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Distorter genes of the mouse *t*-complex impair male fertility when heterozygous

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Summary

Male mice heterozygous for two distorter genes, *Tcd-1* and *Tcd-2*, of the mouse *t*-complex but homozygous wild type for the responder, were generated by crossing animals carrying the partial *t*-haplotypes t^{h51} and t^{h18} to inbred strains. The fertility of these males was then compared with that of their brothers carrying normal chromosome 17s. On three of the inbred backgrounds used, C3H/HeH, C57BL/6J and TFH/H, the $t^{h51}t^{h18}+/+++$ males were significantly less fertile than their normal sibs. With the fourth inbred strain used, SM/JH, both types of male were normally fertile. This confirmed earlier preliminary findings that when both homologues of chromosome 17 carry wild-type alleles of the responder, heterozygosity for the distorter genes is sufficient to impair fertility, but the effect varies with genetic background. These results are consistent with the concept that both the transmission ratio distortion and the male sterility caused by the *t*-complex are due to harmful effects of the distorter genes on wild-type alleles of the responder.

1. Introduction

The *t*-complex on chromosome 17 of the mouse has two major effects on sperm function (Frischauf, 1985; Silver, 1985). The first is distortion of transmission ratio from males such that males heterozygous for a complete *t*-haplotype transmit this homologue of chromosome 17 to far more than 50% of their offspring. The second is sterility in males homozygous for semi-lethal *t*-haplotypes or doubly heterozygous for lethal ones. Lyon (1984, 1986) studied the genetics of these phenomena. She concluded that distortion of transmission ratio depended on the presence of a factor called the responder (*Tcr* or R) in the centre of the complex. The *t*-complex form of the responder must be present and heterozygous for distortion to occur. The responder was acted on by three or more trans-acting distorter genes (*Tcd-1*, *Tcd-2*, etc., or D1, D2, etc.) in such a way that the chromosome carrying the *t*-form of the responder was preferentially transmitted. Male sterility again depended on multiple factors. These were located in the same partial *t*-haplotypes as the distorters and were believed to be identical with them. Homozygosity for at least one of the distorters was necessary for male sterility to occur, but the *t*-form of the responder was not required. The interpretation of these data was that both the male sterility and the ratio distortion were due to harmful effects of the distorter genes on the wild-type form of the responder. The *t*-form of the responder could thus be visualized as a gene(s) conferring resistance to the distorters.

In line with these results was an incidental finding that, in animals homozygous for the wild-type form of the responder, the distorters appeared able to impair male fertility when heterozygous (D1D2/++), as opposed to the usual requirement for homozygosity of at least one distorter to produce male sterility (e.g. D1D2/D1+). However, this impairment of fertility in heterozygotes for distorters was apparent in only two of three groups of animals studied, suggesting an effect of genetic background. Further work was needed to establish the validity of the apparent effect.

In the present work, animals carrying D1 and D2 were crossed to inbred strains. The fertility of F1 sons heterozygous for D1 and D2 was then compared with that of their full brothers, carrying normal chromosome 17s. The results confirm the earlier preliminary findings that, in the absence of the *t*-form of the responder, heterozygosity for D1 and D2 can indeed impair male fertility, and that the effects vary with genetic background.

2. Materials and Methods

The source of the distorters D1 and D2 was a chromosome carrying the partial *t*-haplotypes t^{h51}

and $t^{h\ 18}$, used in the earlier studies (Lyon 1984, 1986). This chromosome was maintained by crossing to the inbred strain TFH (genetically $Ttf/+tf$) using the crosses $t^{h\ 51}t^{h\ 18}+/T+tf \times ++tf/++tf$ and $t^{h\ 51}t^{h\ 18}+/++tf \times T+tf/++tf$ in alternate generations.

For the crosses to inbred strains $t^{h\ 51}t^{h\ 18}+/T+tf$ animals were used. The four inbred strains chosen were C3H/HeH, C57BL/6J, TFH/H and SM/JH. In the first two cases $T+tf/t^{h\ 51}t^{h\ 18}+$ males were crossed to inbred females, whereas for the strains TFH/H and SM/JH the reciprocal outcross was used. For each strain a minimum of 5 F1 sons of each type, $t^{h\ 51}t^{h\ 18}+/+++$ or $T+tf/+++$, were collected and tested. For the fertility tests, males aged 7–12 weeks were placed with two females each (aged 7–12 weeks) of the TFH/H strain; $t^{h\ 51}t^{h\ 18}+/+++$ males with $T+tf/++tf$ females and $T+tf/+++$ males with $++tf/++tf$ females. The females were observed regularly for signs of pregnancy and for live young. If no young or pregnancy had been seen after one month the females were killed and dissected for signs of pregnancy. If young were born the trios were continued for a further two months, or occasionally longer, and the number of liveborn and weaned young were recorded. The young were classified for tail-length and for tufted (*tf*) as a check on the genotype of the males.

3. Results

With three of the four inbred strains studied there was clear evidence that the fertility of the $t^{h\ 51}t^{h\ 18}+/+++$ males was impaired with respect to that of their $T+tf/+++$ brothers (Table 1). No $T+tf/+++$ male was sterile in any of the crosses, but with each of strains C3H/HeH, C57BL/6 and TFH/H at least one $t^{h\ 51}t^{h\ 18}+/+++$ male was completely sterile. With strain C57BL/6 4 of 7 such males were sterile (Table 1). In addition, among those males which were fertile, the number of young born per mated female per month was statistically significantly lower than that from $T+tf/+++$ males. This was true not only in the first month of mating, but also in the later months (except with strain TFH/H, where in later months the difference was significant only at the 10% level). Thus, there are no grounds for supposing that the sterility of some $t^{h\ 51}t^{h\ 18}+/+++$ males was due to a delayed onset of fertility. The numbers of young born to the $T+tf/+++$ males were normal for the TFH/H females used.

It might be argued that the impaired fertility of the $t^{h\ 51}t^{h\ 18}+/+++$ males was due to some property of the chromosome 17 they carried other than the distorter genes. Some evidence on this comes from a few animals which proved on testing not to be of the desired genotype, due to recombination in the outcross

Table 1. *Fertility of* $t^{h51}t^{h18}+/+++$ *males compared to that of* $Ttf/++$ *sibs*

Outcross strain	Male type	No. males		Young/female/month	
		Total	Sterile	Month 1	Later
C3H/HeH	t	5	1	1·38** ±0·57	1·75** ±0·32
	T	5	0	5·50 ±0·43	6·81 ±0·90
C57BL/6J	t	7	4	2·17* ±1·08	2·63* ±1·04
	T	5	0	6·00 ±0·94	6·40 ±1·05
TFH/H	t	7	2	1·40* ±0·54	2·85 ±0·78
	T	5	0	4·10 ±0·78	6·38 ±1·35
SM/JH	t	5	0	4·60 ±0·92	6·95 ±0·89
	T	4†	0	6·29 ±0·61	5·71 ±1·51

Young/female/month includes only females mated to known fertile males.
Significance was tested using Student's *t* test, except that for strain C3H/HeH Welch's *t* was used.
* Significantly different from $Ttf/++$ brothers at 5% level.
** Significantly different from $Ttf/++$ brothers at 1% level.
† In addition there was one recombinant $Tt^{h\ 18}+/+++$ male, which was normally fertile.

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parent. One animal in the C3H/HeH cross, and one in the C57BL/6 cross, proved to be not $t^{h\,51}t^{h\,18}+/+++$, but instead carried *tf*, and hence were $t^{h\,51}+tf/+++$. Both these animals were fully fertile.

Results with the fourth inbred strain SM/JH were different. None of the tested $t^{h\,51}t^{h\,18}+/+++$ males was sterile (Table 1). Furthermore, there was no difference between the $t^{h\,51}t^{h\,18}+/+++$ males and their $T+tf/+++$ sibs in young born per female per month, either in the first month or in later months, all the males siring normal numbers of young. In addition to the males of the desired genotypes there were two crossover males in this group, one $t^{h\,51}+tf/+++$ and one $Tt^{h\,18}+/+++$, and these too were full fertile.

4. Discussion

The results of this work confirm and extend the earlier preliminary findings. After crosses to three different inbred backgrounds, males heterozygous for the distorter genes D1 and D2 (carried on the $t^{h\,51}t^{h\,18}+$ chromosome), and homozygous wild type at the responder locus, showed clearly impaired fertility, when compared with their normal sibs. The fourth inbred strain, SM/JH, gave different results, with the fertility of the heterozygous D1D2 males unimpaired. Thus both of the preliminary findings are confirmed. Firstly, heterozygosity for D1 and D2 in the presence of wild-type alleles at the responder locus can result in impaired male fertility. Secondly, the effect can vary with genetic background.

At present it is not known whether the genetic background effect involves chromosome 17. The group of inbred strains used for outcrossing differed in alleles at various loci in the proximal part of chromosome 17, including those of *Tcp-1*, *Tas*, *Ggm-1*, *H-2* and *Neu-1* (Willison, Dudley & Potter, 1985; Washburn & Eicher, 1983; Hashimoto *et al.* 1983; Klein, Figueroa & David, 1983; Peters *et al.* 1981; Womack, Yan & Potier 1981) so there is ample scope for an effect of this chromosome region, but further work is needed to elucidate this point.

Although the harmful effects are attributed to the distorter genes D1 and D2, the recombination suppression caused by the *t*-complex makes it impossible to exclude the possibility that the genes responsible were in fact others closely linked to D1 and D2 and not yet separated by recombination. It seems appropriate to assume that D1 and D2 are responsible, until proved otherwise, since such an assumption makes testable scientific predictions possible.

The evidence obtained here shows that D1 and D2 are producing deleterious effects on male fertility when heterozygous, and are thus co-dominant rather than recessive in their effects on fertility. This fits well with the concept that transmission ratio distortion (where the distorters are typically again heterozygous) is due to deleterious effects of the distorter genes on the wild-type form of the responder gene. When both homologues of chromosome 17 carry such wild-type alleles, both would be equally affected and thus there would be no transmission distortion, but instead some impairment of fertility. The results thus agree with those of Seitz & Bennett (1985) and Olds-Clarke & Peitz (1986), who both showed either by study of chimaeric mice (Seitz & Bennett, 1985) or by mixed artificial insemination (Olds-Clarke & Peitz, 1986) that the distortion was due to malfunction of normal sperm, rather than intrinsic superiority of *t*-bearing sperm.

The *t*-form of the responder should thus, as stated earlier, be regarded as some factor conferring resistance to the harmful action of the distorter genes. It may therefore involve an alteration in the regulatory elements of the gene, or there may be an amplification of gene number. Changes in the structural elements of the gene could, however, be involved if the interaction between distorters and responder included steric interactions of gene products to form multimeric compounds.

The deleterious effect of heterozygosity for the distorter genes is interesting from an evolutionary point of view. Naturally occurring *t*-complexes as they exist at present are maintained in the population by the selective advantage in heterozygotes conferred by distorted transmission from males (Lewontin & Dunn, 1960; Lewontin, 1962). However, the various component parts of the complex are either neutral or deleterious. The responder, if present alone without distorters, shows low transmission and so is deleterious (Lyon, 1984). No effect has yet been detected if a single distorter is present alone. However, the present work shows that if two distorters are present without the responder the effect is deleterious. Only if the complete set of distorter and responder factors are present together on the same chromosome are they advantageous. It is possible that the individual factors were less disadvantageous when they first arose, and the *t*-complex was first formed. During evolution there has undoubtedly been selection among *t*-complexes for enhanced transmission distortion (Lewontin & Dunn, 1960; Lewontin, 1968), and this may well have resulted at the same time in individual components of the complex becoming more disadvantageous.

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