Restoration of the Mendelian transmission ratio by a deletion in the mouse chromosome 1 HSR

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Summary
The house mouse, Mus musculus, harbours a variable cluster of long-range repeats in chromosome 1. As shown in previous studies, some high-copy clusters such as the MUT cluster are cytogenetically inconspicuous as a homogeneously staining region (HSR) and are associated with a distortion of the Mendelian recovery ratio when transmitted by heterozygous females. The effect is caused by a decreased viability of +/+ embryos. It is compensated by maternal or paternal MUT. In this study, a deletion derivative of MUT, MUT^del, shows normal transmission ratios and no compensating capability. In this respect, MUT^del behaves like a wild-type cluster. Hence, both properties – transmission ratio distortion and compensating capability – map to the deleted region. The deletion comprises three-quarters of the MUT HSR and does not extend to the nearest markers adjacent to the HSR.

1. Introduction
Chromosome 1 of the house mouse (Mus musculus) contains a cluster of long-range repeats (LRR; ~ 100 kb repeat length) with variable copy numbers (locus D1Lub1, 53±1 cM; for review see Traut et al., 1994). High-copy cluster variants of more than 200 LRRs are C-band positive (Kunze et al., 1996) and appear as homogeneously staining regions (HSRs; Traut et al., 1984). Most clusters, however, such as that in laboratory strain C57BL/6, are low-copy clusters of about 60 repeats and cytogenetically inconspicuous. Such clusters are considered ‘wild-type’ (+) clusters. LRRs harbour the Sp100-rs gene the function of which is unknown (Weichenhan et al., 1995, 1997).

Individuals with chromosome 1 HSRs occur in many feral mouse populations (Winking et al., 1991; Agulnik et al., 1993a). Two of the high-copy clusters studied in more detail – one from a population in Mutten, Switzerland (MUT) and one from a population in Siberia – displayed non-Mendelian inheritance: they were preferentially transmitted from heterozygous females to viable offspring (Agulnik et al., 1990; Weichenhan et al., 1996). Preferential recovery of maternal MUT, i.e. MUT/+ embryos, was shown to be caused by preferential post-implantation lethality of the +/+ embryos (Weichenhan et al., 1996). Normal Mendelian ratios were restored with the introduction of paternal MUT. Distortion of the Mendelian transmission ratio (a maternal effect) and restoration of the Mendelian transmission ratio (a zygotic effect) are therefore properties of the high-copy cluster or of loci linked to the high-copy MUT cluster.

Here we investigate transmission of a cluster variant, MUT^del, which arose by spontaneous deletion of a considerable number of repeats from MUT (Kunze et al., 1996). We show that MUT^del is transmitted at a normal Mendelian ratio; it has lost both properties of MUT: maternal distortion and zygotic restoration of the Mendelian ratio. Molecular characterization of the deletion shows that these properties map to a chromosome region that includes the cluster but does not extend to the closest adjacent markers proximal or distal to the cluster.

2. Materials and methods
A cytogenetically inconspicuous LRR cluster in chromosome 1 is the prevailing type in feral mice and thus termed the + cluster. As representative with a
+ cluster, outbred NMRI-animals were used. The cytotogenetically conspicuous cluster \textit{MUT} (Weichenhan et al., 1996) was introduced by six backcross generations into an NMRI background. Subsequently a homozygous \textit{MUT} stock was established. In that stock, a deleted version of \textit{MUT}, termed \textit{MUT}\textsuperscript{del}, was detected cytotgenetically. Since homozygous \textit{MUT}\textsuperscript{del} females were subfertile, a homozygous \textit{MUT}\textsuperscript{del} stock could not be established. \textit{MUT}\textsuperscript{del} was maintained by backcrossing and intercrossing in an NMRI background.

Pre-implantation loss was determined by comparing the number of implantation sites with the respective number of corpora lutea. Post-implantation 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.) were determined by C-bandoning of metaphase chromosomes (Sumner, 1972), exploiting the C-band positive staining of \textit{MUT} and \textit{MUT}\textsuperscript{del} and the C-band negative staining of the + cluster (Traut et al., 1984; Kunze et al., 1996).

Genomic DNA was isolated from mouse liver either as described by Blin & Stafford (1976) or with the Genomic DNA Extraction Kit (Talent, Trieste, Italy) as recommended by the manufacturer. Plasmid DNA for probes was prepared by the CTAB method (del Sal et al., 1988). Southern blotting, hybridization and autoradiography were performed as described previously (Kunze et al., 1996). Total and poly(A)\textsuperscript{+} RNAs were prepared from mouse liver and hybridized as described by Weichenhan et al. (1995).

\textit{MmHSRc10e-c3} is a cDNA probe from the \textit{Sp100-rs} gene that is amplified in the \textit{D1Lub1} cluster (Eckert et al., 1991; Weichenhan et al., 1997). The probe consists of six exons scattered over a genomic region of 23 kb or up to 40 kb, depending on the LRR copy (Eckert et al., 1991; Plass et al., 1995). Further cDNA probes were from the \textit{M. musculus} genes \textit{Acrg} (1-8 kb cDNA; Schurr et al., 1990), \textit{Sag} (1-5 kb cDNA; Tsuda et al., 1988) and from the \textit{M. caroli} \textit{Sp100} gene (1-0 kb cDNA; probe comprises exons 6 to 17 which are not present in the partially homologous \textit{Sp100-rs} gene; Weichenhan et al., 1997).

MapPair primers for the anonymous chromosome 1 markers \textit{D1MIT8}, \textit{−10}, \textit{−11}, \textit{−44}, \textit{−48}, \textit{−50}, \textit{−51} and \textit{−53} were purchased from Research Genetics (Huntsville, USA). PCR was performed with Taq polymerase (Life Technologies, Eggenstein, Germany) on a Perkin Elmer GeneAmp 9600 apparatus using the standard protocol recommended by the distributor of the MapPairs. In brief, 10 \textmu l reaction mixes contained 40 ng mouse DNA and 0-4 pmol of each primer. Denaturation was at 94 °C for 45 s, annealing at 50 °C for 45 s and extension at 72 °C for 60 s in 30 cycles. PCR products were analysed on 2% agarose gels using \textit{HpaII}-digested Bluescript SK + (Stratagene, Heidelberg, Germany) as a length standard. Map positions of anonymous and gene markers were retrieved from the Mouse Genome Database (8, 1997).

3. Results

(i) Transmission ratios of maternal \textit{MUT}\textsuperscript{del}

We have previously shown that ++ offspring were underrepresented amongst the progeny of \textit{MUT}/+ females and ++ males (see Table 1, line 1; Weichenhan et al., 1996). Here we examine a deletion derivative of \textit{MUT}, \textit{MUT}\textsuperscript{del}, which is a viable condition in both heterozygous and homozygous form.

In crosses of \textit{MUT}\textsuperscript{del}/+ females with ++ males, the ratio of \textit{MUT}\textsuperscript{del}/+ to ++ offspring was close to the 1:1 expectation (Table 1, line 2). Post-implantation losses were considerably lower in \textit{MUT}\textsuperscript{del}/+ × ++ crosses than in crosses of \textit{MUT}/+ females with ++ males (\chi\textsuperscript{2} test, \(P < 0.001\)). Accordingly, in crosses of \textit{MUT}\textsuperscript{del}/+ females with ++ males, \textit{MUT}\textsuperscript{del} shows the property of the wild-type cluster and not that of \textit{MUT}.

Progeny of \textit{MUT}\textsuperscript{del}/\textit{MUT} females mated with ++ males deviated from the Mendelian 1:1 ratio, \textit{MUT}\textsuperscript{del}/+ embryos being underrepresented (\(P < 0.001\); Table 1, line 3). Post-implantation mortality exceeded 50\% and could be mainly ascribed to the death of embryos with maternal \textit{MUT}\textsuperscript{del}. When \textit{MUT}\textsuperscript{del}/\textit{MUT} females were mated with \textit{MUT}\textsuperscript{del}/\textit{MUT}\textsuperscript{del} males, the results were similar (Table 1, line 4): postimplantation mortality was high and not significantly different from that in the aforementioned cross (\(0.1 < P < 0.5\)), and embryos with maternal \textit{MUT}\textsuperscript{del} were underrepresented. Thus, with respect to the Mendelian recovery ratio and post-implantation loss, these crosses resembled the \textit{MUT}/+ × ++ cross.

(ii) Impaired fertility of homozygous \textit{MUT}\textsuperscript{del} females

In crosses of \textit{MUT}\textsuperscript{del}/\textit{MUT}\textsuperscript{del} females and ++ males, 5 of 12 females did not become pregnant. In the pregnant females, post-implantation mortality was much higher than in heterozygous females (Table 2, line 2; compare with Table 1, line 2; \(P < 0.001\)) and in homozygous \textit{MUT} females (Table 2, line 1; \(P < 0.001\)). Accordingly, fertility of homozygous \textit{MUT}\textsuperscript{del} females in crosses with wild-type males is significantly reduced. Fertility of \textit{MUT}\textsuperscript{del}/\textit{MUT}\textsuperscript{del} males, in contrast, appeared to be normal as deduced from high litter sizes in matings with ++ and \textit{MUT}\textsuperscript{del}/+ females. Those males were used to generate the homozygous \textit{MUT}\textsuperscript{del} females.
### Table 1. Embryos from heterozygous females

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Implantation loss (%)</th>
<th>Live embryos</th>
<th>Post-implantation loss (%)</th>
<th>Deviation from 1:1 (χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. n</td>
<td>Female</td>
<td>Male</td>
<td>MUT&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>MUT/MUT&lt;sup&gt;+/+&lt;/sup&gt;</td>
</tr>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12</td>
<td>MUT/+</td>
<td>+/+</td>
<td>152</td>
</tr>
<tr>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11</td>
<td>MUT&lt;sup&gt;del&lt;/sup&gt;/+</td>
<td>+/+</td>
<td>144</td>
</tr>
<tr>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11</td>
<td>MUT&lt;sup&gt;del&lt;/sup&gt;/MUT&lt;sup&gt;del&lt;/sup&gt;/+</td>
<td>+/+</td>
<td>145</td>
</tr>
<tr>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>MUT&lt;sup&gt;del&lt;/sup&gt;/MUT&lt;sup&gt;del&lt;/sup&gt;/MUT&lt;sup&gt;del&lt;/sup&gt;</td>
<td>+/+</td>
<td>41</td>
</tr>
<tr>
<td>5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6</td>
<td>MUT/+</td>
<td>MUT/MUT</td>
<td>88</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data in lines 1 and 5 taken from Weichenhan et al. (1996).

<sup>b</sup> Two recognized triploids were excluded from genotyping.

<sup>c</sup> One recognized triploid was excluded from genotyping.

*<sup>P < 0.001</sup>; **<sup>0.01 < P < 0.005</sup>.

### Table 2. Embryos from homozygous MUT and MUT<sup>del</sup> females

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Implantation loss (%)</th>
<th>Live embryos</th>
<th>Post-implantation loss (%)</th>
<th>Deviation from 1:1 (χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. n</td>
<td>Female</td>
<td>Male</td>
<td>MUT/MUT&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>MUT/MUT&lt;sup&gt;del&lt;/sup&gt;/+</td>
</tr>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>MUT/MUT</td>
<td>+/+</td>
<td>43</td>
</tr>
<tr>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MUT&lt;sup&gt;del&lt;/sup&gt;/MUT&lt;sup&gt;del&lt;/sup&gt;/+</td>
<td>+/+</td>
<td>76</td>
</tr>
<tr>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MUT&lt;sup&gt;del&lt;/sup&gt;/MUT&lt;sup&gt;del&lt;/sup&gt;/MUT/MUT</td>
<td>+/+</td>
<td>108</td>
</tr>
<tr>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>MUT&lt;sup&gt;del&lt;/sup&gt;/MUT&lt;sup&gt;del&lt;/sup&gt;/MUT/+</td>
<td>+/+</td>
<td>135</td>
</tr>
</tbody>
</table>

<sup>a</sup> Five of the 12 females did not become pregnant.

<sup>b</sup> Four of the 12 females did not become pregnant.

*<sup>P < 0.001</sup>.  

P.S: The table appears to be truncated or cut off, possibly due to formatting issues. The values provided seem to be in percentages, indicating the success or failure rates in certain conditions.
Table 3. Sizes of PCR products (in bp) of strains MUT\textsuperscript{del} and MUT with D1MIT MapPairs

<table>
<thead>
<tr>
<th>Marker\textsuperscript{a}</th>
<th>D1MIT44</th>
<th>D1MIT8</th>
<th>D1MIT53</th>
<th>D1MIT50</th>
<th>D1MIT51</th>
<th>D1MIT48</th>
<th>D1MIT10</th>
<th>D1MIT11</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUT\textsuperscript{del}</td>
<td>190</td>
<td>200</td>
<td>150</td>
<td>130</td>
<td>250</td>
<td>140</td>
<td>140</td>
<td>100</td>
</tr>
<tr>
<td>MUT</td>
<td>190</td>
<td>200</td>
<td>150</td>
<td>130</td>
<td>250</td>
<td>140</td>
<td>140</td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The figure below each marker indicates the map position in centimorgans.
53.1 cM; markers from 50.3 cM to 58.7 cM were selected. None of them was deleted in homozygous MUT del animals (Table 3). The closest markers were D1MIT50 and D1MIT51, which map 0.1 cM proximally to the cluster, and D1MIT48, which maps 0.9 cM distally.

The presence of the genes Aegr (52.3 cM), Sag (53.6 cM) and Sp100 (distally adjacent to the cluster; see Section 4) was tested by hybridization of cDNA probes to EcoRI-digested DNA. All probes recognized genomic fragments in homozygous MUT del animals, some of the same size as in homozygous MUT animals, some with restriction fragment length polymorphisms (not shown). Thus, the deletion does not encompass the genes Aegr, Sag or Sp100; the respective genes map outside the borders of the MUT del deletion.

4. Discussion

(i) Phenotypic consequences of the MUT del deletion

MUT del was derived from the MUT LRR cluster by a deletion (Kunze et al., 1996). The deleted segment comprised roughly 640 of the 920 LRRs from the MUT cluster and little, if anything at all, from the adjacent proximal or distal chromosome regions. As we report in this paper, homozygous male carriers of the deletion are viable and fertile, whereas homozygous females carriers are viable but show impaired fertility.

MUT is associated with transmission ratio distortion in MUT/+ females mated to +/- males. The distortion is due to preferential death of +/- post-implantation embryos while no such disadvantage of +/- embryos is evident in the reciprocal cross (Weichenhan et al., 1996). The MUT genotype confers an adverse maternal effect (AME) on +/- embryos (sensitive) but not on MUT/+ embryos (tolerant). Thus, like AME, AME tolerance is linked to MUT. AME tolerance can be contributed by paternal MUT and, therefore, is a zygotic effect (Weichenhan et al., 1996). MUT del has lost the AME property and acts like the + genotype (Table 1, line 2). In MUT del/MUT females, the Mendelian transmission ratio is distorted, with underrepresentation of MUT del/+ embryos (Table 1, line 3). Paternal MUT del is unable to restore the Mendelian 1:1 ratio (Table 1, line 4). Like +, MUT del does not confer AME tolerance on the embryos. In summary, both AME and AME tolerance of MUT are absent in MUT del and, hence, map to the chromosome region that is deleted in the MUT del genome.

Homozygous MUT del females show reduced fertility in combination with paternal + (Table 2, line 2) while no such impairment is evident in the reciprocal cross. Partial restoration of fertility by paternal MUT but not by paternal MUT del indicates similarity to AME tolerance. We consider it possible but have no further evidence that partial restoration of fertility and AME tolerance are the same phenomenon.

AME and AME tolerance are properties associated with MUT. They map to the MUT del deletion region, which comprises a part of the LRR cluster and little if anything at all of the immediate proximal or distal vicinity. Although we cannot exclude that genes in the immediate vicinity of the cluster or genes entrapped within the cluster are responsible for these properties, the cause may be the cluster itself.

In a similar system, chromosome 1 high-copy LRR clusters from Siberia showed transmission distortion like MUT (Agulnik et al., 1990). The authors considered the effect to be caused by meiotic drive. Mapping of the responsible locus with fZ (44 cM), in (59 cM) and Pep3 (71 cM) (Agulnik et al., 1993b) is consistent with its location in or near the LRR cluster at 53.1 cM (updated map positions from Mouse Genome Database; 8, 1997). The similarity extends to the restoration of the Mendelian transmission ratio by a paternal high-copy cluster which was originally thought to have an effect on segregation during the second meiotic division of the oocyte (Agulnik et al., 1993c).

It is tempting to assign the high LRR copy numbers a causative role in AME and/or AME tolerance, similar to the dosage-dependent action of Enhancer of Segregation Distortion, E(SD), in Drosophila (Brittnacher & Ganetzky, 1984; Temin, 1991). However, one of the low-copy LRR cluster genotypes tested, that of BALB/c, did not show transmission distortion when heterozygous with a high-copy cluster genotype (Agulnik et al., 1993b; H. Winking, unpublished), i.e. BALB/c exhibits AME tolerance according to our terminology.

(ii) Nature of the maternal effect

The nature of AME is not clear. We envisage three possible forms of the inhibiting influence on the development of post-implantation embryos: (1) a maternal effect in the strict sense, (2) an interaction between the meiotic partners, and (3) an interaction between embryo and mother.

(1) A maternal effect in the strict sense, i.e. an RNA or protein with an inhibiting influence produced by the unreduced MUT/+ genome, and delivered to both types of eggs, MUT and +, may be the cause of AME. Zygotic AME tolerance provided by the MUT genome would then make the MUT/+ embryo resistant to that influence. A genetic system with similar components, the mouse DDK syndrome, has been proposed (Renard et al., 1994; de Villena et al., 1996). The inhibited stage in the DDK syndrome, however, is the pre-implantation stage. The post-
implantation stage, at which AME becomes apparent, appears to be rather late for a typical maternal effect, which becomes effective in the early stages of development (Wilkins, 1993).

(2) An interaction between meiotic partners in the t-complex of the mouse was considered the cause for preferential impairment of + sperm in t/+ males (Seitz & Bennett, 1985; review by Silver, 1993). The system resembles that of MUT/+ females. To cause AME, the MUT genotype may either confer some toxic agent on the gamete or imprint the + genotype in some deleterious way. Known cases of imprinting regard differences of the female versus the male germline (reviews by Solter, 1988; and Jaenisch 1997).

There are no reports yet, however, on particular genotypes producing imprints in the meiotic partner genome.

(3) The obvious site for an interaction between mother and post-implantation embryo is the uterus. The maternal and zygotic effects reported here bear some resemblance to a mouse model used to study spontaneous abortion. CBA/J × CBA/J2 crosses – but not the reciprocal crosses – show a high rate of spontaneous resorption (Clark et al., 1980; Kiger et al., 1985). The maternal effect has been attributed to damage caused by tumour necrosis factor-alpha and natural killer cells (Gendron & Baines, 1988; Clark et al., 1991). The effect is abrogated by introduction of a paternal BALB/c genome; CBA/J × BALB/c crosses show normal rates of abortion (Kiger et al., 1985). One can envisage a scenario in which the MUT/+ uterus, unlike the +/+ or MUTdel/+ uterus, offers a hostile environment for the sensitive +/+ and MUTdel/+ embryos but not for the tolerant MUT/+ embryos. Transplantation experiments of MUT/+ and +/+ embryos are under way to decide upon this interpretation.

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