Transmission-Ratio Distortion Through F₁ Females at Chromosome 11 Loci Linked to Om in the Mouse DDK Syndrome


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ABSTRACT

We determined the genotypes of >200 offspring that are survivors of matings between female reciprocal F₁ hybrids (between the DDK and C57BL/6J inbred mouse strains) and C57BL/6J males at markers linked to the Ovum mutant (Om) locus on chromosome 11. In contrast to the expectations of our previous genetic model to explain the “DDK syndrome,” the genotypes of these offspring do not reflect preferential survival of individuals that receive C57BL/6J alleles from the F₁ females in the region of chromosome 11 to which the Om locus has been mapped. In fact, we observe significant transmission-ratio distortion in favor of DDK alleles in this region. These results are also in contrast to the expectations of Waksasugi’s genetic model for the inheritance of Om, in which he proposed equal transmission of DDK and non-DDK alleles from F₁ females. We propose that the results of these experiments may be explained by reduced expression of the maternal DDK Om allele or expression of the paternal DDK Om allele in only a portion of the ova of F₁ females.

The unusual inheritance pattern of the mouse “DDK syndrome” first was described >30 years ago (Tomita 1960). When females from the DDK inbred strain are mated to males of many other inbred strains, ≈95% of the resulting embryos die during pre-implantation development, but offspring from the reciprocal matings, between DDK males and females of other inbred strains, are viable and fertile (Wakasugi et al. 1967; Wakasugi 1973, 1974; Wakasugi and Moriga 1977; Mann 1986; Renard and Babinet 1986; Baldacci et al. 1992; Sapienza et al. 1992). Fertility tests of reciprocal F₁ backcrosses between the DDK strain and the C57BL/6J strain (Wakasugi 1973, 1974; Sapienza et al. 1992) indicate that the lethal trait most likely segregates as a single gene, with the interpretation that a factor of DDK maternal origin interacts with a gene of non-DDK paternal origin to produce the lethal effect (Wakasugi 1974).

The location of a gene with a major effect on embryo survival has been mapped to mouse chromosome 11 by two laboratories using different genetic methods (Baldacci et al. 1992; Sapienza et al. 1992). In the first report on the location of the Om locus (Baldacci et al. 1992), a phenotypic assay was used to evaluate the breeding performance of males derived from the cross [BALB/c females × (BALB/c female × DDK male)] F₁ males—in all subsequent crosses listed in the text, the dam is listed first and the sire listed second when mated to DDK females. Each male was then genotyped at a large number of loci to find the region of the genome for which the concordance between genotype and fertility phenotype (either BALB/c or F₁, based on both in vitro and in vivo assays) was greatest. These investigators then confirmed the location of the lethal gene by analyzing recombinant-inbred (RI) strain females constructed between the DDK and BALB/c inbred strains. Females from each strain were scored as “DDK-like” or “BALB/c-like” based on the developmental morphology of pre-implantation embryos resulting from mating these females to BALB/c males. In the second report on the location of Om (Sapienza et al. 1992), offspring that were survivors of DDK × F₁ matings were genotyped at a large number of loci covering the majority of the mouse genome and the region for which the greatest transmission-ratio distortion in favor of male-derived DDK alleles was discovered. Both of these experiments pointed to the same region of chromosome 11 as the location of the Om locus.

The fact that Om has been placed at the same location on chromosome 11, regardless of whether transmission of the lethal gene of non-DDK paternal origin is examined through F₁ males or the “factor” of DDK maternal origin (Wakasugi 1974) is examined through RI females, is in accordance with the prediction of the origi-
nal genetic model (Wakasugi 1974). This model stated that the factor of maternal origin was produced from the same locus (or a closely linked locus) as the lethally interacting gene of paternal origin. This conclusion was based on the observation that the factor of maternal origin and the gene of paternal origin did not segregate independently among F1 backcross individuals (Wakasugi 1974).

In our previous report (Sapienza et al. 1992), we analyzed the segregation of alleles at loci on chromosome 11 in the crosses F1 × DDK (as controls) and DDK × F1 (as experiments). The decision to map Om by analyzing these crosses, rather than the strategy of using F1 × C57BL/6J as the alternative experimental mating predicted by our genetic model (Sapienza et al. 1992), was dictated by the endogenous murine provirus marker system used in that experiment. Because the chromosomal locations of endogenous proviral loci in the C57BL/6J strain have been determined (Frankel et al. 1990), any C57BL/6J provirus that does not have a homologue in the DDK strain results in a plus/minus polymorphism between the two strains that may be scored by blot hybridization using one of three oligonucleotide probes (Frankel et al. 1990). In this system, the presence of a hybridization signal at a particular position indicates the presence of the C57BL/6J allele, while the absence of a hybridization signal at that position indicates the presence of the DDK allele. In offspring of matings between F1 hybrids and the C57BL/6J strain, all individuals have at least one C57BL/6J allele at all of the relevant proviral loci. We were unable to make reliable distinctions between one and two copies of the proviral sequence at each locus and could not score offspring from these crosses for the segregation of alleles at chromosome 11 loci using this system.

Since we began our genetic analysis of the DDK syndrome, a large number of polymorphic, microsatellite, marker loci have been mapped in the mouse genome (Love et al. 1990; Hearne et al. 1991; Montagutelli et al. 1991; Copeland et al. 1993; Dietrich et al. 1994). We have tested many of these markers for polymorphism between DDK and C57BL/6J, including a number that could be scored reliably in offspring from all crosses. The loci that map to chromosome 11 (Lossie et al. 1994; Whitehead Institute/MIT Center for Genome Research 1995) can be used to analyze the segregation of the Om region in offspring of F1 females mated to C57BL/6J males.

Because very few offspring are produced when DDK females are mated to C57BL/6J males, but F1 females produce litters that are ~50% of normal size when mated to C57BL/6J males, our previous genetic model for imprinted expression of Om, derived from the experiments described above, predicted that the vast majority of offspring produced by mating F1 females to C57BL/6J males would be homozygous for C57BL/6J alleles at the Om locus (Sapienza et al. 1992). This prediction was based on the fact that almost all embryos derived from mating DDK females with C57BL/6J males die before the end of preimplantation development but we expect embryos that are the product of fertilization of a C57BL/6J-type ovum by a C5BL/6J sperm to survive. We have tested this prediction by genotyping 218 offspring derived from matings between F1 females and C57BL/6J males at polymorphic markers spanning the Om locus. Our results indicate that there is no selection for survival of C57BL/6J homozygotes in this region of chromosome 11. In contrast, we observe significant transmission-ratio distortion for DDK/C57BL/6J heterozygotes among survivors.

MATERIALS AND METHODS

Extraction of DNA from tail or skin biopsies, gel electrophoresis and autoradiography were all performed as previously described (Maniatis et al. 1982; Hogan et al. 1986). In all crosses described in the text, the female is listed first and the male is listed second. All mice used in this experiment were treated according to the recommendations of the Canadian Council on Animal Care.

Genotypes at D11Mit71, D11Mit20, D11Mit5, D11Mit6, D11Mit38, D11Mit67, D11Mit61, and D11Mit168 were determined by polymerase chain reaction as indicated by the manufacturer. Oligonucleotide primers for these loci were obtained from Research Genetics (Huntsville, AL). The sizes of the C57BL/6J alleles were obtained from Research Genetics. The sizes (in base pairs) of the DDK alleles at each locus are: D11Mit71 (>240), D11Mit20 (136), D11Mit5 (191), D11Mit66 (151), D11Mit38 (202), D11Mit67 (140), D11Mit61 (190) and D11Mit168 (126).

RESULTS

We determined the genotypes of the offspring of (C57BL/6J × DDK)F1 × C57BL/6J and (DDK × C57BL/6J)F1 × C57BL/6J matings at the chromosome 11 loci shown on the right side of Figure 1. The seven loci scored span 86% of the total length of chromosome 11. The location of Om, as placed by our laboratory, is shown as a bar on the left side of Figure 1. We were unable to map the trait with greater precision because of the incomplete penetrance of the lethal phenotype (Sapienza et al. 1992). The location of Om, as placed by Baldacci et al. (1992), as given in Lossie et al. (1994), is shown as an arrow on the left side of Figure 1. Baldacci et al. (1992) did not observe any recombination between Om and the Sns2 (Sige) locus.

The chromosome 11 haplotypes of the 218 backcross offspring using all of the loci scored are shown in Figure 2a, and the chromosome 11 haplotypes only in the vicinity of Om are shown in Figure 2b. Our previous genetic model for the inheritance of the DDK syndrome predicted that the vast majority of offspring from F1 × C57BL/6J matings would be homozygous for C57BL/6J alleles at the Om locus (Sapienza et al. 1992). However, we did not observe a significant excess of C57BL/6J alleles at Om.
Transmission Ratio Distortion

**FIGURE 1.**—Genetic map of mouse chromosome 11 (Cope-land et al. 1993; Lossie et al. 1994). Loci scored in this report are shown on the right side of the chromosome and the location of Om is shown on the left side. The arrow indicates the map position of Om as placed by Baldacci et al. (1992) and reported in Lossie et al. (1994). The bar indicates the position of Om as placed by our laboratory (Sapienza et al. 1992; C. Sapienza, unpublished data). Because of the incomplete penetrance of Om (~8% of individuals survive the lethal genotype, Sapienza et al. 1992), our analyses of F1 backcross offspring (Sapienza et al. 1992; C. Sapienza, unpublished data) do not allow the localization of Om with more than the indicated level of precision. The published map position (in centimorgans from the centromere) for each locus is: D11Mit71 (1), D11Mit20 (20), D11Mit5 (37), D11Mit66 (47), D11Mit38 (49), D11Mit67 (58), D11Mit61 (70) and D11Mit168 (70). The most likely gene order correlating the markers used in this study and the markers scored in Sapienza (1992) is Pmv2-D11Mit5-Mpv2-D11Mit33-D11Mit66-D11Mit38/Xmv42-D11Mit67 (Feil et al. 1995; C. Sapienza, unpublished data).

6] homozygotes at any locus (Table 1). In fact, at the loci closest to Om (D11Mit66 and D11Mit38), we observe significant transmission ratio distortion in favor of DDK alleles (Table 1). This result is illustrated best by classifying the offspring according to their recombinant or nonrecombinant status within each interval between each pair of consecutive loci (Table 2). Four of six intervals are distorted in the nonrecombinant classes against the B-B class (C57BL/6] alleles at both the proximal and distal markers that define the interval) and, the maximum distortion is located within the D11Mit66-D11Mit38 interval, (H0: equal transmission; \( \chi^2 = 13.75; P < 0.001 \)). Among the recombinant classes, three inter-

ervals are distorted: the interval immediately proximal to the Om locus (D11Mit5-D11Mit66), in favor of the inheritance of the DDK allele at D11Mit66 (H0: equal transmission, \( \chi^2 = 10.93; P < 0.002 \)); an interval distal to the Om locus (D11Mit67-D11Mit61) in favor of the inheritance of the DDK allele at the proximal locus (H0: equal transmission, \( \chi^2 = 6.10; 0.01 < P < 0.025 \)); and in the D11Mit66-D11Mit38 interval, with distortion in favor of inheriting the DDK allele at the proximal locus (exact binomial test of equal proportions of B-K and K-B haplotypes \( P = 0.03 \)).

These observations confirm that there is no selection for survival of individuals that are homozygous for C57BL/6] alleles in the Om region and, conversely, that there is transmission ratio distortion in favor of DDK alleles in the Om region.

**DISCUSSION**

Our previous genetic model sought to explain the polar-lethal character of the Om trait by invoking a "re-verse imprinting" of the Om gene (Sapienza et al. 1992). In this model, Om was proposed to be an imprinted locus at which most mouse strains expressed only the maternal allele, but the DDK strain was proposed to express only the paternal allele. This model explained not only the directional lethality of the cross, but also the observed loss of approximately one-half of the offspring of F1 females backcrossed to C57BL/6] males. This model made the prediction that surviving offspring of F1 × C57BL/6] backcrosses would be homozygous for C57BL/6] alleles at the Om locus. We have tested this prediction by determining the genotype of >200 such offspring in the vicinity of Om and find that we may reject this model. Among the 181 individuals that may be scored as homozygous or heterozygous at Om (i.e., those that have nonrecombinant chromosomes in the interval D11Mit5-D11Mit38, see Figure 2b), our previous model predicted that 167 individuals would be homozygous and only 14 would be heterozygous (homozygous = 181 × 0.92; heterozygous = 181 × 0.08. These figures are based on the survival of only 8% of zygotes from DDK × C57BL/6], see Sapienza et al. 1992). As shown in Table 2, we found that 79 individuals are homozygous and 133 are heterozygous (H0: reversed imprinting in DDK strain, \( \chi^2 = 260.43, P < 0.001 \)). Our results also provide a test of the genetic model of Wakaugi (Wakaugi 1974), in which equal survival of homozygous and heterozygous offspring of F1 females mated to C57BL/6] males is predicted. We find that this model, too, may be rejected (H0: equal transmission, \( \chi^2 = 7.56, 0.005 < P < 0.01 \)).

An unexpected conclusion that may be drawn from our experiments is that individual survival has been partially uncoupled from the segregation of a particular allele at the Om locus through the maternal line. This observation must be considered peculiar to F1 females,
FIGURE 2.—Haplotype analysis of the reciprocal F1 × C57BL/6J progeny. (a) Haplotypes using all the loci shown in Figure 1. (b) Haplotypes in the Om region and closely linked loci. □, inheritance of the DDK allele at the particular locus; ▪, inheritance of the C57BL/6J allele at the particular locus; *, a single (C57BL6/J × DDK) × C57BL6/J male that has not been successfully typed for DllMit71; †, a single (C57BL6/J × DDK) × C57BL6/J male that has not been typed successfully either for DllMit61 or DllMit168. As these animals could have either of the two haplotypes signaled, depending on its genotype at the locus not successfully scored, we decided not to include them in any column.

as it is in contrast to the observed correlation between the transmission of the polar-lethal trait and the genotype of RI females at the Om locus (Baldacci et al. 1992). In the simplest terms, an F1 ovum that segregates the DDK allele at Om does not behave as a DDK ovum (i.e., does not die when fertilized by a C57BL/6J sperm) and an F1 ovum that segregates the C57BL/6J allele at Om does not behave as a C57BL/6J ovum (i.e., does not

**TABLE 1**

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<th>DllMit71</th>
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<th>DllMit66</th>
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<td>(B × K)F1 × B</td>
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<td>(K × B)F1 × B</td>
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<td>K</td>
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<td>(B × K)F1 × B</td>
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<td>(K × B)F1 × B</td>
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Eighty-five males and 70 females were genotyped for DllMit61 and 35 males and 26 females were genotyped at DllMit168, because of the proximity of DllMit61 and DllMit168 (<1 cm) both loci were treated as one in the following analysis. One (B × K)F1 × B individual was not scored for DllMit71 because of repeated failure of PCR reaction. Another (B × K)F1 × B individual was not scored for either DllMit61 or DllMit168 due to the loss of DNA sample.
always survive when fertilized by a C57BL/6J sperm). Furthermore, although a bias in the survival of individuals carrying different Om alleles is observed, the bias is in the direction opposite to that which might be expected from the polar-lethal nature of the DDK syndrome (i.e., many more heterozygotes are observed than homozygotes).

We are unable to provide a simple alternative hypothesis to explain these data, but they may provide some insight into the timing or pattern of expression of the DDK maternal "factor" that interacts with the C57BL/6J paternal genome to result in preimplantation embryo lethality. Renard et al. (1994) have performed an important series of microsurgical and biochemical experiments using the DDK strain. In these experiments, the investigators performed cytoplasm transfers in which they demonstrated a detrimental effect of DDK oocyte cytoplasm on the survival of normally viable embryos. In addition, these authors provided evidence that the component of DDK oocyte cytoplasm that is responsible for the lethal effect is an RNA molecule.

If the timing of expression of the DDK maternal factor is the same for the ova of F1 females as for the ova of DDK females, then the factor must be expressed before the first meiotic division (i.e., it is already present in the ovum at ovulation) and there are two possibilities for the expression of the DDK maternal factor. The first is that both alleles of Om are expressed in each F1 ovum, and all F1 ova must contain the DDK maternal factor. Under this model, the "survival" of an ovum (each of which contain the DDK factor) fertilized by a C57BL/6J sperm cannot be related to which allele of Om is segregated. The fact that ~50% of these fertilizations survive must be ascribed to some other mechanism, such as a reduced amount of DDK maternal factor present in each ovum due to the presence of only one DDK Om allele in each oocyte. This model does not succeed in explaining the preferential survival of the DDK Om allele or the closeness of the average F1 × C57BL/6J litter size (Sapienza et al. 1992) to that predicted by genetic models (Wakasugi 1974; Sapienza et al. 1992).

The second possibility for the expression of the DDK maternal factor is that it is not present in all of the ova ovulated by F1 females, i.e., the DDK Om allele is not expressed in each oocyte. Under this model, those ova in which the DDK Om allele has not been expressed survive when fertilized by a C57BL/6J sperm. Furthermore, although the survival of an ovum is related to whether or not it contains the maternal DDK factor it is not related to which allele of Om is segregated at meiosis (assuming that expression of an allele before meiosis and its segregation at meiosis are independent). This model requires the operation of some mechanism similar to that demonstrated to operate at the autosomal loci encoding olfactory receptors in the mouse (Chess et al. 1994), i.e., monoallelic, but nonimprinted expression. If the choice of which Om allele to express in an oocyte (i.e., whether the DDK maternal factor will be produced in the ovum or not) is stochastic, then the 50% survival of the offspring of F1 females may be explained under this model, but the preferential survival of the DDK Om allele is not expected. It should be noted that Warasugi (1974) also proposed that F1 females produced two phenotypic classes of ova, although no hypothesis for the manner in which this might be addressed was proposed.

The combination of a functional assay for the pres-

| TABLE 2 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | D11Mit71-D11Mit20 | D11Mit20-D11Mit5 | D11Mit5-D11Mit66 | D11Mit66-D11Mit38 | D11Mit38-D11Mit67 | D11Mit67-D11Mit61 |
| B-B             | 67              | 78              | 73              | 79              | 74              | 70              |
| (B × K)F1 × B   | 32              | 38              | 31              | 32              | 30              | 29              |
| (K × B)F1 × B   | 35              | 40              | 42              | 47              | 44              | 41              |
| K-B             | 66              | 103             | 113             | 133             | 124             | 105             |
| (B × K)F1 × B   | 23              | 43              | 54              | 64              | 59              | 44              |
| (K × B)F1 × B   | 43              | 50              | 59              | 69              | 65              | 61              |
| B-K             | 47              | 27              | 26              | 0               | 11              | 13              |
| (B × K)F1 × B   | 27              | 12              | 14              | 0               | 6               | 6               |
| (K × B)F1 × B   | 20              | 15              | 12              | 0               | 5               | 7               |
| K-B             | 37              | 20              | 7               | 6               | 9               | 29              |
| (B × K)F1 × B   | 17              | 7               | 1               | 4               | 5               | 20              |
| (K × B)F1 × B   | 20              | 13              | 6               | 2               | 4               | 9               |
| n               | 217             | 218             | 218             | 218             | 218             | 217             |

Individuals were classified as B-B when they inherited C57BL/6J alleles in both proximal and distal loci of each interval, as K-K when they inherited DDK alleles in both proximal and distal loci of each interval, as B-K when they inherited the C57BL/6J allele in the proximal marker and the DDK allele in the distal marker, and as K-B when they inherited the DDK allele in the proximal marker and the C57BL/6J allele in the distal marker; n, number of offspring scored for each interval.
ence of the maternal DDK factor (Renard et al., 1994) and a detailed physical map (Nehls et al., 1995) in the region of chromosome 11 that contains Om is likely to result in the isolation of the Om gene in the foreseeable future. The above hypotheses may be tested most easily by examination of individual ova from F1 females for the presence of the maternal DDK RNA factor.

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