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The mouse t complex distorter-3 (*Tcd*-3) locus and transmission ratio distortion

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Summary

A novel central partial *t* haplotype was generated by screening for a recombination event between overlapping distal and proximal partial haplotypes. This haplotype contains just two elements – Tcr^t and $Tcd\text{-}3^t$ – involved in the *t*-specific transmission ratio distortion phenotype. Breeding analysis of males that carry this chromosome provides evidence that *Tcd-3* is, indeed, a distorter locus and not a second responder. Furthermore, the data indicate that a single well-defined distorter locus is insufficient to overcome completely the self-destructive, low transmission ratio distortion phenotype expressed by the *t* allele at the *t* complex responder locus, although a small, but highly significant, effect was observed.

1. Introduction

In papers published in 1977 and 1984, Lyon proposed and further developed a general model to explain the genetic basis for the transmission ratio distortion phenotype expressed by male mice that carry one or more complete or partial *t* haplotypes (Lyon & Mason, 1977; Lyon, 1984). A *t* haplotype is a variant form of the proximal portion of chromosome 17 that exists in natural populations of house mice (Silver, 1985; Klein, 1986). The complete *t* haplotype is distinguished from a wild-type homologue by a series of four non-overlapping inversions that act to suppress recombination and maintain the variant chromosomal region as a single genetic entity in its transmission from one generation to the next (Committee for Mouse Chromosome 17, 1991). Complete *t* haplotypes are generally transmitted to the offspring of heterozygous $+/t$ males at very high frequencies – often greater than 95% – and it is this property that provides a *t* haplotype with its 'selfish chromosome' character (Silver, 1988; Silver, 1993).

Although recombination is suppressed across the *t* haplotype in heterozygotes, rare recombination events can occur – most often at sites between inversions (Committee for Mouse Chromosome 17, 1991) – and these yield partial *t* haplotypes that have lost the ability to be transmitted at the very high frequencies characteristic of complete *t* haplotypes (Lyon & Mason, 1977; Lyon, 1984).

Lyon was able to explain the transmission ratio distortion (TRD) data obtained with various *t* haplotype genotypes by hypothesizing the existence of two classes of loci that interacted to produce the effect. The *t* complex responder (*Tcr*) locus – proposed as the only member of the first class – acts in a haploid cell-specific manner to determine the particular homologue of chromosome 17 that will be transmitted at an altered ratio. When an animal is homozygous for either a wild-type or a *t* allele at this locus, distortion of transmission ratio can not occur. The second class of loci contains at least three, and perhaps several more, members that are referred to as *t* complex distorter loci and are numbered sequentially according to the order in which they were identified (*Tcd-1*, *Tcd-2*, and so on). The *Tcd* loci act additively, in either *cis* or *trans*-configuration, to raise the frequency at which Tcr^t is transmitted to offspring. Interestingly, in the extreme case where a Tcr^t allele exists in the absence of any Tcd^t loci, it is transmitted at a frequency far below 50% (Lyon & Mason, 1977).

The general tenets of Lyon's TRD model have been confirmed through the results obtained in many experiments with partial and complete haplotypes (Bennett & Dunn, 1971; Styrna & Klein, 1981; Lyon, 1987; Silver & Remis, 1987; Silver, 1989). Molecular markers have allowed the mapping of each locus to defined DNA regions within the *t* haplotype (Fox *et al.* 1985; Silver & Remis, 1987) and, in several cases, cloned candidates for these loci have been described (Willison, Dudley & Potter, 1986; Schimenti *et al.* 1988; Lader *et al.* 1989; Ha *et al.* 1991; Yeom *et al.*

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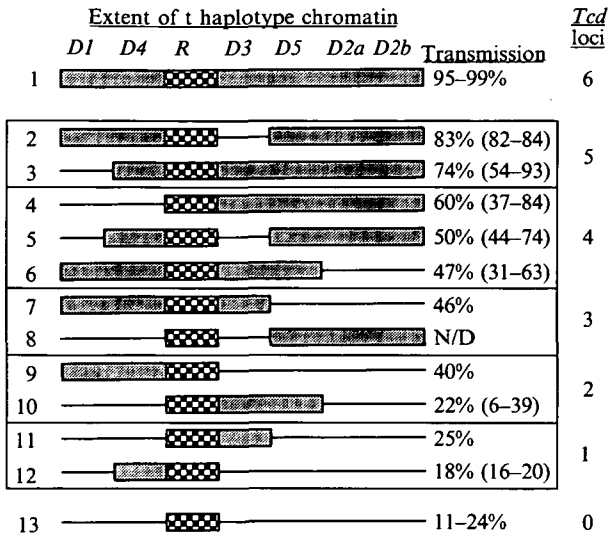

Fig. 1. The effect of different combinations of distorter loci on TRD. Each composite horizontal bar represents a different class of *t* haplotypes or combinations of *t* haplotypes that bear a particular set of TRD elements. The elements are indicated according to their order along the chromosome with the centromere at the left, and are named in abbreviated form: *D1* is *Tcd-1*^{t}, *D4* is *Tcd-4*^{t}, *R* is *Tcr*^{t}, *D3* is *Tcd-3*^{t}, *D5* is *Tcd-5*^{t}; *D2a* and *D2b* represent the postulated sub-loci that together act as *Tcd-2*^{t}. The spacing between elements is not indicative of true genetic distances. The transmission frequency (or range of frequencies in cases of large variation) is indicated to the right of each haplotype/genotype class. The actual *t* haplotypes involved and references to earlier data are as follows: (1) complete *t* haplotypes; (2) t^{h49}/t^{h18} (Lyon, 1984) and t^{h49}/t^{Tu2} (Silver & Remis, 1987); (3) t^6 (Lyon, 1984; Silver & Remis, 1987); (4) T^{s6}, t^{h17} (Silver & Artzt, 1981; Lyon, 1984); (5) t^{h2}/t^{h18} (Lyon & Mason, 1977); (6) t^{w18} (Bennett & Dunn, 1960; Bennett, Dunn & Artzt, 1976; Bennett, Alton & Artzt, 1983; Silver & Remis, 1987; Silver, 1989); (7) t^3 (Dunn & Gluecksohn-Waelsch, 1951) and t^{Tu1} (Styrna & Klein, 1981); (8) t^{low}/t^{h18} (This is the only class of single dose chromosome 17 constructs that has not yet been tested for TRD (N/D: not determined, M. Lyon, personal communication); (9) t^{h49} (Lyon, 1984); (10) multiple recombinant chromosomes derived from crossovers between t^{w18} and distal partial haplotypes of the form illustrated in chromosome 4 of this figure (Bennett, Alton & Artzt, 1983); (11) $t^{Prt1RD3}$ Table 1 of this report); (12) t^{h2} (Lyon & Mason, 1977; Lyon, 1992); (13) t^{low}, t^{lowH}, t^{low3H} (Bennett & Dunn, 1971; Bennett, Alton & Artzt, 1983; Lyon, 1984; Lyon & Zenthon, 1987).

1992). However, as of yet, the data have not allowed a clear demonstration that any cloned candidate is indeed equivalent to any TRD locus. Furthermore, the number and nature of *Tcd* loci and the level at which they interact with *Tcr* all still remain open questions.

The best characterized of the distorter loci are *Tcd-1*, *Tcd-2* and *Tcd-3*. Each is defined within the context of a separate *t* genotype where the *t* allele of this locus alone has been eliminated (chromosomes 3, 6, and 2 in figure 1 which contains references for each). Without *Tcd-2*^{t} or *Tcd-1*^{t}, the transmission of the *Tcr*^{t} bearing chromosome can drop dramatically from the 95–99% level of complete *t* haplotypes. It is also the case that the absence of either of these loci allows genetic background effects to cause wild shifts in the level of TRD that is actually expressed by any individual male (figure 1). In the absence of *Tcd-3*^{t}, transmission frequencies drop from 95% to moderate levels of 82–84%. Each of the three well-characterized distorter loci is associated with large inversions which prevent fine mapping. Each could actually include multiple genes; this is especially true for *Tcd-2* which has only been localized to a 9 cM region and, in general, appears to exert a stronger effect on TRD than any other *Tcd* locus.

Two additional distorter loci (*Tcd-4* and *Tcd-5*) have been defined based on the contribution of each to TRD within the context of complex *t* genotypes (Silver & Remis, 1987; Silver, 1989). The available *t* chromosomes do not allow one to construct genotypes that eliminate only the *t* allele at either of these two loci in isolation, thus, the existence of both is still tentative. In particular, Lyon has questioned the existence of *Tcd-4* because of the observation that chromosomes containing the putative *Tcd-4*^{t} allele in combination with *Tcr*^{t} do not express a higher transmission frequency than *Tcr*^{t} alone (Lyon, 1990).

To further understand the nature of the *Tcd* loci and their interactions with *Tcr*, we constructed a partial *t* haplotype that contains only a central portion of the complete *t* haplotype with the *Tcr*^{t} allele and the single distorter allele *Tcd-3*^{t}. Males that carry this novel haplotype were tested for TRD. Two potential outcomes of this experiment were predicted based on different potential properties of the *Tcd-3* locus. First, since *Tcd-3*^{t} has never been examined for *trans*-interactions with *Tcr*^{t}, it is still formally possible that the *Tcd-3* locus is actually a second member of the responder class. If this were the case, the combination of a double dose of responders together in isolation might produce a rate of transmission even lower than the 10–20% observed with just *Tcr*^{t}. If *Tcd-3* is instead a distorter, the second potential outcome of our breeding experiment is predicated on the general assumption that distorters act additively to increase the transmission of *Tcr*^{t}. In this case, we might expect the [*Tcr*^{t}-*Tcd-3*^{t}] combination to be transmitted at a frequency that was greater than that observed for *Tcr*^{t} alone.

2. Materials and methods

All mice were bred in our colony at Princeton University which is maintained according to NIH standards. The partial *t* haplotypes Tt^{s6} and t^3 are described elsewhere (Committee for Mouse Chromosome 17, 1991). Animals were genotyped based on two visible marker loci – Brachyury (*T*) and tufted (*tf*). *T*/+ animals express a dominant short-tail pheno-

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type. Animals doubly heterozygous for alternative mutant alleles at the *T* locus – T/t – are tailless. The tufted mutation is recessive; tf/tf animals are recognized by waves of hair loss in the adult.

Males that carried the partial haplotype Tt^{s6}, which is tightly coupled to the *tf* mutation, were bred to females homozygous for the partial haplotype t^3. Double heterozygous ($Tt^{s6}\,tf/t^3+$) female offspring were identified as tailless non-tufted animals and were bred to homozygous tufted ($+tf/+tf$) males. Non-recombinant offspring were either short-tailed and tufted ($Tt^{s6}\,tf/+tf$) or normal-tailed and non-tufted ($t^3+/+tf$). A recombinant animal that carried only a central portion of a *t* haplotype was identified based on the observation of a short-tailed non-tufted phenotype ($Tt^{Pri1RD3}+/+tf$).

3. Results

A novel partial *t* haplotype that contained both the Tcr^t and $Tcd\text{-}3^t$ alleles in isolation was created by screening for recombinants between the two overlapping partial haplotypes t^3 and t^{s6}. As shown in figure 2, these partial haplotypes overlap only in the region associated with *Tcr* and *Tcd-3*. In the Tt^{s6}/t^3 female, we only expect recombination to occur within this region of overlap, because both the proximal and distal portions of this *t* complex genotype are heterozygous for inversions that act to prevent viable recombinants. A female with the expected recombinant genotype was recovered. This recombinant chromosome has been named $Tt^{Pri1RD3}$. Eight males were produced that were heterozygous for this chromosome and a wild-type homologue. These eight males were progeny tested to determine the frequency at which this novel partial *t* haplotype is transmitted. Offspring were scored according to tail length: short tails indicated the transmission of $Tt^{Pri1RD3}$ and normal tails indicated the transmission of the wild-type homologue present in the male parent. The results are presented in Table 1. The average transmission ratio was calculated in two ways. By summing up all offspring obtained over all four males, the value was 24·8%. By averaging together the transmission ratios obtained for each male, the value was 24·1%. The lowest transmission frequency observed was 18% and the highest was 31%, however, these two extremes were obtained from males that produced the smallest numbers of total offspring. Upon elimination of the highest and lowest scores the range was 20%–27%. There were no significant differences among the results obtained with the six males that fell into this middle range.

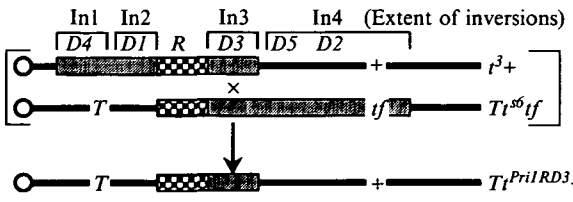

Fig. 2. Generation of a novel partial haplotype with Tcr^t and $Tcd\text{-}3^t$ only. The top two chromosomes are a schematic representation of the *t* complex genotype of the $t^3+/Tt^{s6}\,tf$ female. The extent of the four inversion polymorphisms characteristic of complete *t* haplotypes is indicated above the genotype along with the six TRD elements in the abbreviated notation described in the legend to figure 1. The likely site of the recombination event is indicated with an '*X*'. The structure of the recombinant $Tt^{Pri1RD3}$ chromosome is indicated below the arrow.

4. Discussion

The average transmission ratio obtained with males heterozygous for $Tt^{Pri1RD3}$ was somewhat greater than the average obtained with males that carry an isolated Tcr^t element (figure 1). This result argues strongly against the possibility that the *Tcd-3* region harbours a second *Tcr*-class locus. The *Tcd-3* region does contain a second member (*Tcp-10c*) of the gene family defined by an element proposed as a candidate for *Tcr* which is *Tcp-10b* (Schimenti *et al.* 1988; Cebra-Thomas *et al.* 1991). If *Tcp-10b* does indeed have *Tcr* activity, it would appear that *Tcp-10c* does not.

The $Tcd\text{-}3^t$ allele is well-defined by its effect in absentia where the level of TRD is consistently reduced from the 95–99% range down to 82–84% (chromosome 2, figure 1). The results presented here suggest that $Tcd\text{-}3^t$ in isolation from other distorters can increase the transmission of a Tcr^t element to a small degree. The average TRD value obtained by summing over all $Tt^{Pri1RD3}/+$ offspring – 24·8% – was significantly different ($P < 0{\cdot}004$) from all TRD values of 20% or less, which are commonly observed in breeding experiments with males that contain isolated Tcr^t elements.

As described in the introduction, the putative $Tcd\text{-}4^t$ allele can not act to increase the ratio of an isolated Tcr^t allele to a significant degree (chromosome 12, figure 1). The significant difference in data obtained with chromosomes that contain Tcr^t and either $Tcd\text{-}4^t$ or $Tcd\text{-}3^t$ as single distorter elements (no. 12 and 11 in figure 1) thus suggests that the $Tcd\text{-}3^t$ element has the more powerful effect on TRD. Nevertheless, it seems clear that a single dose of a single distorter locus can have, at most, only a small effect on the transmission of an associated Tcr^t element. In contrast, with the two distorters $Tcd\text{-}1^t$ and $Tcd\text{-}4^t$, the negative effect of Tcr^t can be almost completely overcome with an increase in transmission from 11–24% to 40% (chromosome 9, figure 1). With three distorter elements (chromosome 7, figure 1), the transmission frequency is not significantly different from 50%.

As indicated previously, the *Tcd-2* locus extends across the largest portion of the *t* complex and exerts a disproportionate effect on both the TRD and sterility

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Table 1. *Frequency of transmission of the* $Tt^{Pri1RD3}$ *chromosome from heterozygous males*

Mouse no.	Short[1]	Normal[2]	*R-D3* transmission
25722	5	23	0·18
25909	7	28	0·20
26343	7	27	0·21
26341	6	21	0·22
25723	19	51	0·27
25908	20	54	0·27
26342	10	27	0·27
26339	8	18	0·31
Total	82	249	0·248(0·24)[3]

[1] A short tail indicates transmission of $Tt^{Pri1RD3}$.
[2] A normal tail indicates transmission of the wild-type (+) homologue.
[3] The average of the frequencies determined for each male is indicated in parenthesis.

phenotypes expressed by *t* haplotypes. Thus, as a working hypothesis, and for the purpose of further discussion, I will assume that this locus is actually composed of two sub-loci designated *Tcd-2A* and *Tcd-2b* in figure 1. With this interpretation, one can see that the elimination of a single TRD locus (either *Tcd-1* or *Tcd-3*) drops the transmission frequency down to a moderate level of 74–83%, with some variability in the former value, but the elimination of two loci (*Tcd-1* and *Tcd-4*, or *Tcd-1* and *Tcd-3*, or *Tcd-2a* and *Tcd-2b*) causes a high level of variability in the transmission frequencies observed for individual males with the same genotype from significantly below 50% to significantly above. This highly variable expressivity is presumably due to the interactions of non-chromosome 17 TRD modifier genes (Gummere, McCormick & Bennett, 1986) whose effects are amplified in these moderate ratio *t* genotypes.

A comparison of chromosomes 10 and 11, and of chromosomes 6 and 7 in figure 1 shows that the *Tcd-5* locus does not exert a strong effect on TRD in genotypes that contain single doses of various *Tcd* elements. Although the effect of a putative *Tcd-5* locus is not visible in these comparisons, it has been seen in other comparisons of TRD and fertility with genotypes containing double doses of *Tcd-1* and *Tcd-4* (Silver, 1989). Thus, the evidence for the existence of *Tcd-5* is still tentative, but if it does exist, it would be the weakest-acting of all the *Tcd* loci characterized to date.

The data presented here, along with those published previously (Silver, 1989), provide confirming evidence in support of the view that the distorters *Tcd-1*, *Tcd-3* and *Tcd-4* play similar – although distinguishable – roles in amplifying the strength of the TRD effect (Lyon, 1992). However, it is not yet possible to determine whether these similar genetic effects are caused by similar biochemical mechanisms. An understanding at this level must await the characterization of the true gene products from these loci.

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