RESEARCH ARTICLE

A search for transmission ratio distortions in offspring from crosses between inbred mice

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Abstract

Equal transmission of the two alleles at a locus from a heterozygote parent to the offspring is rarely violated. Beside the differential embryonic mortality, nondisjunction and gene conversion that are rather irregular forms of transmission–ratio distortion (TRD), there are two major forms of departure from Mendelian segregation. The first, found in females, based on the asymmetric nature of female meiosis, is usually referred to as meiotic drive, and has been well documented in a few cases. The second is segregation distortion found in males. There are several known male-related segregation distortion systems that are caused by different fertilizing capacity of sperm cells carrying alternative alleles at a particular locus. Observation of TRD effects requires a sufficient number of offspring produced by a parental pair. As individuals in a population most likely have different genotypes in TRD affecting loci, the total transmission ratio is close to the expected Mendelian ratio and masks potential TRD effects. Highly inbred strains of laboratory mice provide a very good model for studying this phenomenon, because comparing two mice strains is effectively similar as comparison of two individuals in a population. This study tests both forms of TRD in progeny of F_1 hybrids from reciprocal crosses of inbred mice. Three previously unknown instances of TRD in females were observed. Therefore, this study concludes that some genes in females may carry alleles that can cause segregation distortion.

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Introduction

According to the first law of Mendelian, segregation occurs in a manner wherein a gamete has an equal probability to carry either of the segregating alleles. However, rare deviations from the expected segregation have been known for a long time. These so-called distorters can alter transmission ratio in specific cases (Lyttle *et al*. 1991). In this study, we avoid discussing rather irregular forms of segregation distortion like chromosome nondisjunction, gene conversion and embryonic mortality. Instead, we focused more on the two major classes of events affecting segregation ratio: transmission ratio distortion (TRD) and meiotic drive (De-Villena and Sapienza 2001). The need to understand these types of distortion is related to fundamental mechanisms governing the meiotic system, gamete formation and fertilization. TRD is rarely described in males and caused by either an impaired formation or function of sperm cells. In mice, by far the most studied example of TRD in males involves the *t*-complex on chromosome 17, reviewed by Lyon *et al*. (2003). Meiotic drive, on the contrary, is found in females and can come about by preferential entry of one allele into a polar body or an oocyte at the first or second meiotic division (Agulnik *et al*. 1990).

Both female and male related TRD systems, while being of substantial interest, are usually considered as rare specific exceptions from the basic rules. Here we present the data that indicate that loci causing TRD in mice might be more common than often thought. Observation of such effects is possible when a large number of offspring from a pair of parents can be studied. As individuals in a population most likely have different genotypes in TRD affecting loci,

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the total transmission ratio is close to the expected Mendelian ratio and thus masks numerous TRD effects. Highly inbred strains of laboratory mice provide the best available mammalian model for studying this phenomenon, because comparing two mice strains is effectively similar as comparison of two individuals in a population. Our results indicate that high consistency of the first Mendelian law, at least in part might be an outcome of numerous TRD events which negate each other. Here we present segregation data obtained in progeny of F_1 hybrids from two common inbred strains of mice. The hybrids were produced in both direct corosses and reciprocal crosses.

Materials and methods

Animals

The animals used in this study are two inbred strains of laboratory mice C57BL/6J and DBA/2J. The F_1 hybrids were obtained by crossing DBA/2J males with C57BL/6J females. These heterozygous (F_1) males and females were then backcrossed to parental C57BL/6J strain in order to produce progeny, which were studied for possible transmission distortion. The following two crosses were made:

Cross 1: ♀ F_1 (DBA/2J ♂× C57BL/6J ♀) × ♂C57BL/6J

Cross 2: ♀ C57BL/6J × ♂F_1 (DBA/2J ♂× C57BL/6J ♀)

There were two series of experiments. The first series of experiments was done by Dr R. W. Elliott and colleagues (Roswell Park Cancer Institute, Buffalo, NY). In the first series, 93 offspring from cross 1 and 94 offspring from cross 2, as well their parents were genotyped. In the second series, carried out at the University of New England Armidale, NSW, Australia, 182 offspring from cross 1 and 149 offspring from cross 2 were genotyped.

Sample collection and DNA extraction

Pregnant females were sacrificed for sample collection on the 16th or 17th day of pregnancy. The embryos were collected to extract offspring DNA. The excision of liver from the dam and the sire were used to collect samples for extraction of parental DNA. The DNA extraction was carried out using phenol/chloroform/isoamyl alcohol mixture.

Estimation of DNA concentration and quality

DNA extracted from the mice samples were estimated by two techniques: agarose gel electrophoresis and sensitive spectrophotometer. In the first technique, 1% agarose gel was prepared and stained using 1:50 concentration GelStar nucleic acid gel stain. The second technique was done using a sensitive spectrophotometer (NanoDrop Technologies, Wilmington, USA). The concentration of the sample is expressed as ng/μl and is measured at wavelengths of 260 nm and 280 nm. The ratio of the concentration 260/280 indicates the purity of the sample.

Genotyping

In the first series of experiments, 93 female offspring were genotyped for 227 molecular markers randomly distributed over the mouse genome. Also 94 male offspring were genotyped for 210 markers, following the method of Yen *et al*. (1997). The list of markers can be found in table 1 of electronic supplementary material at http://www.ias.ac.in/jgenet/.

The DNA samples obtained in the second series of experiments (25μl of 20 ng/μl of each sample) were sent to Australian Genome Research Facility (AGRF) for microsatellite genotyping. This laboratory employs fluorescently labelled primers for PCR amplification of the desired microsatellite. Seven microsatellite markers were chosen for studying 182 female offspring on the basis of segregation results obtained in the first series of experiments. The following markers were used: *D3Mit159, D4Mit278, D9Mit286, D14Mit101, D14Mit234, D16Mit182* and *D19Mit43*. The basis of selecting these markers is justified in the result section. 149 male offspring were genotyped in the second series of experiments using the following autosomal-molecular markers: *D2Mit266, D3Mit328* and Y chromosome linked marker *Smcy*.

Statistical analysis of the results

A goodness of fit χ^2 test was carried out to identify the presence of TRD in all the markers genotyped in both the crosses. Two thresholds were used to identify those markers that showed a distorted transmission. One was $0.01 < P < 0.05$ and the other $P < 0.01$. The first threshold was adequate to reject the null hypothesis (H_o) and to conclude that segregation distortion would likely occur whereas the second threshold level confirms that segregation distortion would most likely occur.

Results

The genotyped data obtained in the first series of experiments were subjected to statistical analysis. Figure 1 shows distribution of deviations (χ^2 values) from the expected 1:1 ratio in offspring from the cross 1 where F_1 hybrids were females. Several loci from the total 227 studied markers show significant deviations with P values between 0.05 and 0.01. As the number of studied offspring was limited, and multiple tests were performed, a cautious interpretation of these deviations is warranted. Nevertheless, these results might be used as an indication of possible TRD and we have chosen seven microsatellite markers on six chromosomes which show significant χ^2 values for additional study in the second series of experiments.

Table 1 demonstrates transmission ratio of seven chosen loci in cross 1 where F_1 hybrids were females. In the second series of experiments there were no statistically significant deviations from 1:1 ratio. Nevertheless, markers *D3Mit159, D4Mit278* and *D19Mit43* located on chromosomes

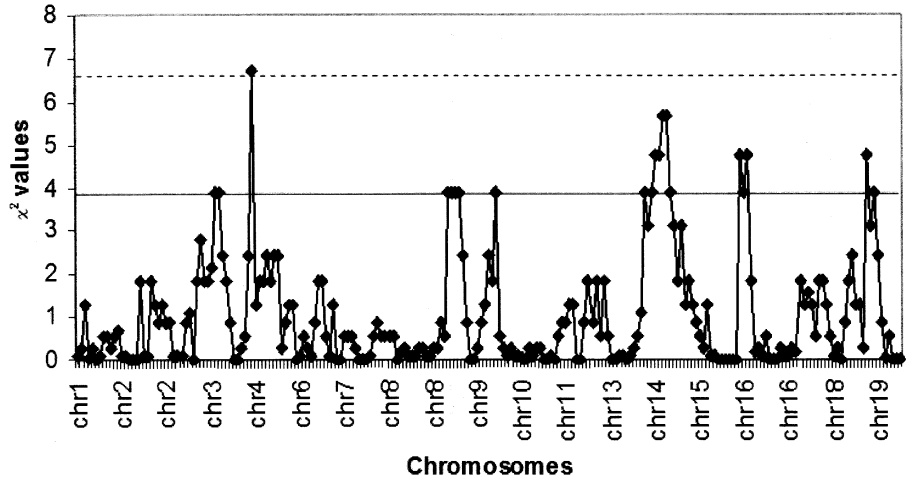

Figure 1. Distribution of deviations (χ^2 values) from 1:1 ratio along autosomes of offspring from crosses ♀♀ F_1 (DBA×C57BL) × C57BL ♂♂. Solid line indicates $P = 0.05$ and broken line indicates $P = 0.01$. Markers on chromosome five were not studied. Few chromosomes which carry numerous markers are depicted twice on X axis, chromosome 17 is not shown.

Table 1. Comparison of transmission data obtained in both series of experiments for cross 1 (♀F_1× ♀C57BL/6J). *B* allele originated from C57BL/6J strain and *D* allele from DBA/2J.

	First series of experiments				Second series of experiments				Total				
	Observed				Observed				Observed				
Marker	*B* alleles	*D* alleles	Exp.	χ^2	*B* alleles	*D* alleles	Exp.	χ^2	*B* alleles	*D* alleles	Exp.	χ^2	*P*
D3Mit159	37	56	46.5	3.88	84	98	91	1.08	121	154	138	3.96	0.047
D4Mit278	34	59	46.5	6.72	82	100	91	1.78	116	159	138	6.72	0.0095
D9Mit286	37	56	46.5	3.88	93	89	91	0.09	130	145	138	0.82	0.365
D14Mit101	35	58	46.5	5.69	96	85	90.5	0.67	131	143	137	0.53	0.467
D14Mit234	35	58	46.5	5.69	96	86	91	0.55	131	144	138	0.61	0.435
D16Mit182	36	57	46.5	4.74	95	86	90.5	0.45	131	143	137	0.53	0.467
D19Mit43	57	36	46.5	4.74	99	82	90.5	1.6	156	118	137	5.27	0.022

3, 4 and 19 showed similar types of TRD in both experiments and the combined data showed statistically significant. Marker *D4Mit278* on chromosome 4 (55.2 cM) had particularly strong TRD. Studied markers located on chromosomes 9, 14 and 16 had inconsistent patterns of deviations in the first and second series of experiments.

Transmission ratios found in the first series of experiments in offspring from the cross 2 where F_1 hybrids were males are shown in figure 2. In the first series of experiments from 210 studied microsatellite loci only in two case deviations from the expected 1:1 ratio were significant. These two markers, *D2Mit266* and *D3Mit328*, located on chromosomes 2 and 3 were chosen for additional testing in the second series of experiments together with Y chromosome linked locus *Smcy*. As table 2 shows, transmission ratio in none of these three markers was different from the expected, the same is correct for the combined data.

Thus we can summarize that there was some degree of volatility in transmission ratios obtained in two series of experiments. Still we have observed three statistically significant TRD in offspring from cross 1.

Discussion

Two facts observed in this investigation can be emphasized. First, the differences observed between crosses 1 and 2. In offspring of F_1 male hybrids no TRD was found. F_1 female hybrids, however, showed a number of TRD, three of which were statistically significant in combined data. While a discussion about the nature of observed TRD is premature,

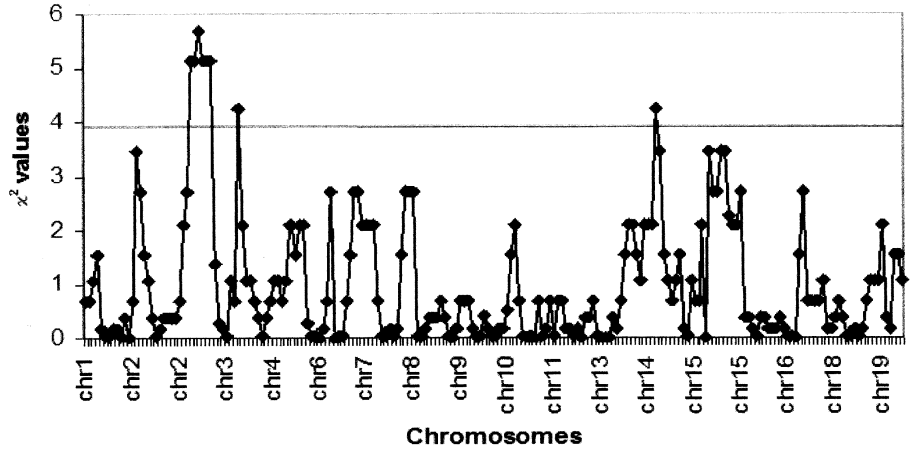

Figure 2. Distribution of deviations (χ^2 values) from 1:1 ratio along autosomes of offspring from crosses ♀♀C57BL × F_1 (DBA × C57BL) ♂♂. Solid line indicates $P = 0.05$. Markers on chromosome five were not studied. Few chromosomes which carry numerous markers are depicted twice on X axis, chromosome 17 is not shown.

Table 2. Comparison of transmission data obtained in both series of experiments for cross 2 (♂F_1 × ♀C57BL/6J). *B* allele originated from C57BL/6J strain and *D* allele from DBA/2J.

	First series of experiments				Second series of experiments				Total				
	Observed				Observed				Observed				
Marker	*B* alleles	*D* alleles	Exp.	χ^2	*B* alleles	*D* alleles	Exp.	χ^2	*B* alleles	*D* alleles	Exp.	χ^2	*P*
D2Mit266	58	36	47	5.15	75	74	74.5	0.01	133	110	121.5	2.18	0.92
D3Mit328	36	58	47	5.15	72	76	74	0.11	108	134	121	2.79	0.74
					XY	XX							
Smcy					73	77	75	0.11	73	77	75	0.11	0.74

it is essential to remember that in other similar cases meiotic drive was involved. The asymmetric nature of female meiosis is a major cause of unequal frequency of gametes carrying certain alleles, haplotypes and chromosomes. Further, investigations are desirable before a firm conclusion about the three cases of observed TRD can be drawn. Based on the previously obtained knowledge, it can be suggested that possible cause for such deviations could be meiotic drive, that increase the probability of an allele or a haplotype to enter oocyte rather then the polar body (Ruvinsky 1995; Pardo-Manuel de Villena *et al.* 2000). Male meiosis is different in this regard and this could be a possible explanation for the differences between results of crosses 1 and 2.

Random nature of the process responsible for formation of transmission ratios provides some explanation for volatility of the results observed in two series of experiments described earlier. Despite all the attempts to standardize genetic background and experimental conditions we saw a great deal of variation between these two series of experiments. Probably, a part of this variation can be attributed to a limited number of studied offspring; the rest should be related to variation of environmental factors as well as less known genetic factors. Certainly, embryonic mortality can not be ruled out as a contributing factor. However, the margins for embryonic mortality in mice are narrow in order to accommodate a significant TRD shifts.

A massive support for Mendelian transmission pattern and a small number of well described TRD caused by distorter loci seems to contradict to the idea that there are numerous genes affecting equal segregation and/or transmission of alleles, haplotypes and chromosomes. However, there is a reason to believe that resolution of typical test crosses is not sufficient in many cases to reveal 'hidden' TRD. In a classical test cross, $Aa \times aa$, attention is usually concentrated on the gene in question and often little or nothing is known about surrounding genes and particular alleles. It opens a possibility that TRD genotypes of different individuals with the same basic genotype, like *Aa* or *aa*, may differ significantly and offspring of some individuals have high probability of getting allele *A*, while others more likely will get

a. The final step of a test cross is adding up all the results, which may in many situations lead to the expected 1:1 ratio; deviations usually are not statistically significant. There are a few models which allow overcoming limitation of this standard approach. For instance, the same parental pair produces a large number of offspring. Unfortunately, there is a limit to this approach in mammals. In this regards highly inbred strains of mice represent another opportunity. Here, one can take as many males and females as practical and add up transmission data in offspring without a large risk of negating the differences simply because, the parental animals have almost exactly the same genotype and fluctuations between individuals should be of random nature. In a few studies of similar kind, which used interspecific backcrosses, TRD were discovered (Siracusa *et al*. 1991).

It is also important to remember that we studied TRD in one particular genotype (effectively only in one individual). Obviously in a population/species a number of active TRD systems must be greater and, if so, this is not a local phenomenon and more genes might be involved. The total number of currently identified genes, that are specifically involved in the TRD affecting processes, is close to 50 (Matzuk and Lamb 2002) and continues to grow.

There is an increasing recognition of the idea that segregation distortion is not necessarily is a 'rare genetic curiosity' (Taylor and Ingvarsson 2003; Zölner *et al*. 2004). Despite a significant interest in a few well-studied TRD systems, a greater role, which TRD causing genes may play in evolution of populations, is not yet generally appreciated. Our data point out that many such TRD systems may remain undetected.

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