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An Analysis of Human Sperm Chromosome Breakpoints

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Summary

Sperm chromosome analysis of 19 sperm donors with either normal or balanced karyotypes was carried out in order to explore the nature of sperm chromosome structural aberrations. A total of 2,389 cells (range 36–298/donor) were karyotyped after in vitro penetration of hamster eggs. The median percentage of sperm structural aberrations was 9.3% (SD ± 4.7; range 0%–17.8%), with a total of 247 breakpoints, of which 220 could be characterized fully. Two sets of donors were studied in two different centers: center 1 (United States) and center 2 (Spain). The frequencies of nonrejoined and rejoined chromosome-type aberrations were very similar between center 1 and center 2: 83.6% and 10.0%, and 75.0% and 10.3%, respectively. Chromatid-type aberrations were more frequent in center 2 (14.7%) than in center 1 (6.4%) ($P = .037$). Chromosome 4 had less than the expected number of breakpoints ($P < .001$). A positive significant correlation was found between sperm breakpoints reported in this study and sites of balanced chromosome de novo rearrangements detected at prenatal diagnosis and reported in the literature ($P = .0001$).

Introduction

In a study of the origin of de novo structural chromosome rearrangements using chromosome heteromorphisms, Olson and Magenis (1988), found that 27 (84%) of 32 cases were paternal in origin. They suggested that environmental insults to a testis undergoing DNA replication and sperm production could be an explanation for the excess of paternally derived de novo structural chromosome rearrangements.

A lack of DNA repair in spermatid and spermatozoa may increase the vulnerability of sperm DNA to damaging agents. It is known that a wide variety of agents damage sperm chromatin, including ionizing radiation (Brandriff et al. 1988), alkylating agents (Matsuda and Tobari 1988), disrupters of nucleotide pools (Ehling and Neuhauser-Klaus 1988*b*), DNA-repair inhibitors (Matsuda and Tobari 1989), and antineoplastic agents (Ehling and Neuhauser-Klaus 1988*a*).

A high frequency of sperm chromosome structural aberrations has been described in normal human males. Taking advantage of the interspecific fusion of hamster egg/human sperm (Rudak et al. 1978), several investigators have reported incidences of structural aberrations, with the majority of donors having 5%–10% of sperm cells with structural aberrations. When intraspecific systems were used to study mouse sperm chromosomes (Santaló et al. 1986) and Chinese hamster-sperm chromosomes (Mikamo and Kamiguchi 1983), much lower incidences of structural aberrations were reported in these two species.

Sperm chromosome structural aberrations may originate at different times during spermatogenesis, after completion of this process and before sperm-egg fusion, or during culture of sperm pronuclei. It has been hypothesized (Brandriff et al. 1988) that translocations, inversions, insertions, marker chromosomes, and small deletions could originate early in spermatogenesis, at the stem-cell stage and, because they are stable rearrangements, could persist during spermatogenesis and spermiogenesis and could ultimately be recovered in the egg. Ring chromosomes, dicentrics, and large chromosome breaks and fragments could only arise after meiosis II; otherwise, they would have been lost from cells at earlier cell divisions. Sperm chromatid-type events would be formed during DNA synthesis in the egg.

The purpose of this paper is to characterize and classify sperm chromosome structural aberrations found in two sets of sperm donors, who include normal men and male heterozygotes for structural rearrangements. A total of 2,389 cells gave an incidence of sperm structural aberrations of 9.3%, with a total of 247 breakpoints. Two hundred twenty breakpoints could be assigned to a two- or three-digit band and, therefore, be fully characterized. The two sets of donors were studied in two different centers. Both institutions used the hamster egg/sperm fusion technique with slight adaptations for each laboratory. The nature of the differences in some sperm structural aberrations found in the two centers of study is explored and the ade-

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Table 1

General Characteristics of the Sample

Donor[a]	Age[b] (years)	No. of Cells	No. (%) of Cells with Structural Abnormalities[c]	95% Confidence Intervals[d] Low (%)	95% Confidence Intervals[d] High (%)
1	24	199	9 (4.5)	3	5.9
2	37	142	5 (3.5)	1.9	5.0
3	30	84	7 (8.3)	5.3	11.3
4	25	92	3 (3.3)	1.4	5.1
5	36	53	2 (3.8)	1.1	6.3
6	34	36	0	0	7.6
7	28	298	26 (8.7)	7	10.3
8	31	205	24 (11.7)	9.4	13.7
9	38	152	19 (12.5)	9.8	15.1
10	33	148	15 (10.1)	7.6	12.6
11	18	281	40 (14.2)	12.1	16.3
12	38	115	13 (11.3)	8.3	14.2
13	40	90	16 (17.8)	13.7	21.8
14	37	51	5 (9.8)	5.6	13.9
15	40	73	3 (4.1)	1.7	6.4
16	34	50	7 (14.0)	9.0	18.9
17	26	75	7 (9.3)	5.9	12.6
18	32	105	16 (15.2)	11.7	18.7
19	38	140	21 (15.0)	11.9	18
Total		2,389			

[a] Donors 12–19 are translocation carriers. Donors 1–7 and 12–16 are from center 1; and donors 8–11 and 17–19 are from center 2.

[b] Median age was 32.5 years.

[c] Median percentge of cells with structural abnormalities was 9.3%.

[d] Calculated for the percentage of structural abnormalities.

quacy of the technique in providing sperm chromosome data is discussed. Correlations between the locations of sperm breakpoints and fragile sites and breakpoints of de novo rearrangements in humans are explored.

Material and Methods

Semen samples were obtained from 11 normal men (donors 1–11); six carriers of reciprocal translocations—t(1;4)(p36.2;q31.3) (donors 12 and 13), t(3;15)(q26.2;q26.1) (donor 14), t(10;12)(q26.1;p13.3) (donor 15), t(5;7)(q13;p15.1) (donor 16), t(2;5)(p11;q15) (donor 17), and t(1;2)(q36;q32) (donor 18); and one carrier of a pericentric inversion, inv(7)(p13q36) (donor 19). All except donor 4 were reported to be free of exposures to any known mutagens, clastogens, radiation, or recreational or medical drugs. Donor 4 had used LSD for 5 years prior to sperm donations, but he had been free of drug exposure for 1 year before the study began. Sperm chromosome studies of donors 1–10, 12, 13, and 17–19 have already been reported elsewhere (Templado et al. 1988, 1990; Benet et al. 1991, 1992; Estop et al. 1991, 1992; Navarro et al. 1993). There were no men studied at both centers. The age of the semen donors at the time of producing the samples was 18–40 years. Donors 1–7 and 12–16 were studied in center 1, and donors 8–11 and 17–19 were studied in center 2.

Sperm chromosomes were obtained after culture of zona-free hamster oocytes penetrated by human sperm, according to the methods described by Martin (1983) and modified by Benet et al. (1989) and Estop et al. (1991). Sperm metaphases were analyzed after solid Leishman's stain and were sequentially reanalyzed after G-banding (center 2) or were directly G-banded (center 1). The scoring criteria followed the classification of chromosome aberrations as described by the ISCN (1985), with the following adaptations: (1) Chromatid gaps were recorded only when their width was more than the width of one chromatid. (2) Centromere gaps were not recorded, as they appear to be rather common events in this system. The gaps vary in length even among chromosomes of the same spread and are not restricted to chromosomes with variable heterochromatic regions. (3) The centromeric area of each chromosome (bands p11 and q11) was considered as one band, because of the difficulty in assigning breaks to either p11 or q11. All the analyses and comparisons between sperm

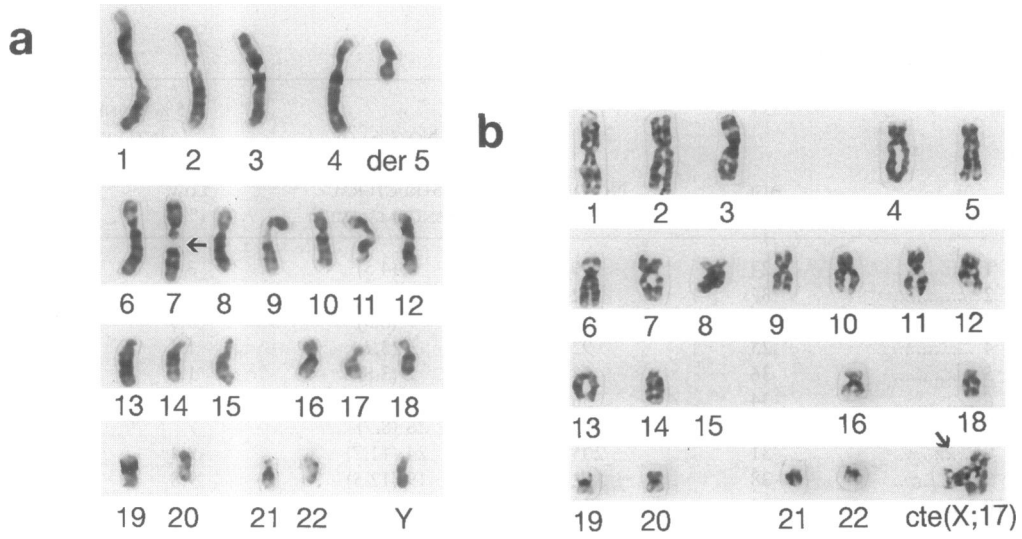

Figure 1 *a*, Spermatozoa with a 23,XY,−5+der(5)t(5;7)(q13;p15.1),csb(7)(q21) karyotype. *b*, Spermatozoa with a 21,−15,cte(X;17)(q27.2;p13.3) karyotype.

breakpoints/fragile sites and sites of de novo rearrangements were carried out at the ~400-band karyotype (ISCN 1985). Three hundred ninety-four bands were reduced to 370 because of the simplification of considering the centromeres (p11 and q11) one single band. For correlation between breakpoints of sperm chromosome rearrangements and fragile sites described for human lymphocytes, the fragile sites described by Human Gene Mapping 11 (1991) were used. Correlations between sperm breakpoints and the breakpoints of de novo chromosome rearrangements were carried out, using the data of Warburton (1991) on de novo rearrangements detected at prenatal diagnosis. Statistical analyses are described as they appear in the text.

Results

Table 1 summarizes age, number of cells analyzed per donor, and frequency of cells with structural aberrations found in the 2,389 sperm cells from 19 donors analyzed. A single cell may contain more than one structural aberration. The number of cells analyzed per donor was between 36 (donor 6) and 298 (donor 7). The percentage of cells with structural aberrations ranged from 0 (donor 6) to 17.8 (donor 13), with a median percentage of 9.3 (SD ± 4.97). The observed percentages of cells with structural aberrations were found to be normally distributed (Shapiro and Wilk 1965). However, there was a statistical difference between the median rate of structural aberrations for donors studied in center 1 and the median value for donors studied in center 2 (P = .018). This was a reflection of difference in the median aberration rates between normal donors (center 1 = .038; center 2 = .121; P = .019). Comparison of data for translocation carriers shows that such differences do not exist. Center 1's rate for abnormal translocation carriers is .113, and the rate for center 2 is .151 (P = .665). Ninety-five percent confidence intervals were calculated for the percentage of structural aberrations in each individual donor and are included in table 1.

Structural abnormalities were classified as nonrejoined and rejoined. Nonrejoined structural abnormalities consist of such chromosome aberrations as breaks, gaps, deletions, and, mostly, acentric fragments. Chromatid breaks, gaps, deletions, and chromatid exchanges are nonrejoined chromatid-type aberrations. Inversions, translocations, insertions, dicentrics, rings, and markers are rejoined chromosome structural abnormalities (fig. 1).

Table 2 depicts the sperm chromosome structural aberrations as classified according to type and to center of study. Seventy-eight percent of sperm structural anomalies are nonrejoined chromosome-type aberrations, chromosome breaks being the most frequent type of anomaly found (37.4%), followed by chromosome fragments, which appear most of the time as acentric (23%). There are no statistical differences between the frequencies of nonrejoined chromosome aberrations found in center 1 (83.6%) and those found in center 2 (75%).

Rejoined-type aberrations are less frequent (10.2%). No statistical differences were observed between the frequencies of rejoined-type events of center 1 (10.0%) and those of center 2 (10.3%). It is interesting to note the remarkable similarities between the frequencies of inversions, translocations, insertions, dicentrics, rings, and chromosome markers found in the two independent centers (see table 2).

As far as chromatid-type aberrations—namely, chromatid breaks, gaps, deletions, and exchanges—are concerned, center 1 found an incidence of 6.4%, and center 2 found a higher incidence, 14.7% (P = .037). Although the fre-

Table 2

Classification of Structural Abnormalities

Structural Abnormality	No. (%)		
	Center 1	Center 2	Total
Nonrejoined:			
Chromosome:			
Breaks	50 (45.4)	60 (32.6)	110 (37.4)
Gaps	9 (8.2)	22 (12.0)	31 (10.5)
Fragments	23 (20.9)	45 (24.5)	68 (23.2)
Deletions	10 (9.0)	11 (6.0)	21 (7.1)
Total	92 (83.6)	138 (75.0)	230 (78.2)
Chromatid:			
Breaks	4 (3.6)	5 (2.7)	9 (3.1)
Deletions	1 (.9)	0 (0)	1 (.3)
Exchanges	0 (0)	8 (4.3)	8 (2.7)
Gaps	2 (1.8)	14 (7.6)	16 (5.5)
Total	7 (6.4)	27 (14.7)	34 (11.6)
Rejoined chromosomes:			
Inversions	1 (.9)	2 (1.1)	3 (1.0)
Translocations	3 (2.7)	4 (2.2)	7 (2.4)
Dicenentrics	3 (2.7)	5 (2.7)	8 (2.7)
Markers	4 (3.6)	4 (2.2)	8 (2.7)
Rings	0 (0)	3 (1.6)	3 (1.0)
Insertions	0 (0)	1 (0.5)	1 (.3)
Total Rejoined	11 (10.0)	19 (10.3)	30 (10.2)
Grand total	110	184	294

quencies of chromatid breaks are very similar (center 1, 3.6%; and center 2, 2.7%) chromatid gaps were more frequent in center 2 (7.6%) than in center 1 (1.8%); and chromatid exchanges were not described in center 1, whereas center 2 found a frequency of 4.3%.

The chromosome distribution of the identified chromosome anomalies pooled from both centers is presented in table 3. Note that the breakpoints of acentric fragments and marker chromosomes are not listed, because they are of chromosomally unknown origin. For translocations, inversions, dicentrics, rings, and insertions, the two breakpoints are listed in the table. The number of expected breaks per chromosome was calculated, using the formula of chromosome relative length (Lubs et al. 1985) (i.e., expected no. of breaks for chromosome p = chromosome p relative length/Σ length for 24 chromosomes [=1] $\times$ total no. of breaks). The relative lengths of chromosomes X and Y were corrected for the number of X and Y sperm metaphases (1:1). Ninety-five percent confidence intervals were calculated for the expected values and were compared with the observed number of anomalies. When a Bonferroni procedure was used to adjust for multiple comparisons, it was found that only one comparison (chromosome 4) was statistically significant. Chromosome 4 has less than the expected number of breakpoints ($P < .001$).

A total of 220 breakpoints could be localized to a specific chromosome band or subband. Of those, 134 fall in different bands; therefore, 86 break events fall into bands where there is more than one breakpoint. Breakpoints may fall into G-light, G-dark, and variable regions (such as stalks, satellites, and heterochromatic areas of the chromosome). Of the 220 localized breakpoints of sperm chromosome, 136 (61%) were confined to G-light bands, a much higher number than the expected 64.4 (29%) ($P < .001$). The expected number, 64.4 (29%), was estimated by simplifying and by assuming all bands to be of the same length. The same predominance of G-light breakpoints is found both for fragile sites on human chromosomes (66 of 103 fragile sites are in G-light bands) and for breakpoints of de novo rearrangements identified at prenatal diagnosis (Warburton 1991). One hundred twenty-two of 207 different breakpoints of de novo rearrangements are in G-light bands.

Association between Fragile Sites and Sperm Breakpoints

Both common and rare fragile sites were included in the analyses. The criteria for collecting data for table 4 were as follows: if both the reported location of the fragile site (fra^+) and that of the sperm breakpoint were at the band level (i.e., 2q32), 2q32 was considered a band with both a fragile site and one or more sperm breakpoints (≥ 1, in table 4). If the sperm breakpoint was at the band level (i.e., 1p31) and that of the fragile site was reported at the subband level (i.e., 1p31.2), 1p31 was considered a band with both a fragile site and one or more sperm breakpoints. If a sperm breakpoint was present at a specific band (i.e., 1p13) and no fragile site was reported in that particular location, that band was accounted for as a band with one or more sperm breaks but fragile negative (fra^-) (table 4). There were 103 chromosome bands with a fragile site (Human Gene Mapping 11).

In an attempt to rule out false associations between fragile sites and breakpoints of sperm rearrangements, the analyses were carried out for G-light and G-dark bands separately, as well as in all chromosome bands together, using Fisher's exact test (see table 4). Contingency tables were created for each scenario. There was a statistically significant ($P = .0053$) positive association (odds ratio = 1.98) between fragile sites and sperm breakpoints across all of the chromosomal bands when looked at together. However, when the data were stratified according to G-light and G-dark bands, the strongest positive association between fragile sites and sperm breaks occurred on the G-dark bands ($P = .0158$). For the G-light bands, the association between fragile sites and sperm breakpoints was still positive (odds ratio = 1.48), but this association did not reach a level of statistical significance ($P = .2149$).

Association between Breakpoint of De Novo Rearrangements and Sperm Breakpoints

The same criteria were followed for collecting data for table 5 as for table 4. There were 207 different chromosome

Table 3

Chromosome Distribution of Breaks and Gaps

Chromosome	Chromosome Break	Chromosome Gap	Chromosome Deletion	Chromatid Break	Chromatid Gap	Chromatid Exchange	Inversion	Translocation	Insertion	Dicentric	Ring	Total	Expected Interval[a]	Significance
1	10	5	1	1	2	1	...	1	...	2	...	23	11–28	...
2	12	4	1	1	4	...	...	1	...	...	...	24	11–27	...
3	11	2	5	1	1	2	...	1	...	1	...	24	9–23	...
4	2	...	...	...	...	...	...	...	...	1	...	3	8–22	($P < 0.001$)
5	7	3	4	...	1	1	...	...	...	1	...	17	7–21	...
6	6	...	1	...	2	2	...	...	...	1	...	12	7–21	...
7	7	1	1	1	...	...	4	1	2	1	...	18	6–19	...
8	2	...	2	...	...	1	...	1	1	...	...	7	5–18	...
9	11	3	1	1	2	1	2	...	...	...	...	21	5–18	...
10	3	3	2	1	1	1	...	...	...	...	...	11	5–17	...
11	5	2	...	1	1	...	...	...	...	...	...	9	5–17	...
12	3	...	...	0	1	1	...	3	...	...	...	8	5–17	...
13	5	...	...	...	...	1	...	2	...	...	...	8	3–15	...
14	2	3	...	1	...	1	...	...	...	...	...	7	3–14	...
15	3	...	...	...	...	1	...	...	...	...	...	4	3–14	...
16	2	...	1	...	...	...	...	1	...	...	...	7	3–13	...
17	8	...	1	...	...	1	...	1	...	2	...	13	3–13	...
18	...	...	...	...	...	...	...	2	...	...	...	2	2–12	...
19	2	...	1	...	...	...	...	...	...	...	...	3	2–11	...
20	1	...	...	...	...	...	...	...	...	1	...	2	2–11	...
21	1	...	...	...	...	...	...	...	...	2	...	3	1–9	...
22	3	...	...	...	...	...	...	...	...	1	...	4	1–9	...
X	3	1	...	...	...	1	...	...	...	1	...	6	2–11	...
Y	1	2	...	1	...	...	...	...	...	...	...	4	0–6	...
Unknown	...	...	...	...	1	1	...	...	...	2	6	...	...	...
Total breaks	110	29	21	9	16	16	6	14	3	16	6			
Total structural abnormalities	110	29	21	9	16	8	3	7	1	8	3			

[a] Expected no. of breaks = relative length of chromosome/Σ length of 24 chromosomes (=1) × total no. of breaks; expected interval = interval containing 95% of expected breaks.

breakpoints cited in the series of de novo rearrangements detected at amniocentesis (Warburton 1991, appendix). Fisher's exact test was also used to investigate associations between de novo rearrangements and sperm breakpoints.

There was a statistically significant ($P < .0001$), positive association (odds ratio = 2.59) between chromosome de novo rearrangements and sperm breakpoints across all of the chromosomal bands when looked at together (table 5). When the data were stratified according to G-light and G-dark bands, the strongest positive association between de novo rearrangements and sperm breakpoints occurred on the G-light bands ($P = .0014$). For the G-dark bands, the association between de novo rearrangements and sperm breakpoints was still positive (odds ratio = 1.90), and this association reached a marginal level of statistical significance ($P = .0578$).

Discussion

There is a wide variability in the frequencies of sperm chromosome structural aberrations among the 19 studied donors. While one donor did not have any structural aberrations, 17.8% of another donor's cells had structural abnormalities. The median percentage was 9.3 (SD ± 4.97). The percentages of sperm structural abnormalities in the 19 donors studied follow a normal distribution. The statistical differences observed between the median abnormal rate from center 1 and that from center 2 ($P = .018$) may be mostly due to differences between the two center's median abnormal rates of normal donors ($P = .019$), whereas the translocation carriers did not contribute to differences between the respective median rates reported for the two centers. Three possible factors may have caused these differences: (1) real differences between the rates of chromosome structural abnormalities in normal donors; (2) artifactual differences, as a result of smaller populations of cells studied for normal donors in center 1, where four of seven normal donors had <100 cells studied, whereas center 2 analyzed >150 cells per donor; and/or (3) differences in staining protocols (Material and Methods and Discussion) that may result in center 2 scoring a significantly higher number of chromatid events.

Most of the structural abnormalities found were chromosome breaks and fragments and small deletions, supporting earlier studies and confirming that the sperm is a very vulnerable cell type. The frequency of stable aberrations (such as translocations, inversions, small deletions, and markers) remains consistent in both centers 1 and 2.

The ages of the donors ranged from 18 to 40 years. Whereas the donors for this study were not selected on the basis of age, when they are grouped into younger (18–30; $n = 6$) and older (31–40; $n = 13$), there is no statistical difference between the frequencies of sperm structural aberrations between the two age groups (8.05% and 9.90%, respectively). However, a trend toward higher numbers of structural rearrangements with older ages does exist.

Another report (Martin and Rademaker 1987) had shown a significant increase in the frequency of structural chromosome abnormalities with age, from 2.8% in a 20–24-year-old age group to 13.6% in donors >45 years of age. Thirty-two normal men and a total of 1,582 sperm cells were studied in that report. Olson and Magenis (1988) did not find a paternal age effect in their series of de novo chromosome rearrangements of paternal origin (including balanced and unbalanced), nor was it seen in other previously published cases of obligate paternal de novo Y chromosome rearrangements (Magenis et al. 1985). Very little data on the paternal origin of de novo rearrangements exist in the literature. Transit through the epididymis (the site where stabilizing disul-

Table 4

Contingency Tables for Sperm Breakpoints and Fragile Sites

	No. of Chromosome Bands with Sperm Breaks	
	≥1	0
Fra^{+}[a]	49	54
G-light[b]	31	35
G-dark[c]	18	19
Fra^{-}[a]	84	183
G-light[b]	45	73
G-dark[c]	39	110

NOTE.—Fra^{+} = number of bands with fragile site; Fra^{-} = Number of bands with no fragile site.

[a] Odds ratio = 1.98; $P = .005$.

[b] Odds ratio = 1.48; $P = .21$.

[c] Odds ratio = 2.65; $P = .016$.

Table 5

Contingency Tables for Sperm Breakpoints and Sites of De Novo Rearrangements

	No. of Chromosome Bands with Sperm Breaks	
	≥1	0
De novo^{+}[a]	94	113
G-light Bands[b]	61	61
G-dark Bands[c]	33	52
De novo^{-}[a]	40	123
G-light Bands[b]	15	45
G-dark Bands[c]	25	78

NOTE.—De novo^{+} = number of bands with ≥1 de novo rearrangements; de novo^{-} = number of bands with 0 de novo rearrangements.

[a] Odds ratio = 2.59; $P = .0001$.

[b] Odds ratio = 3.00; $P = .0014$.

[c] Odds ratio = 1.9; $P = .058$.

fide bonds between DNA and protamines form) does not seem to decrease with age; however, sperm production does decline significantly, and the frequency of ejaculation decreases, with age in the human male. There is evidence that longer periods of in vitro storage of sperm result in an increase of sperm structural abnormalities (Munné and Estop 1993). The majority of sperm structural abnormalities found in this and other series are chromosome breaks that probably occur during spermiogenesis in spermatids and in mature sperm, the only germ cells lacking DNA repair (Sega 1976; Working and Butterworth 1984). An increase in these anomalies is found when mouse (Munné and Estop 1991) and human (Martin et al. 1992; Munné and Estop 1993) sperm are stored in vitro.

The two laboratories involved in this study observed different frequencies of chromatid gaps events: 1.8% for center 1 and 7.6% for center 2. At least two factors may have contributed to those differences. (1) Minor differences in culture conditions, such as center 2's slightly longer periods of culture time in Ham's F-10 (12–13 h, vs. center 1's 11–12 h). Protocols call for 10–12.5 h culture time prior to transfer to colcemid-containing media. (2) Chromatid events may be detected more accurately when the chromosomes are uniformly stained (center 2) than they are when G-banded (center 1). G-band procedures tend to swell the chromosomes and to mask small chromosome lesions. The actual presence of chromatid-type lesions at first cleavage of the cross between hamster egg and human sperm suggests deficient DNA repair by the hamster after the first S-phase. However, postreplication repair, after the first S-phase, by the hamster egg may actually occur because chromatid-type aberrations increase after inseminated hamster oocytes are exposed to caffeine, a DNA-repair inhibitor (Genescà et al. 1992).

Our report shows a statistically lower than expected number of breaks in chromosome 4 ($P < .001$). Chromosomes 3, 9, and 17 have a higher than expected number of breaks, and chromosomes 18 and 20 have a lower than expected number of breaks; however, the P values of chromosomes 3, 9, 17, 18, and 20 are not statistically significant when adjusted for multiple comparisons. An excess of chromosome breaks in chromosome 9, in both lymphocytes and sperm, has been reported elsewhere. Aula and van Koskull (1976) noted 9q1 as a site where breakpoints clustered in lymphocytes, whereas Mattei et al. (1979) also found an excess of chromosome breaks in chromosome 9. In spermatozoa, breaks were found most frequently in chromosomes 3, 5, and 9, by Brandriff et al. (1988). Moreover, both our study and this latter report coincide in that chromosome 9 had twice as many breakpoints as expected by chance alone, and both studies also coincide in that half of the breakpoints in chromosome 9 are between the 9cen and 9qh+ area. Most of the excess breaks for chromosomes 3 and 17 are also breaks around the pericentromeric area.

Association between Sperm Breakpoints, Fragile Sites, and Sites of De Novo Rearrangements

Some investigators have claimed that most, if not all, chromosome rearrangements result from breakage within light (rather than dark) G-bands. This would also include fragile sites and rearrangements found in neoplasia (Sutherland and Simmers 1988).

Sperm breakpoints fall preferentially in G-light bands, as do fragile sites. When the locations of fragile sites and of all sperm breakpoints are analyzed statistically, a significant association is found ($P = .0053$). Because of the preferential involvement of breakpoints in G-light bands, the data were reanalyzed, this time with a separation between breakpoints and fragile sites in G-light bands and breakpoints and fragile sites in G-dark bands (table 4). Here, the statistical association is found between sperm breakpoints and fragile sites in G-dark bands ($P = .0158$). Presently, no association is found between sperm breakpoints and fragile sites in G-light bands ($P = .2149$), revealing that although they both tend to fall in G-light bands, there is no actual association between the two biological phenomena. However, the weak statistical association between sperm breakpoints (G-dark) and fragile sites (G-dark) remains to be explained.

Possible relationships between sperm breakpoints and sites of balanced de novo rearrangements were looked at. For that purpose, the series of chromosome de novo rearrangements detected at prenatal diagnosis, published by Warburton (1991), was chosen because it is relatively unbiased in terms of breakpoints, since the only selection was for survival until the time of amniocentesis. Since each rearrangement is a de novo event, the breakpoints represent the actual breakpoints of chromosome breakage and rearrangement, in viable germ cells. However, it must be noted that balanced rearrangements represent only 10% of chromosome breakpoints in human male sperm cells (see table 2). A positive association ($P < .001$) between the de novo chromosome rearrangements and sperm breakpoints, across all chromosomal bands, is present. Again, because both types of breakpoints tend to fall in G-light bands, a statistical analysis was carried out independently in G-light and G-dark bands, revealing (*a*) a strong correlation between the number of sperm breakpoints and de novo rearrangements that coincide in the same G-light bands and (*b*) a marginal significance in breakpoints in G-dark bands.

These data show an association between the breakpoints of de novo rearrangements and the breakpoints of chromosome breakage in human sperm cells. They both tend to occur at the same sites, and are statistically associated. Therefore, the same breakpoints detected in spermatozoa are reflected in de novo rearrangements detected at amnio-

centesis. The results of this study suggest a paternal origin of de novo balanced chromosome rearrangement present at amniocentesis and are in agreement with data from Olson and Magenis (1988), who demonstrated a paternal origin of 27 of 32 cases with de novo chromosome rearrangements.

Because of the absence of surveys on unbalanced chromosome rearrangements at prenatal diagnosis, no further inferences can be drawn on the potential contribution of sperm to pregnancies with unbalanced chromosome complements. The breakpoints were recorded at a low level of resolution, and the same band location for two breakpoints may represent positions several megabases apart in the genome. Molecular analysis of breakpoints may shed more light on the true relationship between breakage in human sperm cells and human conception with de novo chromosome rearrangements.

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