**X Chromosome Effect on Maternal Recombination and Meiotic Drive in the Mouse**

Elena de la Casa-Esperón,*1 J Concepción Loredo-Osti,†1 Fernando Pardo-Manuel de Villena,‡ Pardo-Manuel de Villena* and Carmen Sapienza*###2

*Fels Institute for Cancer Research and Molecular Biology and **Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, †Department of Genetics and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599-7264 and ‡Department of Human Genetics and Department of Medicine, McGill University, and the Research Institute of the McGill University Health Centre, Montreal, Quebec H3G 1A4, Canada

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**ABSTRACT**

We observed that maternal meiotic drive favoring the inheritance of DDK alleles at the *Om* locus on mouse chromosome 11 was correlated with the X chromosome inactivation phenotype of (C57BL/6-Jpgk1a1 × DDK)F1 mothers. The basis for this unexpected observation appears to lie in the well-documented effect of recombination on maternal drive that results from nonrandom segregation of chromosomes. Our analysis of genome-wide levels of meiotic recombination in females that vary in their X-inactivation phenotype indicates that an allelic difference at an X-linked locus is responsible for modulating levels of recombination in oocytes.

**HOMOLOGOUS** chromosome pairing and recombination contribute significantly to the processes of DNA repair, fidelity of chromosome segregation, and the generation of genetic diversity. In general, each pair of chromosomes (or, more precisely, each pair of chromosome arms; Dutrillaux 1986; Pardo-Manuel de Villena and Sapienza 2001) undergoes at least one recombination event in most organisms (Mather 1936; reviewed in Pardo-Manuel de Villena and Sapienza 2001). Although some variation in the frequency of recombination is observed as a result of differences in sex, chromosome size, DNA sequence, or chromatin structure (reviewed in Robinson 1996), genetic changes that dramatically alter the frequency of recombination are associated with decreased fitness, increased nondisjunction, and aneuploidy (reviewed in Kleckner 1996; Roeder 1997; Moore and Orr-Weaver 1998).

Meiotic recombination also has a well-documented effect on genetic systems in which there is unequal segregation of homologous chromosomes between the ovum and polar bodies during female meiosis (maternal meiotic drive; Rhoades and Dempsey 1966; Pardo-Manuel de Villena et al. 2000a). This form of meiotic drive requires heterozygosity for “sensitive” and “insensitive” alleles at a “Responder” locus (Lyttle 1991) such that the alleles can be distinguished from one another and one allele segregated preferentially to the polar body. If meiotic drive occurs at the first meiotic division, preferential segregation of chromosomes carrying a sensitive allele at a Responder can take place only if both chromatids of each homolog bear the same allele. In contrast, unequal segregation at the second meiotic division can occur only if the members of each dyad carry different alleles at a Responder (*i.e.*, when a recombination event has taken place proximal to the Responder; Pardo-Manuel de Villena et al. 2000a).

We used these requirements to formulate a general genetic test to determine whether any instance of maternal transmission ratio distortion (TRD, defined as a significant departure from the Mendelian inheritance ratio expected, regardless of the cause) is the result of meiotic or postmeiotic processes (MII; Pardo-Manuel de Villena et al. 2000a). We used the test to demonstrate that maternal TRD at the *Om* locus on mouse chromosome 11 is the result of meiotic drive at the second meiotic division (Pardo-Manuel de Villena et al. 1996, 1997, 2000a). Although the overall level of meiotic drive was similar in all of our experiments, the level observed among the offspring of individual females was variable. We reasoned that, because the F1 females used in our experiments were genetically identical, differences between individual females in the transmission of alleles at *Om* must be ascribed either to chance or to some epigenetic difference between the females.

We investigated whether one potentially highly variable epigenetic character of F1 females, their X chromosome inactivation phenotype (defined as the proportion of cells with a particular X chromosome active in an
individual female), influenced levels of meiotic drive at *Om*. Our results indicate that allelic differences at one or more X-linked loci affect the level of meiotic drive at *Om* and that this effect is mediated by modulation of the overall level of meiotic recombination in oocytes. The existence of X chromosome-linked genetic variability in the overall frequency of meiotic recombination has potential implications for the mechanism by which sex-specific differences in recombination are obtained.

**MATERIALS AND METHODS**

**Mouse crosses:** The backcross used in this study is (C57BL/6-*Pgrpl* × DXK) F1 × C57BL/6 (dams were listed first and sires were listed second). Of the 555 offspring analyzed, 490 have been described previously (de la Casa-Esperón et al. 2000; Pardo-Manuel de Villena et al. 2000a). All offspring were obtained before the first female was 1 year old. All animals described in this report were treated according to the recommendations of the Institutional Animal Care and Use Committee of Temple University School of Medicine.

**X-inactivation assay:** Total RNA extracted from tail biopsies was analyzed for the expression of *Pgrpl* alleles at the single nucleotide primer extension assay, as described (Singer-Sam et al. 1992; Latham et al. 2000). We found that the DKK strain carries the *Pgrpl* allele by DNA sequencing and by detecting the “b” allele polymorphism described previously (Boer et al. 1990). RT-PCR was performed using primers 5’-AGCTGAGCCTCACTGTCCA-3’ (upstream) and 5’-TCGCCAAAATTGAT-3’ (downstream) followed by a primer extension step using primer 5’-TCCGAGCCTCACTGTTCAC-3’ (Singer-Sam et al. 1992). The allele-specific products were separated by electrophoresis and the amount of radioactivity incorporated in each allele was quantified by phosphorimaging. The analysis of each female was done in triplicate and the data presented represent the average values for the relative expression of the alleles.

**Genotype determination:** DNA extraction from tail biopsies, gel electrophoresis, and autoradiography were performed as described previously (Maniatis et al. 1982; Hogan et al. 1986). The following genetic markers [and their map position in cytogenetic maps] were scored: D1Mit166 (9); D1Mit251 (18.5); D1Mit251 (38.1); D1Mit132 (43.1); D1Mit390 (63.3); D1Mit424 (81.6); D1Mit270 (92.3); D1Mit293 (109.6); D2Mit117 (53); D2Mit83 (16); D2Mit244 (33); D2Mit37 (45); D2Mit276 (65); D2Mit285 (86); D2Mit200 (107); D3Mit164 (24.3); D4Mit203 (11.2); D5Mit63 (22); D5Mit74 (41); D5Mit254 (64.1); D5Mit163 (87.6); D5Mit227 (3.2); D4Mit286 (14.5); D4Mit164 (28.6); D4Mit58 (48.5); D4Mit37 (56.5); D4Mit339 (65.7); D4Mit256 (82.7); D5Mit334 (1); D5Mit79 (26); D5Mit15 (39); D5Mit314 (59); D5Mit31 (78); D5Mit143 (86); D6Mit236 (3.1); D6Mit384 (27.5); D6Mit65 (46); D6Mit25 (65); D6Mit201 (74.1); D7Mit178 (0.5); D7Mit117 (11); D7Mit310 (18); D7Mit30 (37); D7Mit323 (50); D7Mit291 (66); D7Mit223 (72.4); D8Mit157 (2); D8Mit191 (21); D8Mit348 (44); D8Mit166 (56); D8Mit222 (61); D9Mit26 (6); D9Mit90 (99); D9Mit97 (29); D9Mit270 (45); D9Mit212 (61); D9Mit281 (68); D10Mit298 (3); D10Mit214 (19); D10Mit40 (29); D10Mit66 (49); D10Mit233 (62); D10Mit269 (70); D11Mit71 (1.1); D11Mit131 (13); D11Mit20 (20); D11Mit70 (37); D11Mit66 (47); D11Mit67 (57); D11Mit168 (71); D12Mit182 (2); D12Mit112 (22); D12Mit260 (45); D12Mit150 (59); D13Mit16 (10); D13Mit63 (26); D13Mit99 (40); D13Mit107 (48); D13Mit78 (75); D14Mit10 (3); D14Mit54 (12.5); D14Mit234 (22.5); D14Mit162 (44.3); D14Mit170 (63); D15Mit12 (4.7); D15Mit100 (21); D15Mit234 (34.2); D15Mit189 (48.5); D15Mit16 (61.7); D15Mit79 (66.2); D16Mit182 (34.3); D16Mit166 (21); D16Mit140 (42.8); D16Mit191 (57.8); D16Mit106 (71.45); D17Mit78 (8.2); D17Mit175 (17.7); D17Mit7 (32.3); D17Mit39 (45.5); D17Mit123 (56.7); D18Mit67 (4); D18Mit60 (16); D18Mit123 (31); D18Mit47 (50); D18Mit144 (57); D19Mit42 (5); D19Mit111 (15); D19Mit66 (41); D19Mit137 (55.7); DXMit124 (2.8); DXMit166 (15.5); DXMit210 (29.5); DXPs29 (42.5); DXMit117 (50.8); and DXMit135 (69). Markers were selected to maximize the detection of recombination events. For each chromosome, polymorphic markers in the most proximal and distal regions were selected, as well as the number of additional markers to cover most of the length of the chromosome at intervals of 25 cM [in which multiple crossovers are rare (Lawrie et al. 1995; Silver 1995)]. On average, the genetic distance between consecutive loci is 14 cM (range 3–27 cM). In some cases markers were typed more than once, particularly to confirm multiple recombinants. Genotype information at loci on the X chromosome was reported previously (with the exception of data for DXMit135), as well as the majority of the data for markers D11Mit71 and D11Mit66 (de la Casa-Esperón et al. 2000; Pardo-Manuel de Villena et al. 2000a). D11Mit66 is tightly linked to *Om* and was used to infer the genotype at this locus (Pardo-Manuel de Villena et al. 2000b). Oligonucleotide primers for “D_Mit” loci (Dietrich et al. 1994) were purchased from Research Genetics (Huntsville, AL) and oligonucleotide primers for DXPs29 were synthesized as described (Simmler et al. 1995). PCR reactions were performed as suggested by the manufacturer.

**Statistical analysis of the X-inactivation effect on TRD at *Om* and X chromosome recombination:** To test for an effect of X-inactivation on TRD at *Om*, the probability of inheriting a maternal DDK allele at *Om* vs. a B6 allele was analyzed on 555 offspring of F1 females by using generalized linear models (GLM) with a logit link function. A two-sided test was performed under the null hypothesis that the *Om* genotype of offspring was unrelated to the X-inactivation phenotype of the mothers.

We also used GLM to test for an effect of X-inactivation on recombination on the X chromosome in the same 555 offspring. The number of recombination events was assumed to be Poisson distributed and a one-sided test was performed under the hypothesis that females with “more DDK X-active” X-inactivation phenotypes have higher levels of recombination than females with “less DDK X-active” phenotypes.

**Tetrad frequency estimation and statistical analysis:** A simple comparison of the distribution of recombination class frequencies between the two groups (Table 1) might not detect real changes in recombination rates because only a single product from each meiosis is analyzed. We used the following biological considerations to promote the analysis of recombination in the two groups of females:

1. Recombination takes place in meiotic tetrads but only one product of each tetrad is recovered from each female meiosis (Figure 1). Under the assumption of no chromatic interference, this means that when a single crossover occurs on a pair of homologs (so-called E tetrads, in which a single crossover occurs between two non-sister chromatids), two of the potential meiotic products will be recombinant, two will be nonrecombinant, and recovery of either type of product will occur with equal probability (Weinstein 1996). If two crossovers occur in a tetrad (E tetrads, in which at least one crossover occurs involving two, three, or four chromatids), a double recombinant chromosome will be recovered one-quarter of the time, a single recombinant will be recovered one-half of the time, and a nonrecombin-
RESULTS

X-inactivation phenotype of (C57BL/6-Pgk1<sup>a</sup> × DDK)F<sub>1</sub> females: Although F<sub>1</sub> females are identical in terms of their genome, the presence of two different X chromosomes and the stochastic component of the X chromosome inactivation process provides ample opportunity to generate potentially large epigenetic differences between individual females if those differences reflect the expression of X-linked genes (Heard et al. 1997). We derived F<sub>1</sub> females by mating C57BL/6-Pgk1<sup>a</sup> (PGK) females with DDK males. PGK is a C57BL/6 (B6) congenic strain in which the central region of the B6 X chromosome has been replaced by the homologous region from a Danish wild mouse (Nielsen and Chapman 1977). This strain carries the "a" allele at the X-linked Pgk1 locus and the "c" allele at the X chromosome controlling element locus, Xce (Nielsen and Chapman 1977; Johnston and Cattanach 1981), while the DDK strain carries the "b" allele at Pgk1 (see materials and methods). The Xce allele carried by the DDK strain had not been characterized previously, but the X chromosome haplotype of this strain in the vicinity of Xce is the same (our unpublished data) as that of strains that carry the Xce<sup>a</sup> allele (Simmler et al. 1993). F<sub>1</sub> females constructed between these strains are thus Xce<sup>a</sup>-Pgk1<sup>a</sup> / Xce<sup>a</sup>-Pgk1<sup>b</sup>.

We quantified the allele-specific expression of Pgk1 mRNA in tail biopsies from 38 F<sub>1</sub> females using a single nucleotide primer extension assay (Singer-Sam et al. 1992; Latham et al. 2000; Figure 2). Tail biopsies were selected as the tissue for X-inactivation analysis because they could be obtained without sacrificing the animal and because they contain cells derived from all primary germ layers and so might be representative of the animal as a whole. Each circle in Figure 2 represents the X-inactivation phenotype of an individual female, expressed as the percentage of each individual’s cells in which the DDK X chromosome remains active (see materials and methods).

The X-inactivation phenotype of the population of females is shifted in the direction of <50% of cells having the DDK X chromosome active because the PGK X chromosome carrying the "strong" Xce<sup>a</sup> allele has a higher probability of remaining active than the DDK X...
chromosome that carries a “weak” Xce* allele (Johnston and Cattanach 1981). The mean X-inactivation phenotype of the population of females is 25% of an individual’s cells in which the DDK X chromosome remains active (Figure 2). This observation is in agreement with previous estimates obtained for F1 females that are also Xce*/Xce* heterozygotes (Johnston and Cattanach 1981; Ploenje et al. 2000). The mean and variability observed among the population of F1 females is what one would expect if the X-inactivation phenotype of an individual were the result of a combination of genetic factors (heterozygosity for Xce alleles of different strength) and stochastic factors (some randomness in the choice of which X chromosome to inactivate).

TRD at Om as a function of the X-inactivation phenotype of the mother: Because we observed apparent differences in the level of TRD at Om among the offspring of individual F1 females (data not shown), we reasoned that such differences must be the result of chance or epigenetic differences between these females. We tested whether the level of TRD in favor of DDK alleles at Om was related to a female’s X-inactivation phenotype.

With data from 535 offspring, we fitted a logistic model to the inheritance of maternal alleles at Om as a function of the X-inactivation phenotype of their F1 mothers. The analysis shows a positive regression of TRD at Om on the X-inactivation score (P < 0.025). Offspring of mothers having a low percentage of cells with an active DDK X chromosome have lower levels of TRD at Om than offspring of mothers having a higher percentage of cells with an active DDK X chromosome (Figure 3).

Recombination on chromosomes 11 and X as a function of the X-inactivation phenotype of the mother: Because TRD at Om is the result of MI meiotic drive, which is, in turn, dependent on the occurrence of recombination between the Responder and the centromere of chromosome 11 (Pardo-Manuel de Villena et al. 2000a), the effect of a mother’s X-inactivation phenotype on TRD could be due to differences between the two groups of females in the level of recombination, either on chromosome 11 in particular or over all of the chromosomes. As a preliminary measure of whether recombination on chromosome 11 was affected by X-inactivation, the genotype of the 535 offspring used in the analysis in Figure 3 was analyzed at D11Mit71 (which is closely linked to the centromere of chromosome 11) and Om. We found that the recombination fraction observed in the offspring of mothers with <25% (the mean of the population; see Figure 2) of cells with an active DDK X chromosome is 0.419, while 0.466 is the observed recombination fraction in the offspring of the rest of the females (>25% of cells with the DDK X chromosome active). Although this simple comparison of the chromosome 11 recombination fraction is not significant, the direction of the difference is consistent with the hypothesis that females with a lower percentage of cells having an active DDK X chromosome have lower levels of meiotic recombination on chromosome 11 and, consequently, lower levels of meiotic drive.

As an additional measure of whether a female’s X-inactivation phenotype had a genome-wide effect on recombination, we analyzed the number of recombination events on chromosome X in all 535 offspring as a function of the X-inactivation phenotype of their mothers using a generalized linear model (see MATERIALS AND METHODS). Chromosome X was chosen for analysis because most of the chromosome haplotype data was already available from a previous study (De la Casa-Esparón et al. 2000). The one-sided test for the regression coefficient was significant (P < 0.04).

Genome-wide recombination as a function of X-inactivation: Chromosome haplotypes inherited by offspring of F1 females with a different X-inactivation phenotype: Because our preliminary analysis indicated that any effect of X-inactivation on recombination was not limited to a single chromosome, we extended our analysis of chromosome haplotypes to all 20 chromosomes (see MATERIALS AND METHODS), using only offspring of F1 females from the lower and upper tails of the X-inactivation distribution (less DDK Xactive females, having ≤13% of cells with the DDK X chromosome active, and more DDK Xactive females, having ≥37% of cells with the DDK X chromosome active, respectively; Figure 2). These lower and upper values were chosen because each group would then contain the same number of F1 females (5) and a similar number of offspring (80–81). In addition, these groups showed a substantial difference in the level of TRD observed at Om among offspring (58% in the less DDK Xactive group vs. 75% in the more DDK Xactive group; Figure 3). The haplotypes of all 20 chromosomes inherited by the offspring of these females were classified according to the number of recombination events observed, per chromosome
X-inactivation Effect on Recombination

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**Figure 3.**—Effect of X-inactivation on TRD at Om. Analysis of the inheritance of maternal alleles at Om in 535 offspring as a function of the X-inactivation phenotype of their F1 mothers. The trend line is given by \(1/[1 + \exp(0.03304 - 0.02026 X_i)]\), the predictor equation for the probability of inheriting a maternal DDK allele at Om as a function of the F1 females’ X-inactivation phenotype (Xi is measured as the percentage of cells having an active DDK X chromosome.) The coefficients of this equation were obtained from a GLM with a logit link function. Superimposed circles represent the percentage of DDK alleles at Om observed in four groups of offspring with respect to the average X-inactivation phenotype of their mothers and vertical lines represent the 95% confidence intervals. Groups were pooled according to the X-inactivation phenotypes of the F1 females (see Figure 2) and data for the transmission of DDK alleles at Om to their offspring are shown below the graph.

<table>
<thead>
<tr>
<th>X-inactivation group average (%)</th>
<th>No. offspring that inherit DDK alleles at Om</th>
<th>Total no. offspring</th>
<th>% DDK alleles at Om</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less DDK X-active (≤13%)</td>
<td>9.3</td>
<td>47</td>
<td>81</td>
<td>58</td>
</tr>
<tr>
<td>14–26%</td>
<td>20.1</td>
<td>132</td>
<td>230</td>
<td>57</td>
</tr>
<tr>
<td>27–36%</td>
<td>30.6</td>
<td>88</td>
<td>141</td>
<td>62</td>
</tr>
<tr>
<td>More DDK X-active (≥37%)</td>
<td>41.9</td>
<td>62</td>
<td>83</td>
<td>75</td>
</tr>
</tbody>
</table>

Analysis of meiotic tetrad distributions: Because meiotic recombination is a tightly controlled process, a simple analysis of the number of recombination events observed may not detect true changes in recombination levels, given that only one of the four products of each female meiosis is recovered in the offspring (see materials and methods and Figure 1). To overcome this limitation we have used the method described by Weinstein (1936) to estimate the distribution of chromosome haplotypes among meiotic tetrads. Tetrad frequency distributions (the fraction of \(E_0\), \(E_2\), and \(E_3\) tetrads) were estimated for each chromosome, using the data in Table 1, with the constraint that \(E_0\) tetrads were not permitted (see materials and methods). Average profile values for all of the chromosomes were obtained (Table 2) by dividing the totals of the frequencies by the sum (over chromosomes) of the offspring. The null hypothesis (H0) for this analysis is that the genome-wide level of meiotic recombination is the same in females that have been selected from opposite tails of the distribution of X-inactivation phenotypes. The alternative hypothesis (H1) is that the genome-wide level of recombination is greater in the more DDK X-active group (i.e., that group in which a higher level of meiotic drive at Om is observed). Because an increase in recombination will increase the frequency of \(E_2\) tetrads at the expense of \(E_1\) tetrads (see materials and methods), we have tested this hypothesis by comparing the fraction of \(E_2\) tetrads between less DDK X-active and more DDK X-active females. Significance was assessed by permutation analysis (using 10,000 replicates) in which offspring were assigned randomly to one group or the other and the tetrad frequencies computed and compared with the frequencies obtained in the experiment. The fraction of replicates giving a result more extreme than the experimental result is the \(P\) value given in Table 2 (\(P = 0.01\)), indicating significantly more recombination in the more DDK X-active group of females than in the less DDK X-active group.

**DISCUSSION**

The effect of X-inactivation on recombination and TRD: We observed variability in the level of TRD at Om
TABLE 1

Chromosome haplotypes inherited by offspring of females with different X-inactivation phenotype

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Less DDK X-active (n = 81)</th>
<th>More DDK X-active (n = 80)</th>
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<tbody>
<tr>
<td></td>
<td>NR</td>
<td>SR</td>
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<tr>
<td>1</td>
<td>28</td>
<td>35</td>
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<tr>
<td>2</td>
<td>20</td>
<td>46</td>
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<td>3</td>
<td>19</td>
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<td>4</td>
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<td>5</td>
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<td>6</td>
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<td>18</td>
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<td>37</td>
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<tr>
<td>19</td>
<td>52</td>
<td>28</td>
</tr>
<tr>
<td>X</td>
<td>29</td>
<td>45</td>
</tr>
</tbody>
</table>

The data represent the number of offspring inheriting nonrecombinant (NR), single (SR), double (DR), or triple (TR) recombinant chromosomes from less DDK X-active and more DDK X-active F1 females (see Figure 2).

One additional offspring of more DDK X-active females was available for chromosomes 2, 3, 8, 12, 14, and 16, and three additional offspring were available for chromosome X and for TRD analysis at Om. These extra DNA samples were lost during the course of the experiment and could not be analyzed for the other chromosomes.

among the offspring of (PGK × DDK)F1 females. We tested the hypothesis that this variability was due to epigenetic differences between the females in X-inactivation phenotype. Among 535 offspring of 38 females, we found that the level of TRD at Om was correlated with the X-inactivation phenotype of the female (Figure 3). Because TRD at Om is the result of MII meiotic drive and MII meiotic drive is affected by recombination (Pardo-Manuel de Villena et al. 2000a), we examined whether a female’s X-inactivation phenotype might affect TRD by altering the overall level of meiotic recombination in her oocytes. Analysis of available genotype information on all 535 offspring at X chromosome loci (de la Casa-Esperón et al. 2000) indicated that the level of recombination on chromosome X was correlated with the X-inactivation phenotype of the F1 females. This observation argued that any effect on recombination was not specific to chromosome 11. Therefore, we extended the haplotype analysis to all 20 chromosomes, using only the offspring of females from the two extremes of the X-inactivation distribution. Comparison of estimates of tetrad distributions (E1, E2, E3) over all 20 chromosomes indicates that there are significantly more crossovers in oocytes from more DDK X-active females than in oocytes of less DDK X-active females.

We interpret these data to mean that an allelic difference at an X-linked locus (or loci), subject to X-inactivation, is responsible for the observed difference in recombination. The expression of a DDK allele at this locus increases levels of meiotic recombination relative to the PGK allele and, as a consequence, also increases levels of MII meiotic drive favoring the inheritance of DDK alleles at Om (Pardo-Manuel de Villena et al. 2000a).
Our analysis assumes that the X-inactivation phenotype measured on tail biopsies from each female is representative of the population of primordial germ cells that gave rise to the offspring in which recombination was analyzed. Although there is no way to conduct a post hoc analysis of this assumption, previous studies have shown similar X-inactivation phenotypes among different tissues of a given individual, while larger differences may be observed between females with the same genotype (Plenge et al. 2000). In any case, if the X-inactivation phenotype of a female does have an effect on recombination, misclassification of a female is expected to obscure such an effect rather than enhance it.

One descriptive measure of the level of difference between the two groups of females is that there are ~20% more multiple crossover tetrads ($E_2 + E_3$) in more DDK X-active females than in less DDK X-active females. Although a 20% increase in the fraction of tetrads having more than one crossover may not seem large, one must keep in mind that the number and distribution of recombination events during meiosis is very well controlled. More than three crossovers are rarely observed (reviewed in Kleckner 1996; Roeder 1997) because the presence of one crossover interferes with the occurrence of additional events on the same chromatid in a distance-dependent manner [crossover or chiasma interference (Sturtevant 1915; Muller 1916)]. While chiasma interference suppresses the number of crossovers on a chromosome, multiple studies also indicate that a minimum of one crossover per chromosome arm is required to ensure proper chromosome segregation (Hulten 1974; Dutrillaux 1986; Pardo-Manuel de Villena and Sapienza 2001). In other words, one crossover is required but more than one is suppressed by chiasma interference. In fact, >80% of the total variance in recombination rates among mammals can be explained by a requirement for one recombination event per chromosome arm (Dutrillaux 1986; Pardo-Manuel de Villena and Sapienza 2001). In this light, a 20% difference between the two groups of females does not seem insignificant.

Implications of the tetrad model for testing differences in recombination: A major premise of our analysis is that an increase in the level of recombination is accomplished by increasing the proportion of tetrads that have multiple crossovers. In practice this is accomplished by increasing the proportion of $E_2$ tetrads at the expense of $E_1$ tetrads. This results in the counterintuitive prediction that the largest class of chromosome haplotype recovered (single recombinants) is uninformative for discerning differences in recombination because one-half of the products of both $E_1$ and $E_2$ tetrads are single recombinants (Figure 1). If the great majority of tetrads are $E_1$ and $E_3$, no difference in the number of single recombinants recovered is predicted (or observed; Table 1) by shifting $E_1$ tetrads to $E_2$ tetrads. An increase in the overall level of recombination as a result of shifting tetrads from the $E_1$ to the $E_2$ class will appear as the loss of chromosomes from the nonrecombinant class and the addition of an equal number of chromosomes to the double recombinant class (Table 1).

In a related vein, we note that our estimates of $E_1$ tetrad frequency in the two groups of females (Table 2) are counter to what might be expected; i.e., there is a larger estimated fraction of $E_1$ tetrads in the less DDK X-active females. However, the significance of this observation, in both the statistical and biological senses, is unclear. We recovered only 12 triple recombinant chromosomes (and 3 of these occurred within a single individual) among >3200 examined. [We note, however, that recovery of this number does not appear to be low. In comparison, Broman et al. (2002) identified only 3 triple recombinant chromosomes in an experiment of similar size (3760 chromosomes examined) and with much higher density map coverage.] Both the relative rarity of triple recombinant chromosomes and the large uncertainty associated with the estimation of $E_2$ tetrads (because it is based on recovery of triple recombinant chromosomes in one-eighth of the products of all $E_1$ tetrads) make it difficult to draw any robust conclusions about the effect of X-inactivation on the proportion of $E_1$ tetrads. In spite of the errors resulting from including triple recombinants in our tetrad estimation, recombination differences are still statistically significant between less DDK X-active and more DDK X-active females.

A role for the X chromosome in sex-specific recombination rates? While some studies have focused their interest on variability in recombination over particular intervals in different organisms (Reeves et al. 1991; Yu et al. 1996; Simianer et al. 1997), our study addresses the control of overall recombination. Studies on genome-wide recombination in humans have shown interindividual variation in female recombination (Broman et al. 1998), as we observe in the mouse. Additional differences in the number and distribution of recombination events have been described between different chromosomes (Karack et al. 1992, 1999; Lawrie et al. 1995; Anderson et al. 1999), between different inbred mouse strains (Speed 1977; Anderson et al. 1999), and between male and female meiosis (Polani 1972; Speed 1977; Jagiello and Fang 1979; Lawrie et al. 1995). The latter observation has led some authors to propose that levels of recombination are affected by one or more genes on the X chromosome (Lynn et al. 2000; Broman et al. 2002). Because the only X chromosome in mammalian spermatoctyes is thought to be inactive, in contrast to the two active X chromosomes in oocytes, the product(s) of X-linked gene(s) could be responsible for changes in the baseline rate of recombination.

How does X-inactivation affect a process that takes place in a cell with two active X chromosomes? An important question that remains unanswered by our study concerns the mechanism by which the X-inactivation phenotype of a female may affect the expression of an X-linked gene involved in meiotic recombination. X
chromosome reactivation occurs in the germ cells of the fetal ovary at or shortly before the onset of meiosis (Kratzer and Chapman 1981; McLaren 1983). Although the reported timing of these two processes varies among different studies (Kratzer and Chapman 1981; Monk and McLaren 1981; Tam et al. 1994), it seems plausible that the inactive X chromosome must be reactivated before pairing and recombination (Kratzer and Chapman 1981). If the gene product affecting recombination levels is synthesized by the oocyte itself, it is possible that a delay in the reactivation of the allele that was inactive in the primordial germ cell, or a comparatively “long-lived” product of the single active allele prior to X chromosome reactivation, would be compatible with our observations. Another possibility is that the product of the X-linked gene is not expressed by the oocyte itself but is provided by adjacent somatic cells. The somatic pattern of X inactivation of an individual female would, in such a case, be reflected in her oocytes by the relative amount of product from each allele that reached the oocytes at the time of meiotic recombination.

Some potential consequences of an unusual maternal effect: The phenomenon we have described indicates that a mother may have unexpected effects on the transmission of genes to her offspring. In this regard, it is worth noting that the definition of a maternal effect is that the genotype of the mother determines the phenotype of the offspring. In the situation we have described in this report, it is the phenotype of the mother (the fraction of her cells with a particular X chromosome active) that influences the genotype of her offspring. Although maternal effects have been described in many organisms, we are unaware of any other examples of this type.

The epigenetic effect that we observe on the level of female meiotic recombination has the potential to alter the behavior of genes in natural populations in several ways: (1) alteration of allele frequencies at loci subject to meiotic drive (Buckler et al. 1999; Paro-Manuel de Villena et al. 2000a); (2) alteration of the linkage relationship between alleles at loci that have been coselected or co-adapted during evolution (Wu and Palopoli 1994); and (3) effects on the level of chromosome nondisjunction during meiosis, with possibly widespread consequences (Jacobs and Hassold 1995; Warnburton 1997; Brown et al. 2000).

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