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Absence of Age Effect on Meiotic Recombination between Human X and Y Chromosomes

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Recombination between the X and Y chromosomes is limited to the pseudoautosomal region and is necessary for proper segregation of the sex chromosomes during spermatogenesis. Failure of the sex chromosomes to disjoin properly during meiosis can result in individuals with a 47,XXY constitution, and approximately one-half of these result from paternal nondisjunction at meiosis I. Analysis of individuals with paternally derived 47,XXY has shown that the majority are the result of meiosis in which the X and Y chromosomes have failed to recombine. Our studies of sperm have demonstrated that aneuploid 24,XY sperm have a decreased recombination frequency, compared with that of normal sperm. Some studies have indicated a relationship of increased paternal age with 47,XXY offspring and with the production of XY disomic sperm, whereas others have failed to find such relationships. To determine whether there is a relationship between paternal age and recombination in the pseudoautosomal region, single-sperm genotyping was performed to measure the frequency of recombination between a sex-specific locus, STS/STS pseudogene, and a pseudoautosomal locus, DXYS15, in younger men (age $\leq$30 years) compared with older men (age $\geq$50 years). A total of 2,329 sperm cells were typed by single-sperm PCR in 20 men who were heterozygous for the DXYS15 locus (1,014 sperm from 10 younger men and 1,315 sperm from 10 older men). The mean recombination frequency was 39.2% in the younger men and 37.8% in the older men. There was no heterogeneity in the frequency of recombination rates. There was no significant difference between the recombination frequencies among the younger men and those among the older men, when analyzed by the clustered binomial Z test ($Z = .69$, $P = .49$). This result suggests that paternal age has no effect on the recombination frequency in the pseudoautosomal region.

Introduction

Approximately 55% of 47,XXY conceptuses in all clinically recognized pregnancies survive to birth (Jacobs and Hassold 1995), and the frequency of newborn boys with a 47,XXY constitution is ~1/1,000 (Hook and Hammerton 1977). In most instances, the error leading to the 47,XXY constitution occurs during meiosis I, and paternal and maternal origins are implicated in similar proportions (Jacobs et al. 1988; Lorda-Sanchez et al. 1992). Birth of individuals with 47,XXY was reported to be associated with advanced paternal age by one molecular study (Lorda-Sanchez et al. 1992), but this association was not confirmed by several other large studies (Jacobs et al. 1988; MacDonald et al. 1994; Thomas et al. 2000). Several sperm FISH studies have been performed to detect whether the age of the donor affects the production of XY disomic sperm in human semen samples, with an approximately even division between finding an age effect (Griffin et al. 1995; Guttenbach et al. 2000; Lowe et al. 2001) and finding no evidence for an age effect (Martin et al. 1995; Robbins et al. 1995). In sperm FISH studies, the frequency of XY disomic sperm is usually higher than disomic frequencies for the autosomes, XX and YY sperm, which is consistent with the concept that the XY bivalent is particularly susceptible to nondisjunction during male meiosis. Pseudoautosomal regions (PAR) 1 and 2, which cover 2.6 Mb and 0.33 Mb, respectively, and which, being located at the tips of the short and long arms, are the homologous DNA sequences available for recombination and synaptonemal complex formation between the X and Y chromosomes (Freije et al. 1992; Rappold 1993). During male meiosis, a single crossover normally occurs within PAR 1 to ensure proper disjunction (Burgoyne 1982). Genetic maps have established a linear gradient of recombination within PAR 1, with markers close to the

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telomere displaying a recombination frequency of 50% (Henke et al. 1993). The overall frequency of recombination in PAR 1 is exceptionally high, the mean being, on average, 10- to 20-fold higher than in other regions of the genome (Rouyer et al. 1986).

Elsewhere, we have provided the first evidence of the association of reduced recombination with sex chromosome nondisjunction during meiosis I in human males, based on results of single-sperm PCR (Shi et al. 2001). Two markers were selected to measure recombination frequency between the X and Y chromosomes: the STS pseudogene represents the strictly sex-linked portions of the X and Y chromosomes and serves as a genetic marker for the PAR 1 boundary (2.6 Mb from the telomere on the short arm of the sex chromosomes), and DXYS15, located at Xp22.33 and Yp11.3 (~700–750 kb from the telomere on the short arm of the sex chromosomes) (Henke et al. 1993). A significantly decreased recombination frequency was found in XY disomic sperm, compared with that in normal haploid sperm. In the present communication, we extend our study to haploid sperm from 10 men aged $\leq$30 years and 10 men aged $\geq$50 years, to investigate whether there is an association of recombination frequency with advanced paternal age.

Subjects, Material, and Methods

Study Population

A total of 42 men were recruited, and 20 of them were found to be heterozygous for the DXYS15 marker, with 10 men aged $\leq$30 years and 10 men aged $\geq$50 years at the time the semen samples were collected. All donors were healthy and had no history of chronic illness or exposure to known mutagens. Fertility was proven in 9 heterozygotes, (6 men $\geq$50 years old and 3 men $\leq$30 years old); the remaining 11 heterozygotes (4 men in the older group and 7 men in the younger group) were of unproven fertility. Semen samples were obtained by masturbation and were stored in cryopreservative medium in liquid nitrogen until use. We have previously demonstrated that cryopreservation of human sperm does not affect the frequency of chromosome abnormalities (Martin et al. 1991). The present study was approved by our institutional ethics committee, and informed written consent was obtained from all donors.

Preparation of Plates by Use of Micromanipulation and Flow Cytometry

Single sperm cells were placed in individual wells of 96-well plates, either by micromanipulation or by flow cytometry. To prepare plates by use of micromanipulation, frozen semen was thawed and washed three times with 0.01M Tris/0.9% NaCl. The sperm were resuspended in ~750 μl Tris-NaCl, and a 1–2-μl aliquot was diluted ~1:300 in water. On the day that micromanipulation was performed, the solution was further diluted ~1:100 in water, filling a 2.5-mm well in a 4-mm Plexiglas slide. One at a time, sperm were removed from this solution, using a water-filled glass needle on a Leitz micromanipulator (Germany), and were placed into individual wells of a 96-well PCR plate. Plates were stored at 4°C until lysis.

To prepare plates by use of flow cytometry, frozen sperm were washed in water, the pellet was resuspended in 500 μl water, and the suspension was sonicated using the 4-mm probe of a Branson Sonifier Cell Disruptor 185 at minimum intensity, for three or four intervals of 1 min each. After each 1-min interval, the sperm sample was cooled for 5 min in an ice-water bath, and 2 μl of the suspension was examined by phase-contrast microscopy, to monitor the progress of tail removal. When tails were completely removed in >95% of the sperm and the remaining sperm had tails that were not more than twice the length of the sperm head, the sperm were centrifuged at 3,200 *g* for 2 min and were stained by resuspending the pellet in 100 μl of 10 μg/ml propidium iodide for 5 min at room temperature. Stained sperm were centrifuged at 3,200 *g* for 2 min and were resuspended in 200 μl of 70% sucrose. The sample was sonicated at maximum intensity for 5 s, to disperse clumps, and was then stored at −20°C.

A FACStar^{Plus} flow cytometer (Becton Dickinson), equipped with an automated cell-deposition unit and an argon laser (wavelength = 488 nm), was used to sort cells onto 96-well plates containing 5 μl of freshly prepared lysis solution (200 mM KOH and 50 mM dithiothreitol per well). For each plate, 2 wells, which were used for positioning of the plate, contained only buffer, 2 (positive control) wells received 20 sperm, and 7 (negative control) wells contained no sperm; the remaining 85 wells received one sperm each. After flow sorting, sperm plates were heated at 65°C for 10 min in a Techne Genius thermocycler, then 5 μl of neutralization buffer (900 mM Tris-HCl [pH 8.3], 300 mM KCl, and 200 mM HCl) was added to each well. Similarly, micromanipulated XY disomic sperm were lysed and neutralized, as described above, except that lysis time was 12 min. The 96-well plates were stored at –20°C until needed for PCR analysis.

PCR Analysis

All PCR analyses were performed using a Techne Genius thermocycler. Primer sequences of DXYS15 and STS were as described by Schmitt et al. (1994). In the first round of PCR, DXYS15 and STS were coamplified in a 50-μl reaction system containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl, 0.01% gelatin, 0.24 μM of each of four primers (NA26, NA27, 076, and 077), 100 μM of each of the four dNTPs (Pharmacia),

and 1.2 U of recombinant *Taq* polymerase (Gibco) per well. After an initial denaturation at 94°C for 4 min, 12 cycles of 95°C for 50 s and 53°C for 6 min, followed by 32 cycles of 95°C for 50 s and 53°C for 4.5 min were performed.

DXYS15 and STS markers were amplified separately during the second round, using heminested PCR, with STS analyzed first. For the STS, 5 μl of first-round PCR product was further amplified in a 50-μl reaction system containing 1× PCR buffer (Gibco), 40 μM of each of the four dNTPs, 0.25 μM of NA168, 2 μM each of NA26 and NA169, and 1.0 U of recombinant *Taq* polymerase. After an initial denaturation at 94°C for 4 min, 35 cycles of 95°C for 40 s, 65°C for 1 min, and 72°C for 1 min were performed. Primer NA168 is Y specific and amplifies a 153-bp product with NA26; NA169 is X specific and produces a 144-bp product with NA26. For DXYS15, the second-round PCR was performed only on those wells that produced either a 153-bp (Y) or a 144-bp (X) product. A 50-μl reaction mixture, containing 1× PCR buffer, 120 μM of each of the four dNTPs, 0.2 μM of 077, 0.2 μM of 088, 1.0 U of recombinant *Taq* polymerase, and 2–8 μl of first-round PCR product was used to amplify DXYS15. After an initial denaturation at 94°C for 4 min, 31 cycles at 95°C for 40 s, 58°C for 1 min, and 72°C for 1 min were performed. The PCR product is 186 bp.

Between 8 and 13 μl of each second-round DXYS15 PCR product was digested with 1 U of Fnu4HI (New England Biolabs) at 37°C for 3–5 h. A single-nucleotide substitution within the amplified region of one allele creates an Fnu4HI cleavage site, which results in 166-bp and 20-bp fragments after this enzyme digestion (Schmitt et al. 1993) and allows differentiation between alleles with and without this single-nucleotide mutation. All second-round PCR products were analyzed on an 8% nondenaturing polyacrylamide gel. Electrophoresis was performed at 195 V for 40–45 min. Gels were then stained with 0.5 μg/ml ethidium bromide for ~10 min, were viewed using a UV illuminator, and were photographed.

Data Collection

Data were collected only when both positive and negative controls yielded the expected results. Sperm were recorded as X bearing or Y bearing if the 144-bp or 153-bp PCR product, respectively, was found for STS. For the same sperm, the DXYS15 product was recorded as either 186 bp or 166 bp.

Data Analysis

Heterogeneity of recombination proportions was tested using a χ^2 test. A clustered binomial Z test was used to determine whether there was a significant difference in recombination frequencies between the two groups. A regression analysis of recombination frequency versus age was also performed.

Results

At the time of semen collection, the mean age of the younger men was 23.7 years (range 19–30 years), and that of the older men was 52.9 years (range 50–59 years). There were no significant differences in recombination frequencies, or proportion of wells containing 0, 1, or >1 sperm in data obtained by flow cytometry and micromanipulation techniques (data not shown); therefore, we combined the PCR typing results from single-sperm analysis obtained by the two techniques. We obtained ~100 sperm that were informative for both STS and DXYS15 from each donor and analyzed a total of 1,014 sperm in the younger men and 1,315 sperm in the older men (table 1). The mean (± SE) frequency of recombinants was 39.2% (± .015) in the younger men and 37.8% (± .013) in the older men. A mean frequency of recombination of 38.4% was obtained by pooling recombination data from all the men studied. No heterogeneity for the frequency of recombinants was found among either the younger donors (χ^2 with 9 df = 2.04; $P = .99$) or the older donors (χ^2 with 9 df = 2.85; $P = .97$) or both groups combined (χ^2 with 19 df = 5.37; $P = .99$). Since there was no donor heterogeneity within groups, the intradonor correlation of recombination occurrence was determined to be slightly negative for both the younger and the older group. In light of these facts, for the purpose of calculating power and effect size, a conservative estimate of the intradonor correlation in each group is zero. Under the assumption of a base recombination frequency of 38%, a sample of 100 sperm from each of 10 men in each of two groups (1,000 sperm per group) would have 80% power to detect a difference of 6%, assuming a two-tailed test with a type I–error rate of 5%. A one-tailed test would have 80% power to detect a 5% difference. There was no significant difference in recombination frequency between the two age groups, according to a clustered binomial Z test ($Z = .69$; $P = .49$). A regression analysis of recombination frequency versus age was performed by pooling the two age groups: no association between age and recombination was noted (slope [SE] = .06 [.037], correlation = .36, $P = .12$).

Linkage analysis was performed with software developed by Lazzeroni et al. (1994). This program accounts for potential errors in single-sperm typing, such as more than one sperm per tube, contamination, and different amplification efficiencies of loci and alleles. It was assumed that only one recombination occurred between the markers. Results from this analysis gave an estimate of 38.8% recombination in the younger men (vs. the manual estimate of 39.2%) and 37.5% in the older men (vs. the manual estimate of 37.8%).

Table 1

Number and Frequency of Recombinant and Nonrecombinant Sperm

Age Group (years) and Donor	Age (years)	Recombinant Sperm	Nonrecombinant Sperm	Total Sperm	Recombinant Frequency (%)
19–30:					
1	22	44	64	108	40.74
2	23	41	64	105	39.05
3	29	44	59	103	42.72
4	28	36	63	99	36.36
5	20	38	65	103	36.89
6	19	43	61	104	41.35
7	21	35	57	92	38.04
8	30	40	70	110	36.36
9	24	39	55	94	41.49
10	21	38	58	96	39.58
Overall	23.7 ± 3.9[a]	398[b]	616[b]	1,014[b]	39.3 ± 2.30[a]
50–59:					
11	51	126	203	329	38.30
12	54	41	64	105	39.05
13	53	32	70	102	31.37
14	55	43	72	115	37.39
15	50	44	67	111	39.64
16	55	50	80	130	38.46
17	50	43	69	112	38.39
18	51	39	66	105	37.14
19	51	40	62	102	39.22
20	59	37	67	104	35.58
Overall	52.9 ± 2.9[a]	495[b]	820[b]	1,315[b]	37.5 ± 2.4[a]
19–59:					
Overall	38.3 ± 15.3[a]	893[b]	1,436[b]	2,329[b]	38.4 ± 2.5[a]

[a] Overall values for age and recombinant frequency are mean ± SD.
[b] Overall values for sperm are totals.

Discussion

Linkage Heterogeneity among Donors

Studies of a variety of nonhuman species have revealed genetic variation for recombination within and among both natural and laboratory populations (Brooks 1988; Lindahl 1991). In mice, sizable differences in the recombination fraction across the same interval have been observed in different interspecies and intersubspecies crosses (Reeves et al. 1990). In humans, cytogenetic studies of bivalent chiasma frequencies have suggested that there is individual variation in the position of crossovers among men (Laurie and Hultén 1985). Individual variation has also been inferred from limited human family data supporting linkage heterogeneity on the basis of allele-specific effects on recombination between the markers Gm and α-1-antitrypsin (Babron et al. 1990). Broman et al. (1998) observed striking individual variation in the overall extent of recombination in the 22 autosomes among female subjects, a result that did not appear in the male subjects. Using single-sperm genotyping, Yu et al. (1996) demonstrated that the recombination fraction between D6S291 and D6S109 for two donors (5001 and 50043) differed by more than twofold (5.1% vs. 11.2%), whereas no significant difference was detected in the recombination fraction between two chromosome 19 markers (Mfd232 and Mfd11) for the same two donors. Our results on the recombination fraction over the interval from STS to DXYS15 on human X and Y chromosomes do not show any linkage heterogeneity among 20 donors, which is consistent with the observation on the same interval by Schmitt et al. (1994), using a similar sperm-typing method to analyze sperm from two donors. Recently, a comparable recombination frequency (37%–47.5%) was reported for the interval STS–DXYS15, with the use of sperm-typing analysis for five donors, despite a statistically significant linkage heterogeneity (5.1%–11.2%) among donors for the interval DXYS218–GGAT3F08, which was located within the interval STS–DXYS15 (Lien et al. 2000). This confirmed a previous hypothesis that the variation in recombination is not genomewide and may be restricted to some chromosomes, some regions, or some specific intervals of chromosomes (Yu et al. 1996).

Recombination versus Paternal Age

The present study is, to date, the largest (2,329 sperm from 20 men) to examine the association between recombination and increased donor age. No statistically significant correlation between donor age and recombination

fraction for the interval STS–DXYS15 was detected. In agreement with these results, Schmitt et al. (1994) found a similar recombination frequency in the PAR, including the STS–DXYS15 interval, in two donors of unknown age by use of single-sperm typing. Interestingly, Lien et al. (2000) found a significant variation in recombination among four donors for the GGAT3F08–DXYS218 interval (which is located within the STS–DXYS15 interval) but no sign that the variation is the result of an age-dependent effect, because donor ages were comparable (42–48 years). No age effect was found for recombination between D6S291 and D6S109 by single-sperm typing (Yu et al. 1996), and a thorough analysis for the ABO-NPS1 linkage, performed in 15 large families of 289 individuals, did not show any evidence of an age effect in either male or female subjects (Elston et al. 1976). Also, studies have not demonstrated an association between paternal age and recombination for chromosome 21 (Tanzi et al. 1992; Lynn et al. 2000). Similarly, no age-related recombination variation was detected in the study of approximately one million genotypes from eight CEPH families based on >8,000 STRPs (Broman et al. 1998). In conclusion, results presented here and data published in the literature provide little evidence of age-dependent changes in recombination.

Nondisjunction versus Paternal Age

Although the association of nondisjunction and increased maternal age is well established, the relationship, if any, between increasing paternal age and trisomy is unclear, with conflicting results from epidemiological and molecular studies (Hassold 1998). Lorda-Sanchez et al. (1992) reported a significant increase in the paternal age of individuals with paternally derived 47,XXY, when compared with individuals who had maternally derived 47,XXY, whereas two other large studies did not show any evidence for an increase in paternal age among individuals with paternally derived 47,XXY (MacDonald et al. 1994; Thomas et al. 2000), which is consistent with an early report by Jacobs et al. (1989). Similarly, sperm FISH studies performed to investigate an age effect on XY disomy yielded conflicting results. Some studies observed a statistically significant increase in the frequency of XY-bearing sperm among older men, compared with their younger counterparts (Griffin et al. 1995; Guttenbach et al. 2000; Lowe et al. 2001), whereas no age effect was found by other studies (Martin et al. 1995; Robbins et al. 1995). Thus, the answer to the question of whether there is a paternal age effect on nondisjunction of chromosomes X and Y during male meiosis remains equivocal.

Recombination versus Nondisjunction

Recombination occurs frequently during prophase of meiosis I and plays a critical role in homologue segregation. Interhomologue crossovers and sister chromatid cohesion hold homologues together at metaphase of meiosis I, providing correct orientation on the spindle and ensuring spindle integrity (for a review, see Dej and Orr-Weaver 2000). In *Saccharomyces* and *Drosophila,* it has long been recognized that nonrecombinant chromosomes are at increased risk of undergoing nondisjunction during meiosis I (Koehler et al. 1996) and that mutants with reduced meiotic recombination undergo massive missegregation of homologues (Baker et al. 1976; Shonn et al. 2000; Molnar et al. 2001). Furthermore, two naturally occurring polymorphisms at the *nod* (a chromokinesin required for proper achiasmate chromosome segregation) locus (Zhang and Hawley 1990; Zhang et al. 1990) were demonstrated to be associated with increased rates of nondisjunction in *Drosophila melanogaster* (Zwick et al. 1999). However, an extended meiosis I, achieved through the delay of anaphase I onset by spindle checkpoint, has been observed in recombination-defective yeast mutants and has been suggested for use in preventing or reducing achiasmate nondisjunction during meiosis I (Shonn et al. 2000; Molnar et al. 2001). Using single-sperm typing, our laboratory provided the first direct evidence to indicate that reduced recombination, in humans, between STS and DXYS15 is associated with nondisjunction of chromosomes X and Y during male meiosis I (Shi et al. 2001). These findings were supported by all molecular studies of individuals with paternally derived 47,XXY wherein a reduced recombination between the X and Y chromosomes was observed (Hassold et al. 1991; Lorda-Sanchez et al. 1992; MacDonald et al. 1994; Thomas et al. 2000). The data presented here, which show no association between recombination for the interval STS–DXYS15 and advanced paternal age, suggest indirectly that there may be no relationship between increasing paternal age and X and Y nondisjunction.

On the basis of the frequency of recombination, nondisjunction of the paternal sex chromosomes during meiosis I can be divided into two categories. The first type, achiasmate nondisjunction, occurs when no recombination has taken place in a paternal meiosis in which there is nondisjunction of X and Y chromosomes; it is the most common nondisjunctional event in human spermatogenesis and has been found to be unrelated to age. Achiasmate nondisjunction examples include those in a study published elsewhere (Shi et al. 2001) which showed that ~75% of XY disomic sperm resulted from paternal meiosis I, in which no XY recombination occurred for the STS–DXYS15 interval, and studies which showed that >80% of 47,XXY males of paternal origin resulted from meioses in which the X and Y chromosomes failed to recombine (33 [85%] of 39 [Hassold et al. 1991]; 10 [100%] of 10 [Lorda-Sanchez et al. 1992]; and 53 [83%] of 64 [Thomas et al. 2000]). Achiasmate nondisjunction can result from pairing fail-

ure of the homologous chromosomes or from normal pairing that occurs in the absence of recombination. Current methodology does not allow discrimination between these two possibilities, but the latter may be more plausible if unpaired sex chromosomes are associated with meiotic arrest and spermatogenic failure (Burgoyne and Mahadecaish 1993).

The second category, normochiasmate nondisjunction, is nondisjunction of chromosomes X and Y during paternal meiosis, in which no demonstrably aberrant recombination has occurred between the X and Y chromosomes. Normochiasmate nondisjunction accounts for the remainder of the nondisjunction observed in the studies mentioned above: ~25% of XY sperm were the result of meioses in which recombination took place between the X and Y chromosome for the STS–DXYS15 interval (Shi et al. 2001), and 0%–17% of individuals with paternally derived 47,XXY were in this category in the family studies (6 [15%] of 39 [Hassold et al. 1991]; 0 [0%] of 10 [Lorda-Sanchez et al. 1992]; 11 [17%] of 64 [Thomas et al. 2000]). The proportion of normochiasmate nondisjunction is most likely higher than observed, given that some recombination events that actually occurred during the prophase of meiosis may not have been detected, because of double or multiple recombination or because of the markers used. Even though the frequency is very low, double recombination for the human PAR 1 does occur (1 [1.5%] of 64 individuals with 47,XXY in a family study [Thomas et al. 2000]; 21 [1%] of 1,912 haploid sperm by single-sperm typing [Lien et al. 2000]). Furthermore, since the DXYS15 marker is located 700–750 kb from the telomere of the short arm of the human X and Y chromosomes (Henker et al. 1991), any recombination occurring between the telomere and the DXYS15 marker will not be detected in a study that uses STS and DXYS15 markers. On the other hand, normochiasmate nondisjunction has shown that, although normal recombination is necessary for proper segregation of homologues during meiosis, it does not ensure proper segregation. The reason for this phenomenon remains unknown.

Recombination Frequency for the STS–DXYS15 Interval

On the basis of sperm-typing analysis of large numbers of sperm (2,329) from 20 men, we obtained a mean recombination frequency of 38.4% (SD 2.5%; range 31.4%–42.7%), between DXYS15 and the STS pseudogene. This is consistent with the frequencies previously reported from family studies (31.4% [Rouyer et al. 1986]; 34% [Page et al. 1987]). By use of single-sperm typing, very similar recombination frequencies between STS and DXYS15 were detected by Schmitt et al. (1994) using two donors (mean 38%) and by Lien et al. (2000) using 4 donors (mean 40.8%; range 37%–47.5%). Linkage analysis was also performed using statistical software developed by Lazzeroni et al. (1994), which takes into account potential errors such as more than one sperm per tube, contamination, and different amplification efficiencies of loci and alleles. Results from this computer analysis yielded recombination estimates of 38.8% in younger men and 37.5% in older men. These estimates are very close to our manual estimates of 39.2% and 37.8%, respectively, suggesting that these potential errors were not significant in our data.

It is well established, in yeast, that meiotic recombination can take place in the absence of the synaptonemal complex (SC) (McKim et al. 1998). Perhaps yeast uses the initiation of recombination to align homologous chromosomes and uses the SC merely to stabilize pairing of homologues. Conversely, *Drosophila* needs the SC for the initial alignment of the homologues, and recombination will not be initiated without the SC (McKim et al. 1998; Page and Hawley 2001). Thus, yeast and *Drosophila* control meiotic recombination between homologues by two quite different mechanisms. The question of whether one of these two mechanisms is followed by human beings and other organisms, or whether yet another mechanism is involved, remains unanswered.

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References

Babron MC, Constans J, Dugoujon JM, Cambon-Thomsen A, Bonaiti-Pellie C (1990) The Gm-Pi linkage in 843 French families: effect of the alleles PiZ and PiS. Ann Hum Genet 54:107–113

Baker BS, Carpenter ATC, Esposito MS, Esposito RE, Sandler L (1976) The genetic control of meiosis. Ann Rev Genet 10: 53–134

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL(1998) Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 63:861–869

Brooks LD (1988) The evolution of recombination rates. In: Michod RE, Levin BR (eds) The evolution of sex: an examination of current ideas. Sinauer Associates, Sunderland, MA, pp 87–105

Burgoyne PS (1982) Genetic homology and crossing over in the X and Y chromosomes of mammals. Hum Genet 61: 85–90

Burgoyne PS, Mahadevaiah SK (1993) Unpaired sex chromosomes and gametogenic failure. In: Sumner AT, Chandley

AC (eds) Chromosomes today. Vol 11. Chapman & Hall, London, pp 243–260
Dej KJ, Orr-Weaver TL (2000) Separation anxiety at the centromere. Trends Cell Biol 10:392–399
Elston RC, Lange E, Namboodiri KK (1976) Age trends in human chiasma frequencies and recombination fractions. II. Method for analyzing recombination fractions and applications to the ABO:nail-patella linkage. Am J Hum Genet 26:69–76
Freije D, Helms C, Watson MS, Donis-Keller H (1992) Identification of a second pseudoautosomal region near the Xq and Yq telomeres. Science 258:1784–1787
Griffin DK, Abruzzo MA, Millie EA, Sheean LA, Feingold E, Sherman SL, Hassold TJ (1995) Non-disjunction in human sperm: evidence for an effect of increasing paternal age. Hum Mol Genet 4:2227–2232
Guttenbach M, Kohn FM, Engel W, Schmid M (2000) Meiotic nondisjunction of chromosomes 1, 17, 18, X, and Y in men more than 80 years of age. Biol Reprod 63:1727–1729
Hassold TJ (1998) Nondisjunction in the human male. Curr Top Dev Biol 37:383–406
Hassold TJ, Sherman SL, Pettay D, Page DC, Jacobs PA (1991) XY chromosome nondisjunction in man is associated with diminished recombination in the pseudoautosomal region. Am J Hum Genet 49:253–260
Henke A, Fischer C, Rappold GA (1993) Genetic map of the human pseudoautosomal region reveals a high rate of recombination in female meiosis at the Xp telomere. Genomics 18:478–485
Hook EB, Hammerton TJ (1977) The frequency of chromosome abnormalities detected in consecutive newborn studies. In: Hook E, Porter IH (eds) Population cytogenetics studies in humans. Academic Press, New York, pp 63–79
Jacobs PA, Hassold TJ (1995) The origin of numerical chromosome abnormalities. Adv Genet 33:101–133
Jacobs P, Hassold T, Harvey J, May K (1989) The origin of sex chromosome aneuploidy. In: Hassold TJ, Epstein CJ (eds) Molecular and cytogenetic studies of non-disjunction. Proceedings of the 5th Annual National Down Syndrome Society Symposium, 1988. Liss, New York, pp 135–151
Jacobs PA, Hassold TJ, Whittington E, Butler G, Collyer S, Keston M, Lee M (1988) Klinefelter's syndrome: an analysis of the origin of the additional sex chromosome using molecular probes. Ann Hum Genet 52:93–109
Koehler KE, Hawley RS, Sherman S, Hassold T (1996) Recombination and nondisjunction in humans and flies. Hum Mol Genet 5:1495–1504
Laurie DA, Hultén MA (1985) Further studies on bivalent chiasma frequency in human males with normal karyotypes. Ann Hum Genet 49:189–201
Lazzeroni LC, Arnheim N, Schmitt K, Lange K (1994) Multipoint mapping calculations for sperm-typing data. Am J Hum Genet 55:431–436
Lien S, Szyda J, Schechinger B, Rappold G, Arnheim N (2000) Evidence for heterogeneity in recombination in the human pseudoautosomal region: high resolution analysis by sperm typing and radiation-hybrid mapping. Am J Hum Genet 66: 557–566
Lindahl KF (1991) His and hers recombinational hotspots. Trends Genet 7:273–276
Lorda-Sanchez I, Binkert F, Maechler M, Robinson WP, Schinzel AA (1992) Reduced recombination and paternal age effect in Klinefelter syndrome. Hum Genet 89:524–530
Lowe X, Eskenazi B, Nelson DO, Kidd S, Alme A, Wyrobek AJ (2001) Frequency of XY sperm increases with age in fathers of boys with Klinefelter syndrome. Am J Hum Genet 69:1046–1054
Lynn A, Kashuk C, Petersen MB, Bailey JA, Cox DR, Antonarakis SE, Chakravarti A (2000) Patterns of meiotic recombination on the long arm of human chromosome 21. Genome Res 10:1319–1332
MacDonald M, Hassold T, Harvey J, Wang LH, Morton NE, Jacobs P (1994) The origin of 47,XXY and 47,XXX aneuploidy: heterogeneous mechanisms and role of aberrant recombination. Hum Mol Genet 3:1365–1371
Martin RH, Chernos JE, Rademaker AW (1991) Effect of cryopreservation on the frequency of chromosomal abnormalities and sex ratio in human sperm. Mol Reprod Dev 30: 159–163
Martin RH, Spriggs E, Ko E, Rademaker AW (1995) The relationship between paternal age, sex ratios, and aneuploidy frequencies in human sperm, as assessed by multicolor FISH. Am J Hum Genet 57:1395–1399
McKim KS, Green-Marroquin BL, Sekelsky JJ, Chin G, Steinberg C, Khodosh R, Hawley RS (1998) Meiotic synapsis in the absence of recombination. Science 279:876–878
Molnar M, Bahler J, Kohli J, Hiraoka Y (2001) Live observation of fission yeast meiosis in recombination-deficient mutants: a study on achiasmate chromosome segregation. J Cell Sci 114:2843–2853
Page DC, Bieker K, Brown LG, Hinton S, Leppert M, Lalouel J-M, Lathrop M, Nystrom-Lahti M, de la Chapelle A, White R (1987) Linkage, physical mapping, and DNA sequence analysis of pseudoautosomal loci on the human X and Y chromosomes. Genomics 1:243–256
Page SL, Hawley RS (2001) c(3)G encodes a Drosophila synaptonemal complex protein. Genes Dev 15:3130–3143
Rappold GA (1993) The pseudoautosomal regions of the human sex chromosomes. Hum Genet 92:315–324
Reeves RH, Crowley MR, O'Hara BF, Gearhart JD (1990) Sex, strain, and species differences affect recombination across an evolutionarily conserved segment of mouse chromosome 16. Genomics 8:141–148
Robbins WA, Baulch JE, Moore DN, Weier HU, Blakey D, Wyrobek AJ (1995) Three-probe fluorescence in situ hybridization to assess chromosome X, Y, and 8 aneuploidy in sperm of 14 men from two healthy groups: evidence for a paternal age effect on sperm aneuploidy. Reprod Fertil Dev 7:799–809
Rouyer F, Simmler M-C, Johnsson C, Vergnaud G, Cooke HJ, Weissenbach J (1986) A gradient of sex linkage in the pseudoautosomal region of the human sex chromosomes. Nature 319:291–295
Schmitt K, Lazzeroni LC, Foote S, Vollrath D, Fisher EM, Goradia TM, Lange K, Page DC, Arnheim N (1994) Multipoint linkage map of the human pseudoautosomal region, based on single-sperm typing: do double crossovers occur during male meiosis? Am J Hum Genet 55:423–430
Schmitt K, Vollrath D, Foote S, Fisher EM, Page DC, Arnheim N (1993) Four PCR-based polymorphisms in the pseudoautosomal region of the human X and Y chromosomes. Hum Mol Genet 2:1978

Shi Q, Spriggs E, Field LL, Ko E, Barclay L, Martin RH (2001) Single sperm typing demonstrates that reduced recombination is associated with the production of aneuploid 24,XY human sperm. Am J Med Genet 99:34–38

Shonn MA, McCarroll R, Murray AW (2000) Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. Science 289:300–303

Tanzi RE, Watkins PC, Stewart GD, Wexler NS, Gusells JF, Haines JL (1992) A genetic linkage map of human chromosome 21: analysis of recombination as a function of sex and age. Am J Hum Genet 50:551–558

Thomas NS, Collins AR, Hassold TJ, Jacobs PA (2000) A reinvestigation of non-disjunction resulting in 47, XXY males of paternal origin. Eur J Hum Genet 8:805–808

Yu J, Lazzeroni L, Qin J, Huang MM, Navidi W, Erlich H, Arnheim N (1996) Individual variation in recombination among human males. Am J Hum Genet 59:1186–1192

Zhang P, Hawley RS (1990) The genetic analysis of distributive segregation in Drosophila melanogaster. II. Further genetic analysis of the nod locus. Genetics 125:115–127

Zhang P, Knowles BA, Goldstein LS, Hawley RS (1990) A kinesin-like protein required for distributive chromosome segregation in Drosophila. Cell 62:1053–1062

Zwick ME, Salstrom JL, Langley CH (1999) Genetic variation in rates of nondisjunction: association of two naturally occurring polymorphisms in the chromokinesin nod with increased rates of nondisjunction in *Drosophila melanogaster*. Genetics 152:1605–1614