Distortion of Mendelian recovery ratio for a mouse HSR is caused by maternal and zygotic effects

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Summary

An HSR in chromosome 1 which is found in many feral populations of Mus musculus domesticus was shown in previous studies to consist of a high-copy long-range repeat cluster. One such cluster, MUT, showed distorted transmission ratios when introduced by female parents. MUT/+ offspring were preferentially recovered at the expense of +/+ embryos in the progeny of MUT/+ x cJ +/+ but were found at the expected 1:1 ratio in reciprocal crosses. Preferential recovery of maternal MUT was due to lethality of postimplantation +/+ embryos. There was no distortion of the recovery ratio in MUT/+ x MUT/MUT progeny: maternal MUT and + clusters were present among live implants at a 1:1 ratio. Maternal and zygotic effects therefore contribute to the phenomenon. The mechanism of their interaction is unknown.

1. Introduction

There are various causes of non-Mendelian recovery ratios of alleles in offspring from a heterozygous parent. Biased recovery is due to viability differences between different types of zygotes or gametes (reviews: Lyon, 1991; Lyttle, 1993; Silver, 1993) or to preferential transmission of one allele to more than 50% of gametes at the expense of the other allele ('meiotic drive' sensu strictu; review: Ruvinsky, 1995).

Preferential transmission of a chromosome 1 double-band homogeneously staining region (HSR) was found in heterozygous females of Mus musculus musculus (Agulnik et al., 1990). The biased recovery of the double-band HSR has been attributed to preferential segregation of the HSR chromosome to the oocyte rather than the polar bodies in female meiosis.

Standard chromosomes 1 harbour a low-copy cluster of long-range repeats (LRRs; ~ 50 copies, repeat length ~ 100 kb, locus D1Lub1; Purmann et al., 1992; Traut et al., 1992). Chromosome 1 HSRs are high-copy LRR clusters (> 300 copies; Kunze et al., 1996). They occur as single-band HSRs in M. m. domesticus and as double-band HSRs in M. m. musculus. The double-band HSR was derived from a single-band HSR by a paracentric inversion during evolution of the semispecies (Winking et al., 1991).

In the present study, transmission of a M. m. domesticus HSR was examined. We show that segregation distortion of the HSR results from post-implantation loss of embryos due to maternal and zygotic effects.

2. Materials and methods

Mouse strains AKR, C57BL/6 (abbreviated B6 in the following) and NMRI contain chromosomes 1 with a low-copy LRR cluster (Kunze et al., 1996 and unpublished data), designated wild-type (+) cluster. The high-copy LRR cluster MUT originated from a feral M. m. domesticus mouse trapped near Mutten, Switzerland. MUT consists of about 900 LRRs (Kunze et al., 1996).

To facilitate identification of chromosome 1, a strain homozygous for the Robertsonian Rb(l-18)10Rma chromosome (abbreviated Rb in the following) in an NMRI background was used in some crosses. Rb harbours the + cluster and, thus, the strain was Rb+/Rb+. We created a new strain by introducing MUT into the Rb strain. After six backcross generations, homozygous Rb MUT/Rb MUT mice were generated by interbreeding and maintained as a stock.

Rb homozygous animals do not show enhanced levels of non-disjunction. Non-disjunction is enhanced, however, in Rb heterozygous animals (Gropp...
& Winking, 1981). In crosses with these animals, only euploid near-term foetuses were counted.

Preimplantation loss was determined by comparing either the number of blastocysts (day 4 p.c.; plug day = 1 p.c.) or the number of implantation sites with the respective number of corpora lutea. Postimplantation loss was determined by comparing the number of live embryos (day 10 to day 13 p.c.) with that of implantation sites. Genotypes of live embryos (day 10 to day 13 p.c.) and live near-term foetuses (day 18 to day 19 p.c.) were determined by C-banding of metaphase chromosomes (Sumner, 1972), exploiting the C-band positive staining of MUT and the C-band negative staining of the + cluster. (Traut et al., 1984; Kunze et al., 1996). For typing, blastocysts were transferred to slides in a 1% sodium citrate solution and fixed with methanol–acetic acid (3:1, v:v). We performed fluorescence in situ hybridization (FISH) with a digoxigenin-labelled probe, MmHSR1015-3 (Weichenhan et al., 1995), detecting the signal with a fluorescein-conjugated antibody (Boehringer, Mannheim) and counterstaining in propidium iodide as described in Traut et al. (1992). The MUT and the + cluster were distinguished from each other in interphase nuclei by the signal intensity and the size of the signal area. The two signals were different because of the considerable differences in target LRR copy numbers. To test the reliability of our FISH typing, 36 randomly chosen MUT/ + or +/+ blastocysts from MUT/MUT × +/+ and +/+ × +/+ crosses were examined by FISH. The genotypes of all 36 blastocysts, previously unknown to the investigator, were scored correctly, indicating that genotyping of blastocysts by FISH is reliable.

3. Results

(i) Preferential recovery of MUT from heterozygous females

Transmission of the M. m. domesticus high-copy LRR cluster MUT from heterozygous females and males was studied in reciprocal crosses between a heterozygous Rb MUT/++ and a homozygous +/+/++ parent. Paternal MUT and + clusters from heterozygous males segregated in a 1:1 ratio, in accordance with Mendelian expectation (Table 1, lines 1, 3 and 5). In the reciprocal cross, however, the maternal MUT cluster was preferentially recovered among live foetuses, independent of the genetic background (Table 1, lines 2, 4 and 6). The deviation from the 1:1 ratio may be stronger with an AKR than a B6 background (0.01 < P < 0.05, χ² test of homogeneity).

The results might have been caused by a biased transmission of the Robertsonian chromosome. Among offspring, MUT was found either at the original location in the chromosome 1 arm of the Robertsonian chromosome, or as a consequence of meiotic recombination proximal to the cluster, in the acrocentric homologue. Similar deviations from the 1:1 ratio in favour of MUT were observed in the reciprocal Rb MUT: ++ and + MUT: Rb + classes (data not shown). This indicated that the Robertsonian chromosome did not influence the recovery ratio of the maternal clusters among euploid foetuses. In the following parts of this section, all parental animals harbouring MUT were homozygous for Rb and, thus, imaginable complications caused by Rb heterozygosity were avoided. (For simplicity, the genetic notation of Rb is no longer given in the text but continued in the titles of tables.)

(ii) Embryonic viability and MUT recovery at different developmental stages

To determine the onset of deviation from the 1:1 ratio, we examined offspring from $ \frac{1}{2} \text{MUT/} + \times \frac{1}{2} \text{MUT/} + $ crosses at the blastocyst stage (day 4 p.c.) and postimplantation stage (days 10 and 13 p.c.). Almost all ovulated oocytes, as counted from the number of corpora lutea, resulted in blastocysts (Table 2).

The MUT cluster consists of about 900 LRR copies while the wild-type cluster consists of only about 50 copies (Kunze et al., 1996). This difference can be made visible by FISH and was exploited to genotype the blastocysts (Plate 1). The ratio of the two types of blastocysts, MUT/+ and +/+ +, did not deviate significantly from 1:1 (Table 2, line 1). Mean cell

<table>
<thead>
<tr>
<th>Line no.</th>
<th>Parental genotypes</th>
<th>Offspring</th>
<th>Recovery of MUT (%)</th>
<th>Deviation from 1:1 (χ² test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td>MUT/ +</td>
<td>+/+</td>
</tr>
<tr>
<td>1</td>
<td>+/+</td>
<td>Rb MUT/ +/+</td>
<td>137</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>+/+</td>
<td>Rb MUT/ +/+</td>
<td>74</td>
<td>57</td>
</tr>
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<td>3</td>
<td>+/+</td>
<td>Rb MUT/ +/+</td>
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<td>Rb MUT/ +/+</td>
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<td>Rb MUT/ +/+</td>
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<td>60</td>
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<tr>
<td>6</td>
<td>+/+</td>
<td>Rb MUT/ +/+</td>
<td>139</td>
<td>87</td>
</tr>
</tbody>
</table>

* P < 0.01; ** P < 0.001.
Plate 1. A MUT/+ blastocyst (A) and a +/+ blastocyst (B) genotyped by FISH using an LLR-specific probe. Signal detection was by a fluorescein-labelled antibody, counterstained with propidium iodide. Large arrowheads, MUT; small arrowheads, +.
Influence of paternal MUT on embryonic viability

*MUT/+ females were mated with +/+ males and MUT/MUT males to examine the influence of paternal MUT. In crosses with +/+ males, preimplantation losses and preferential recovery of MUT/+ among the embryos (Table 3, line 1) were approximately as observed in the previous experiments. In crosses with MUT/MUT males, paternal MUT restored the 1:1 ratio of maternal clusters and decreased postimplantation losses (Table 3, line 2). Preimplantation losses, however, were higher than those in the corresponding crosses with +/+ males. We suspect that this is due to reduced sperm fertility of MUT/MUT males.

4. Discussion

(i) Predominant lethality of +/+ postimplantation embryos

This study describes and analyses the non-Mendelian recovery phenomenon of two LRR clusters: the low-copy wild-type cluster (+) and the high-copy cluster MUT from heterozygous *M. m. domesticus* females. Among offspring from MUT/+ females mated to +/+ males, recovery of MUT was significantly higher than that of the maternal + cluster. The reciprocal cross, however, yielded the two classes of +/+ males. We expect that this is due to reduced sperm fertility of MUT/MUT males.
therefore, was lethality of a considerable proportion of postimplantation embryos carrying the maternal + cluster. Survivors of the critical period developed normally: there was no appreciable further change in the MUT/+ to +/+ ratio between days 10–13 p.c. embryos and near-term foetuses.

Distribution of maternal clusters from MUT/+ females was not skewed when combined with a paternal MUT. We found a 1:1 ratio in postimplantation embryos. Thus, a paternal MUT cluster compensates for any adverse effects of the + cluster on progeny from MUT/+ females.

The results of Agulnik et al. (1990, 1993) in a similar investigation of an LRR cluster are compatible with ours with one notable exception: blastocysts showed the same skewed ratio as live progeny. This pointed to a biased meiotic segregation (meiotic drive sensu strictu; Ruvinsky, 1995). In our material, blastocysts presented a normal Mendelian segregation ratio. Due to this difference, we reject meiotic drive as an interpretation of the underlying mechanism in our material.

The difference may lie in the source of animals. Agulnik et al. (1990, 1993) investigated transmission of a M. m. musculus HSR while we used an HSR from a M. m. domesticus population. It is worth noting in this context that high-copy clusters from the two semispecies differ in at least two properties. First, the domesticus cluster occurs as one contiguous cluster while the musculus cluster is split (Winking et al., 1991). Second, the domesticus cluster encodes a family of five, the musculus cluster a family of six LRR transcripts, visible in Northern blots (Weichenhan et al., 1995). We cannot be sure, however, whether it is the cluster itself or genes closely linked to it that produce the observed effects.

The methods of genotyping may also have contributed to the different results. Agulnik et al. (1990) typed blastocysts from mitotic chromosomes while we used FISH on interphase nuclei and thus were able to produce the observed effects.

(ii) Models for +/+ postimplantation lethality

The observed interaction of maternal and zygotic factors may take place at different stages of development. We envisage three possible modes of action: genomic imprinting, incompatibility between uterus and embryo, interference with a maternal gene product.

Genomic imprinting is a mechanism which leads to differential expression of genes depending on their passage through the female or male germ line (Solter, 1988). In the MUT/+ female germ line, imprinting might affect the + cluster (or a linked gene; for simplicity, this reservation is omitted in the following text) to make it less able to sustain normal development. While paternal MUT is able to provide the necessary functions, paternal + is unable to do so. In a search for imprinted chromosome regions, disomies of paternal chromosome 1 have been recovered significantly less frequently than disomies of maternal chromosome 1 (review: Cattanach & Beechey, 1989). No clear-cut imprinting, however, has been discovered yet for chromosome 1 (Beechey & Cattanach, 1995).

Postimplantation lethality might alternatively be caused by incompatibility between the embryo and the uterus. This implies a uterine environment of MUT/+ females different from that of +/+ females. In this scenario, +/+ embryos have a reduced prospect of survival in the uterus of MUT/+ mothers compared with the uterus of +/+ mothers. Zygotic MUT, transmitted either from the mother or from the father, allows the embryo to accommodate to the MUT/+ uterine environment, leading to normal viability.

Maternal effects may also be caused by maternal gene products transmitted to the developing embryo via the oocyte or by the lack of such a product. This type of mechanism occurs in Drosophila (review: St Johnston & Nüsslein-Volhard, 1992). In the mouse, the ‘DDK syndrome’ provides a similar case (Renard et al., 1994). In our study, a MUT-derived, detrimental cytoplasmic factor, an RNA or a protein, might be the source of the maternal effect in MUT-associated postimplantation lethality. If accumulated prior to the second meiotic division in female meiosis, the factor would be incorporated in both types of oocytes from heterozygous MUT/+ females. Conceivably, a zygotic MUT of maternal or paternal origin inactivates the detrimental factor.

Which of the models really applies to the described phenomena remains to be elucidated. Embryo transfer experiments which are currently under way, such as transfer of +/+ zygotes of MUT/+ mothers into +/+ foster mothers, will distinguish between the different hypotheses for the distortion phenomenon among the offspring of MUT/+ mothers.

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References


Recovery distortion for a mouse HSR


