest an association between the presence of heteromorphic variants and infertility. Moreover, the presence of a heteromorphism in one member of an infertile couple appears to have a detrimental effect on the outcome of assisted reproduction treatments and has been related to an increased frequency of miscarriage.[3]

The underlying mechanisms behind the relationship between male infertility and the presence of such chromosomal variants are not fully understood. One of the current hypotheses has been related to a possible deleterious effect of the heteromorphisms on meiotic chromosome pairing and segregation. In this sense, some studies have shown that heteromorphisms in a heterozygous state can disturb homologous chromosome pairing during prophase I.[4–6] This situation has been related to abnormal recombination that could promote chromosome missegregation during meiosis,[5,6] leading to higher frequencies of sperm aneuploidies.[7–10]

In this study, we provide additional information about the impact of chromosomal variants on the production of sperm chromosomal aneuploidies in carriers of different chromosome heteromorphisms.

Semen samples from 16 infertile heteromorphism carriers were obtained (**Table 1**). Written informed consent was obtained from all carriers, and the study was approved by the Ethics Committee on Animal and Human Experimentation of the Autonomous University of Barcelona, Bellaterra (Cerdanyola del Vallès), Spain (reference number: 1883). Spermatozoa were spread on slides and processed for fluorescence *in situ* hybridization (FISH) following the four main steps of the methodology:[11] sperm chromatin decondensation (5 mmol l^{-1} dithiothreitol solution, Merck, Darmstadt, Germany), DNA denaturation (70% formamide solution, Merck), probe and target DNA hybridization, and posthybridization washes (sodium citrate solution, Abbott Molecular Inc., Des Plaines, IL, USA). In each case, sperm diploidies and aneuploidies for chromosomes 1, 9, 13, 18, 21, 22, X, and Y were evaluated by performing three hybridizations in parallel with different combinations of probes (Abbott Molecular Inc., Des Plaines, IL, USA). The first probe combination included three Chromosome Enumeration Probes (CEP): CEP18 (D18Z1, Spectrum Aqua), CEPX (DXZ1, Spectrum Green), and CEPY (DYZ3, Spectrum Orange); the second probe combination included two Locus-Specific Identifier (LSI) probes: LSI 13 (13q14, Spectrum Green) and LSI 21 (21q22.13–21q22.2, Spectrum Orange); and probe combination three included Tel1q (VIJyRM2123, Spectrum Orange), CEP9 (9p11q11 alpha satellite, Spectrum Aqua), and LSI22 BCR (22q11.2, Spectrum Green). This broad panel was established to cover those chromosomes affected by heteromorphisms in the study population, as well as the most prone chromosomes to produce sperm with numerical imbalances in infertile patients.[12] Sperm chromosome analysis was done using an Olympus BX60 epifluorescence microscope (Olympus Iberia, L'Hospitalet de Llobregat, Spain) equipped with filter sets for fluorescein isothiocyanate (FITC), Texas Red, Aqua, and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI)/Texas Red/FITC using standard assessment criteria.[13] We analyzed 2000 spermatozoa per probe combination giving a total number of 6000 spermatozoa per individual. Sperm aneuploidy and diploidy data from each heteromorphic carrier were compared using a Fisher's exact test with internal cutoff values obtained from a control population constituted by six fertile individuals with normal karyotypes and normal seminal parameters (**Table 1**).

Among the 16 heteromorphism carriers analyzed, 11 (68.8%) presented significantly increased rates of numerical abnormalities for at least one of the analyzed chromosomes (**Table 1**). This result suggests the existence of a genetic reproductive risk associated with this population, supporting previously published data.[7–10] We would like to highlight the high heterogeneity regarding the chromosomes with significant aneuploidy increases among the carriers of the same chromosome heteromorphism. This is clearly observable in inv(9) carriers, which include cases without any significant increase (*i.e.*, 940z) and carriers presenting significant increases affecting up to three chromosomes (*i.e.*, 929z) (**Table 1**).

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Table 1: Individuals included in the study. In every case, karyotype, seminogram, age, and frequencies of chromosome anomalies detected are indicated

Carrier	Karyotype	S	Age (year)	Probe combination 1					Probe combination 2					Probe combination 3							Σ
				Dis 18	Nul 18	Dis sex chr	Nul sex chr	Dip	Dis 13	Nul 13	Dis 21	Nul 21	Dip	Dis 1	Nul 1	Dis 9	Nul 9	Dis 22	Nul 22	Dip	
917z	46,XY,Yq-	A	33	0.00	0.10	0.30	0.20	0.10	0.00	0.05	0.15	0.00	0.10	0.05	0.10	0.05	0.05	0.05	0.10	0.15	0
919z	46,XY,9qh+	N	27	0.05	0.05	0.50#	0.15	0.00	0.10	0.20	0.10	0.10	0.20	0.24	0.00	0.00	0.14	0.29#	0.00	0.19	2
924z	46,XY,Yqh+	N	36	0.10	0.05	0.25	0.25	0.05	0.00	0.10	0.05	0.15	0.25	0.20	0.10	0.05	0.05	0.05	0.00	0.10	0
923z	46,XY,22ps+	N	46	0.05	0.30#	0.30	0.15	0.15	0.10	0.20	0.10	0.10	0.10	0.05	0.00	0.34#	0.34#	0.20#	0.05	0.20	4*
925z	46,XY,13pss	N	30	0.00	0.00	0.15	0.00	0.60#	0.00	0.05	0.05	0.10	0.30	0.15	0.05	0.05	0.25#	0.05	0.15	0.05	2
911z	46,XY,inv(1qh)	N	31	0.00	0.15	0.10	0.20	0.15	0.00	0.05	0.15	0.00	0.10	0.30#	0.34#	0.05	0.25#	0.15#	0.10	0.25	4*
927z	46,XY,inv(9)(p11q13)	N	35	0.00	0.05	0.20	0.15	0.05	0.20#	0.20	0.10	0.00	0.10	0.30#	0.05	0.15	0.20	0.10	0.10	0.25	2
928z	46,XY,inv(9)(p11q13)	N	32	0.00	0.05	0.25	0.25	0.10	0.10	0.10	0.05	0.25	0.10	0.15	0.15	0.00	0.15	0.05	0.00	0.20	0
931z	46,XY,inv(9)(p11q13)	N	34	0.00	0.25#	0.30	0.25	0.00	0.10	0.25	0.15	0.05	0.00	0.15	0.10	0.25#	0.00	0.10	0.10	0.00	2*
939z	46,XY,inv(9)(p11q13)	N	35	0.05	0.00	0.30	0.15	0.15	0.00	0.05	0.15	0.05	0.10	0.15	0.05	0.20	0.00	0.15#	0.00	0.00	1
935z	46,XY,inv(9)(p11q13)	T	30	0.05	0.25#	0.25	0.15	0.10	0.05	0.20	0.10	0.15	0.15	0.10	0.25#	0.00	0.00	0.05	0.00	0.05	2
937z	46,XY,inv(9)(p11q13)	N	48	0.15#	0.10	0.15	0.05	0.10	0.10	0.05	0.05	0.00	0.05	0.00	0.10	0.15	0.05	0.10	0.05	0.05	1
940z	46,XY,inv(9)(p11q13)	N	37	0.00	0.05	0.05	0.10	0.20	0.10	0.15	0.05	0.10	0.15	0.00	0.10	0.15	0.05	0.05	0.15	0.05	0
929z	46,XY,inv(9)(phqh)	O	29	0.10	0.05	0.10	0.05	0.25	0.30#	0.05	0.15	0.10	0.45	0.10	0.10	0.45#	0.00	0.20#	0.00	0.25	3*
932z	46,XY,inv(9)(phqh)	N	39	0.00	0.15	0.20	0.05	0.05	0.10	0.05	0.10	0.10	0.05	0.15	0.00	0.20	0.00	0.05	0.05	0.05	0
938z	46,XY,inv(9)(phqh)	N	39	0.10	0.25#	0.15	0.10	0.05	0.05	0.00	0.15	0.10	0.10	0.16	0.05	0.16	0.16	0.05	0.05	0.00	1
Control	46,XY	N	20–25	0.03	0.07	0.07	0.53	0.13	0.04	0.18	0.04	0.13	0.15	0.03	0.03	0.02	0.03	0.03	0.03	0.07	

#Significantly increased frequencies compared to the control population (Fisher's exact test, $P<0.05$); *carriers with significantly increased aneuploidy rates for the chromosome involved in the heteromorphic form. Dip: diploid; Dis: disomic; Nul: nullisomic; Chr: chromosome; A: asthenozoospermia; T: teratozoospermia; N: normozoospermia; O: oligozoospermia; S: seminogram; Σ: number of chromosomes with increased frequencies

It is also noticeable that in 4 (36.4%) of the 11 individuals with increased frequencies of numerically abnormal sperm, one of the chromosomes involved in the aneuploidies was the heteromorphic chromosome itself (cases 923z, 911z, 931z, and 929z; **Table 1**). Similar to our results, a previous sperm-FISH study performed on a carrier of a heteromorphic chromosome 9 inversion detected increased frequencies of sperm with numerical abnormalities for several chromosomes also including the rearranged chromosome itself.[10] All these data reinforce the hypothesis that in some cases, the establishment of heterosynapsis during prophase I might entail subsequent nondisjunction events at anaphase I that would affect both the segregation of the rearranged chromosomes and the segregation of other bivalents. This bidirectional phenomenon has been previously described in carriers of chromosomal translocations,[14,15] and this article extends its occurrence to heteromorphism carriers.

In the remaining 7 (63.6%) individuals with altered sperm FISH results in which the heteromorphic chromosome was unaffected by these increases, chromosomes 18 and 22 were the most frequent sperm anomalies detected (**Table 1**). This finding argues in favor of the participation of other factors besides heterosynapsis in the production of such numerical anomalies. That is, in a scenario of meiotic disturbances produced by the heteromorphism with other unsynapsed regions, X and Y chromosomes (which contain large nonhomologous segments) would be among the most suitable candidates involved.[16] Thus, a preferential increase in the incidence of sex chromosome aneuploidies should be expected. Nevertheless, only one individual (919z) showed increased incidences of sex chromosome aneuploidies.

Other factors besides heterosynapsis may also influence the frequency of chromosome imbalances in sperm. For example, abnormal seminal parameters have been generally associated with the presence of increased frequencies of sperm aneuploidies.[12] Accordingly, one could say that the increased sperm aneuploidies observed in some cases would be the consequence of an abnormal seminogram, rather than a heterosynapsis phenomenon derived from the presence of a heteromorphic form. However, in our study, only two of the 11 individuals (*i.e.*, 935z, 929z) that displayed increased frequencies of abnormal sperm had an altered seminogram (**Table 1**). This low incidence of seminal anomalies among the carriers analyzed also agrees with other previous studies which indicated that the presence of heteromorphisms is not something directly related to the presence of altered seminal parameters.[7]

Another factor that has been suggested to have a possible influence in the production of aneuploid/diploid sperm is paternal age. Up to present, controversial data have been obtained in this area with some studies reporting a correlation of age with certain types of numerical abnormalities while others do not observe such effect.[17] In our study, individuals without altered FISH results ranged 32–39 years old, while individuals with increased frequencies of aneuploid/diploid sperm ranged 27–48 years old. The presence of such a broader age range in individuals with higher ratios of abnormal sperm does not support an effect of this factor on the production of sperm chromosomal abnormalities by heteromorphisms carriers.

Regardless of the origin of the observed sperm alterations, published data suggest a detrimental effect of heteromorphisms on assisted reproductive treatments[8,18–21] Ultimately, this means that in reproductive counseling, significant differences in sperm chromosome anomaly rates should be taken into consideration. Accordingly, we suggest that a risk assessment through sperm FISH should be offered to these individuals, at least for cautionary purposes. Future studies examining larger populations of heteromorphism carriers would be of high interest to divide the individuals into "at-risk" and "without-risk" categories and clarify the effects of these variants over assisted reproductive treatments.

AUTHOR CONTRIBUTIONS

EA, MS, and JB conceived, designed, and coordinated the study. EGG and MS were involved in sample collection. MRM and AG carried out the sperm FISH analysis. EA, EGG, MRM, AG, MS, and JB were

involved in data analysis and interpretation; EA and JB wrote the manuscript with support from EGG and MS. All authors provided critical feedback, read, and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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