

Sex-restricted non-Mendelian inheritance of mouse Chromosome 11 in the offspring of crosses between C57BL/6J and (C57BL/6J × DBA/2J)F₁ mice

Jay Shendure,* Justine A. Melo,* Kara Pociask, Rachel Derr, Lee M. Silver

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544-1014, USA

Received: 19 December 1997 / Accepted: 10 June 1998

Abstract. We report on the observation of sex-restricted, non-Mendelian inheritance over a region of mouse Chromosome (Chr) 11, occurring in the offspring of crosses between two commonly used *Mus musculus*-derived inbred strains, C57BL/6J and DBA/2J. In the surviving backcross progeny of reciprocal matings between (C57BL/6J × DBA/2J)F₁ hybrids and the C57BL/6J parental strain, we observed the preferential appearance of C57BL/6J alleles along a region of Chr 11. The deviation from Mendelian predictions was observed only in female offspring from both reciprocal backcrosses, and not in males from either cross. The sex-specificity of the observed non-Mendelian inheritance points to an explanation based on embryonic or neonatal lethality. Our data add to previously obtained evidence for a Chr 11 locus or loci with sex-specific and allele-specific effects on viability.

Introduction

The first law of genetics developed by Gregor Mendel states that alleles at a locus will segregate equally to offspring of a heterozygous parent. In recent years, however, various examples of “non-Mendelian inheritance” have been uncovered. Based on current biological understanding, there are three situations in which allelic frequencies in the population of surviving offspring could deviate significantly from a one-to-one ratio.

The first is classical segregation distortion, in which the departure of allelic frequencies from an even ratio is a consequence of unequal meiotic segregation in a heterozygous parent. An example of this phenomenon is provided by the segregation distortion observed from females of wild *Mus mus musculus* populations that are carriers of an aberrant form of Chr 1 (*In*+; Agulnik et al. 1990; Ruvinsky 1995). During meiosis, the aberrant Chr 1 is preferentially transmitted (~85%) to the secondary oocyte and then to the egg (rather than to either polar body), leading to distorted allelic frequencies in the female animal’s offspring.

The second situation is a consequence of post-meiotic, but pre-fertilization effects on gamete functionality. A well-studied example of this phenomenon is the *t*-haplotype system on mouse Chr 17 (Silver 1985). Spermatids bearing the variant *t*-haplotype form of this chromosome post-meiotically inactivate their wild-type competitors and, as a consequence, gain a relative advantage in fertilizing ability (Silver 1993).

The third situation is a consequence of post-fertilization effects with the differential survival of embryos, neonatal progeny, or newly born pups that carry a particular allelic combination at a particular locus. An example of this phenomenon is the “DDK

syndrome,” in which an incompatibility between DDK maternal cytoplasm and non-DDK alleles at a Chr 11 locus (*Om*) leads to differential embryonic survival (Babinet et al. 1990; Sapienza et al. 1992; Renard et al. 1994; de Villena et al. 1996). When DDK females are crossed to (C57BL/6 × DDK)F₁ males, the inheritance of the C57BL/6 allele at the *Om* locus is semilethal at an early embryonic stage, leading to the preferential appearance of the homozygous DDK genotype at *Om* in the population of surviving offspring (Sapienza et al. 1992).

It is difficult to estimate the frequency with which any of these types of non-Mendelian inheritance occur in even the best-studied animal species, because none produces a visible phenotype that can be readily interpreted. Nonetheless, through the process of performing whole-genome scans in the pursuit of unrelated goals, numerous examples of non-Mendelian inheritance in the mouse have been uncovered. In particular, through the course of various interspecific backcross matings between animals of the murine species, *Mus musculus musculus* and *Mus spretus*, non-Mendelian inheritance has been observed on regions of Chr 2 (Siracusa et al. 1989, 1991), Chr 4 (Ceci et al. 1989), Chr 10 (Justice et al. 1990), and Chr X (Biddle 1987; Montagutelli et al. 1996). Several of these reports (Siracusa et al. 1991; Biddle 1987; Montagutelli et al. 1996) proposed sex-specific differences in allelic transmission ratios.

In the study reported here, outcross-backcross matings between closely related members of a single species, the *Mus musculus*-derived C57BL/6J and DBA/2J inbred strains, were used to generate several hundred second-generation animals. Incidental to the mapping of loci affecting alcohol consumption levels (Melo et al. 1996), genotypic analysis of the N2 population led to the observation of female-specific, non-Mendelian inheritance along a region of Chr 11.

Materials and methods

C57BL/6J (B6), DBA/2J (DBA), and (B6 × DBA)F₁ animals were purchased from The Jackson Laboratory. N2 animals were bred at Princeton University from reciprocal matings between B6 and F₁ animals. N2 animals were weaned at 3–4 weeks. For all crosses described in the text, the convention of placing the maternal strain to the left of the cross sign and the paternal strain to the right is followed.

Genomic DNA was prepared from tail and spleen tissues according to standard protocols. Primers purchased from Research Genetics (Huntsville, Ala.) were used to PCR amplify microsatellite markers as indicated by the manufacturer (Dietrich et al. 1994). When possible, markers were selected with large differences in size between B6 and DBA products so that typing could be performed by ethidium bromide staining. When necessary, markers with product size differences of less than eight base pairs were analyzed with ³²P-labeled primers, and product was separated by electrophoresis on denaturing gels. All data input and analysis was performed with the Microsoft Excel software package on the Macintosh computer.

* These authors contributed equally to this publication.

Correspondence to: J. Shendure at Suite #104, 33595 Bainbridge Rd., Solon, OH 44139, USA

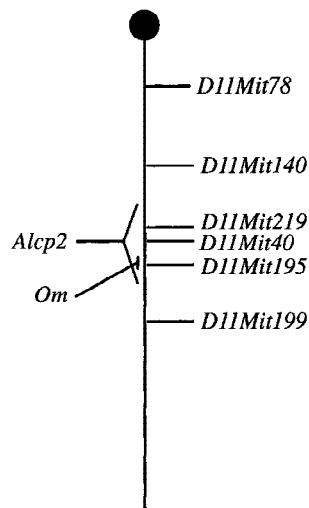


Fig. 1. Relative recombinational distances between six loci on Chr 11. Distances between marker loci are based on the results of this study. The intervals to which *Om* (de Villena et al. 1996) and *Alcp2* (Melo et al. 1996) have been mapped are also indicated. Because *Om* is incompletely penetrant and *Alcp2* is a quantitative trait locus, the precise positions of both are uncertain.

Results

The genotypes of N2 offspring of reciprocal matings between (B6 × DBA) F_1 and B6 mice were determined for six loci spanning a large region of Chr 11. The relative recombinational distances between these loci, calculated from our data set, are shown in Fig. 1. The high density of typed markers on this portion of Chr 11 was a consequence of efforts to map a locus involved in the differential consumption of alcohol by B6 and DBA mice (Melo et al. 1996).

A summary of the allelic frequencies at the loci and intervals tested and the results of statistical analyses are reported in Table 1. For inter-locus intervals, only individuals that are non-recombinant between the flanking loci were counted. Allelic inheritance ratios are reported as the proportion of animals inheriting the B6 allele (equivalent to the proportion of B6 homozygous offspring).

The *t*-test was used to estimate the significance of sex-specific differences in allelic inheritance by comparing the allele ratio of the male offspring subpopulation with that of the female subpopulation. Over the region in which large numbers of both males and females were genotyped (*D11Mit219*–*D11Mit40*–*D11Mit195*), female offspring inherited a significantly higher proportion of B6 alleles from the F_1 parent than did males. The most significant difference between the sexes was calculated for marker *D11Mit195* ($P = 4.0 \times 10^{-4}$).

Chi-squared analysis was also used to determine the significance of the deviation of female allelic frequencies from the Mendelian prediction of a 0.5 value. Deviation from Mendelian inheritance was highly significant in the region between *D11Mit140* and *D11Mit195* but not significant at markers flanking the region *D11Mit78* and *D11Mit199*. The most significant result ($P = 9.7 \times 10^{-4}$) was obtained for the interval flanked by *D11Mit40* and *D11Mit195*. The frequency of the B6 allele in female animals non-recombinant for this interval was 0.64. Allelic frequencies for male and female subpopulations at loci and intervals along the chromosomal region are displayed in Fig. 2.

Approximately 78% of the animals originated from an $F_1 \times B6$ cross, and the remainder from the reciprocal $B6 \times F_1$ cross. When the female population is separated according to cross-of-origin, allelic frequencies do not differ significantly between the reciprocal crosses. At the interval between *D11Mit40* and *D11Mit195*, the B6 allelic frequency in the offspring of the $F_1 \times B6$ cross was

0.634 ($n = 112$), and in the offspring of the $B6 \times F_1$ cross it was 0.657 ($n = 35$). The similarity of distortion ratios in crosses with an F_1 female parent versus crosses with an F_1 male parent is highly suggestive of an effect that occurs at the level of differential offspring viability rather than differential gametic functionality.

Discussion

The occurrence of significant non-Mendelian inheritance involving a specific region of mouse Chr 11 suggests the presence of a locus or linked loci, at which allelic inheritance affects the differential viability of gametes, developing offspring, or newly born pups. Two observations provide support for a post-fertilization effect. First, the sex of the heterozygote parent had no bearing on the expression of the distortion phenotype. In all examples of pre-fertilization distortion phenomena across the animal kingdom, only one parental sex has expressed the phenotype (Lyttle 1993). This appears to result from the very different processes of gametic differentiation in males and females. The second observation providing support for a post-fertilization effect is its limitation to only female offspring. The complicated alternative requires that a pre-fertilization interaction, between alleles at the Chr 11 region and the sex chromosomes, has a significant bearing on gametic ratios or fertilization efficiency. Thus, the reduced viability of female individuals—either at the fetal or early postnatal stages—that carry a heterozygous B/D genotype at a locus on Chr 11 is the best explanation for the results we have obtained.

Assuming that the locus is in the *D11Mit140*/*D11Mit195* interval, we observe a female-specific lethality effect associated with the B/D genotype with a penetrance of 44%, leading to an observed inheritance ratio (the proportion of homozygous B6 offspring) of 0.64 (Table 2). It is interesting that the observed penetrance level is not far from 50%, as a penetrance of 50% could be explained entirely on genetic grounds, by the existence of a second, independently assorting locus with a specific genotype also required for lethality. According to this model, all female embryos with a B/D genotype at the first locus and a B/B genotype at the second locus would not be viable. This would lead to an observed inheritance ratio of 0.67, which is quite close to the value observed. We also observe a sex ratio of 1.19 males: 1 female, while the female-specific lethality effect of the model predicts a sex-ratio of 1.33 males: 1 female. We examined the data from the genome scan for alcohol preference loci (Melo et al. 1996), but no obvious candidate regions for the hypothesized second locus were found. However, as a relatively small number of female animals (<25) were genotyped at all but a few loci, the hypothesis is by no means ruled out. If the hypothesized second locus were on the X Chr, it could potentially account for the observed sex-specificity of the non-Mendelian inheritance as well the level of penetrance. However, genotyping of a larger sampling of ($F_1 \times B6$) N2 females (>80) at loci along the X Chr ruled out this possibility (data not shown).

Because our observation of transmission distortion was incidental to unrelated research on B6 alcohol preference, the number and genotypes of progeny dying between birth and weaning (3–4 weeks after birth) were not recorded, so it is possible that allelic inheritance over this region affects the early post-natal survival of offspring. However, very few animals died after weaning but prior to genotyping ($n < 5$). Thus, the timing of the effect of allelic inheritance at the Chr 11 region on offspring survival is after fertilization, but prior to weaning.

The Ovum mutant locus (*Om*), which maps within the region discussed here (Fig. 1), is responsible for early embryonic lethality or semi-lethality in certain crosses involving the DDK inbred strain (Babinet et al. 1990; Sapienza et al. 1992; Renard et al. 1994; de Villena et al. 1996). Although the DDK × DDK incross and the outcrossing of a DDK male produce normal litter sizes, the

Table 1. Statistical analysis of non-Mendelian inheritance and of sex-specific differences in allelic inheritance in N2 offspring.

Locus ^a	Male IR ^b	Female IR ^b	Sex-specific? ^c	Non-Mendelian? (Female) ^d
<i>D11Mit78</i>	* ^e	0.53 (41/78)	*	n.s. ^e
<i>D11Mit140</i>	*	0.64(82/128)	*	P = 2.0×10 ⁻³
(<i>D11Mit140</i> – <i>D11Mit219</i>)	*	0.65(73/112)	*	P = 1.8×10 ⁻³
<i>D11Mit219</i>	0.47(84/179)	0.62(93/151)	P = 8.0×10 ⁻³	P = 5.7×10 ⁻³
(<i>D11Mit219</i> – <i>D11Mit40</i>)	0.46(79/172)	0.63(91/144)	P = 2.3×10 ⁻³	P = 2.1×10 ⁻³
<i>D11Mit40</i>	0.46(81/177)	0.64(96/151)	P = 1.4×10 ⁻³	P = 1.1×10 ⁻³
(<i>D11Mit40</i> – <i>D11Mit195</i>)	0.45(75/167)	0.64(94/147)	P = 8.5×10 ⁻⁴	P = 9.7×10 ⁻⁴
<i>D11Mit195</i>	0.44(79/180)	0.64(96/151)	P = 4.0×10 ⁻⁴	P = 1.1×10 ⁻³
(<i>D11Mit195</i> – <i>D11Mit199</i>)	*	0.62(79/127)	*	P = 7.8×10 ⁻³
<i>D11Mit199</i>	*	0.58(85/147)	*	n.s.

^a For interval regions, only animals that are non-recombinant between the flanking markers were included in the analysis.

^b Inheritance ratios (IR) are reported as the proportion of N2 offspring inheriting the B6 allele from the F₁ parent (homozygous B6 offspring) at a given locus or interval. The total number of N2 progeny genotyped at each locus or interval is reported in parentheses.

^c The *t*-test was used to determine whether the observed inheritance ratios of males and females were significantly different from each other.

^d The chi-squared test was used to determine whether inheritance ratios for male and female subpopulations significantly deviated from 1:1 Mendelian inheritance. No inheritance ratios for the male subpopulation were significant in this test.

^e *, **, *** indicates that at certain markers, male animals were not genotyped. "n.s." indicates a non-significant result for a statistical test (P < 0.05).

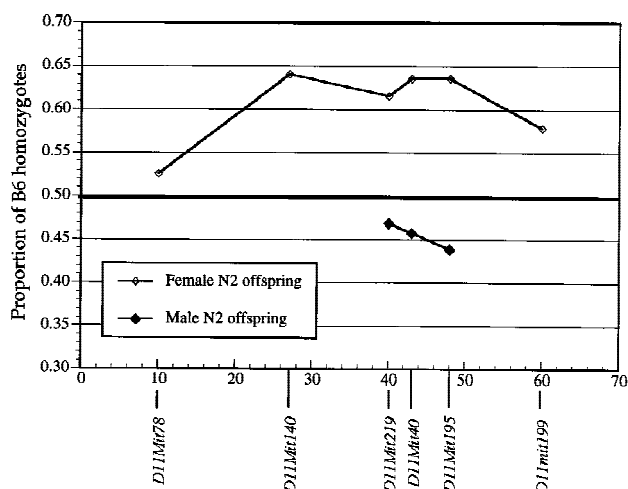


Fig. 2. Allelic frequencies for male and female N2 subpopulations at loci along Chr 11. Allelic frequencies are reported as the proportion of N2 offspring inheriting the B6 allele from the F₁ parent (equivalent to the proportion of B6 homozygous offspring). The centimorgan scale along the X axis starts at the centromere; distances between marker loci are based on the results of this study.

outcrossing of a DDK female to many strains results in >95% lethality for developing embryos. The timing of this lethal effect is post-fertilization but pre-implantation, and the few embryos that survive to implantation seem to develop normally. The accumulated data suggest that the lethality is a consequence of an incompatibility between the paternally inherited non-DDK allele and a maternal DDK cytoplasmic factor, and that both interacting factors (the offspring's genomic locus and the maternal cytoplasmic factor) map to a single locus, *Om*.

If we hypothesize that the *Om* locus is responsible for the transmission distortion reported here, our results could be explained by an incompatibility between a B6-derived maternal cytoplasmic factor [which would be present in eggs produced by both B6 and (B6 × DBA)_{F1} mothers] and a DBA-derived factor produced after fertilization. In its most simple form, however, this hypothesis fails to account for the female specificity of the distortion reported here. However, it is possible to elaborate a hypothesis involving an interaction between the *Om* locus and sex-specific factors produced during gestation.

The phenotypic effect of the *Om* locus is on embryonic survival and allelic frequencies of offspring; the extent of its effect is cross-dependent, and evidence has suggested that it may be genomically imprinted, hypothesized to normally express only the maternal allele (Sapienza et al. 1992; Babinet et al. 1990; de Villena et al. 1997). Allelic inheritance at *Alcp2*, whose maximum likelihood position is nearly identical to the position of *Om* (Fig. 1), affects alcohol consumption levels in the N2 offspring of a [(B6 × DBA)_{F1} × B6] cross, but not in the reciprocal cross (Melo et al. 1996). Genomic imprinting, with expression of the maternal allele, has been hypothesized to control *Alcp2* as well. However, the effect of *Alcp2* is also restricted to female offspring, unlike the *Om* locus, which is unrestricted with respect to sex. The occurrence of non-Mendelian inheritance reported here, over a region containing both loci, is similar to the *Om* locus, in that allelic inheritance ratios are distorted, and similar to *Alcp2* in the female specificity of its effect, but dissimilar to the *Om* locus in that the extent of its effect is not cross-dependent. There is no simple hypothesis that can explain the inconsistent coincidence of sex-specific and allelic-specific effects by loci that map to overlapping regions of Chr 11. However, these loci and this region of Chr 11 may potentially be useful for the further investigation of these relatively uncommon phenomena and their possible relatedness.

Acknowledgments. This research was supported by a grant from the National Institutes of Health to L.M. Silver. We thank Irina Agulnik for excellent technical support.

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