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The *in vivo* and *in vitro* transmission ratio distortion of one complete and two partial *t* haplotypes in mice

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Summary

The effects of different types of insemination (normal and delayed matings and *in vitro* fertilization) on the transmission ratio distortion (TRD) of three *t* haplotypes were determined. The t^{w73} haplotype which contains all of the loci known to affect TRD is transmitted at equivalent frequencies in normal matings and in *in vitro* fertilizations (0·84 and 0·85, respectively) but at a significantly lower frequency (0·62) in delayed matings. The distal partial t^{h18} haplotype is transmitted at equivalent frequencies in all types of insemination (0·66 to 0·70) while the proximal partial t^{w18} haplotype is transmitted in Mendelian frequencies in normal matings and in *in vitro* inseminations but at a significantly lower frequency in delayed matings. The results are discussed with reference to the current genetic model for transmission ratio distortion.

1. Introduction

In mice, *t* haplotypes are classified as being complete or partial depending upon whether they contain all, or only a segment of, the *t*-chromatin region of the 17th chromosome. In a complete *t* haplotype there are four inversions which suppress recombination (Artzt *et al.* 1982; Shin *et al.* 1983; Pla & Condamine, 1984; Herrmann *et al.* 1986; Sarvetnick *et al.* 1986; Hammer *et al.* 1989), a genetic locus (*tct*) which interacts with the *Brachyury* locus (*T*) to affect tail length, and at least one recessive mutation which, in homozygous condition, is deleterious to all embryos (lethal mutations) or to some embryos (semilethal mutations) at specific stages of development (Bennett, 1975; Sherman & Wudl, 1977; Lyon, 1981; Silver, 1985). There are also several genetic loci (distorter loci, symbolized *Tcd*; and a responder locus, symbolized *Tcr*), which act in *cis* or *trans* to distort the transmission ratio of the *t*-chromosome from heterozygous ($+/t^x$) males (Lyon, 1984, 1986; Silver & Remis, 1987). Partial *t* haplotypes, which are referred to as being proximal or distal, vary in length and contain only those *t*-associated loci, including those affecting TRD, within the length of variant DNA retained (Silver, 1981, 1985).

The transmission ratio distortion (TRD) of specific *t*-bearing heterozygous males can be altered by changing the length of time that the spermatozoa remain in the female reproductive tract prior to ovulation and fertilization. For example, the TRDs of some *t* haplotype bearing males are reduced when copulation occurs only 2 h before ovulation and fertilization (delayed matings). This is in contrast to the TRDs of the respective males when their spermatozoa reside in the female reproductive tract for 6–8 h prior to ovulation and fertilization (normal matings) (Braden, 1958, 1972; Yanagisawa *et al.* 1961; Braden & Weiler, 1964; McGrath & Hillman, 1980*a*, *b*). Conversely, the TRDs of males bearing other *t* haplotypes are not affected by delayed matings (Yanagisawa *et al.* 1961; Braden, 1972; Braden & Weiler, 1964; Garside & Hillman, 1989*a*, *b*). Similarly, *in vitro* fertilization (IVF) may change the TRD of males bearing a specific *t* haplotype relative to its TRD during normal and/or delayed matings (McGrath & Hillman, 1980*a*, *b*; Garside & Hillman, 1989*b*), whereas the TRDs of males bearing other *t* haplotypes are not affected by this method of insemination (Garside & Hillman, 1989*a*). In order to further characterize the modification of TRD by the method of insemination, the transmission ratios of males bearing either a complete (t^{w73}), a distal partial (t^{h18}), or a proximal partial (t^{w18}), *t* haplotype were determined following inseminations *in vivo* in normal and delayed matings, and *in vitro*.

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2. Materials and methods

(i) *Matings to obtain experimental and control males*

For these studies, T/t^{w73}, $Tt^{h18}/++$ and T/t^{w18} males were mated with *Rb(16.17)7Bnr* (*Rb7/Rb7*) females to produce the experimental $Rb7/t^{w73}$ and control *Rb7/T* males, the experimental $Rb7/Tt^{h18}$ and control *Rb7/+* males and the experimental $Rb7/t^{w18}$ and the control *Rb7/T* males, respectively. All of the experimental and control males carry a chromosomal complement of 38 acrocentric chromosomes and one metacentric Robertsonian translocation chromosome. The original mating pairs of the T/t^{w73}, $Tt^{h18}/++$ and the T/t^{w18} mice were obtained from Dr L. Silver and the *Rb7/Rb7*, from The Jackson Laboratory. All were maintained by brother–sister matings. At least ten males of each control and experimental genotype were used for the comparative studies.

(ii) *Normal and delayed matings*

(C57BL/6J♀ × BALB/c♂) F_1 females were intraperitoneally injected with pregnant mare serum gonadotropin (PMS; 5 i.u.), followed 48 h later with human chorionic gonadotropin (HCG; 5 i.u.). In normal matings, individual females were caged with either a single experimental, or a single control, male immediately after the second injection in the normal matings. In delayed matings, the females were caged with an individual control or experimental male 12 h after the second hormone injection. On the day following the matings, the females were sacrificed by cervical dislocation, their oviducts excised and flushed, and the experimental and control zygotes placed separately into modified Whitten's medium (Abramczuk *et al.* 1977) according to the protocol of McGrath & Hillman (1980*a*, *b*). The same males were used for both normal and delayed matings. The C57BL/6J mice had been brother–sister mated for 32 generations, and the BALB/c, for 80 generations, in our colony.

(iii) In vitro *fertilization*

The males used to determine the TRDs in the normal and delayed mating studies were also used as the source of cauda epididymal and vasa deferentia spermatozoa for the *in vitro* fertilization studies following the protocol of McGrath & Hillman (1980 *a*, *b*). The eggs were obtained from (C57BL/6J♀ × BALB/c♂) F_1 hormone-stimulated females. After the gametes were coincubated for 6 h, the zygotes were removed and placed into modified Whitten's medium.

(iv) *Embryo culture and karyotyping*

The experimental and control zygotes obtained from the three types of inseminations were allowed to develop until they reached the blastocyst stage using the culture procedure described by McGrath & Hillman (1980*a*). The preimplantation development of these embryos was compared to insure that the conditions of handling and culturing the experimental zygotes did not introduce errors into the TRD determinations.

Experimental blastocysts were karyotyped (Garside & Hillman, 1985) and an embryo without an *Rb7* chromosome was scored as being fertilized by a spermatozoon carrying the *t*-bearing 17^{th} chromosome. Only euploid embryos were included in the results, and at least two chromosome spreads from each embryo were counted to reduce the possibility of error. The TRD of each *t* haplotype was calculated from the ratio of the number of embryos without the *Rb7* marker chromosome to the total number of embryos scored.

(v) *Statistical analysis*

A contingency χ^2 test was used to determine significant differences in the preimplantation development of control and experimental embryos from each method of insemination. The transmission ratios from each method of insemination were arcsine transformed and compared using Student's *t* test. A significance level of 5% was used in all statistical analyses.

3. Results

The data (Table 1) show that there are no significant differences in the *in vitro* preimplantation development of the experimental zygotes and their control counterparts. Neither the method of insemination nor the subsequent handling of the experimental zygotes affects development or causes death above background levels in the corresponding types of insemination.

Karyotypic analyses of experimental blastocysts are reported in Table 2. Blastocysts obtained from the $RB7/t^{w73}$ males show the TRD from normal matings to be 0·84 and from delayed matings, 0·62. These TRDs are significantly different from each other ($P < 0{\cdot}001$). *In vitro*, the TRD of the t^{w73} haplotype is 0·85 which is equivalent to that of normal matings ($P > 0{\cdot}05$) but significantly higher than that of delayed matings ($P < 0{\cdot}001$).

The TRD of the t^{h18} haplotype in normal matings is 0·66 and in delayed matings, 0·70. These transmission ratios are not significantly different ($P > 0{\cdot}05$). The IVF TRD of this haplotype is 0·66 which is not significantly different from the TRD of either of the *in vivo* inseminations ($P > 0{\cdot}05$).

The TRDs of the t^{w18} haplotype are 0·45 and 0·41 in normal and delayed matings, respectively, and 0·54 in *in vitro* fertilizations. The normal and delayed mating TRDs are not significantly different ($P > 0{\cdot}05$), whereas the *in vitro* fertilization TRD is significantly

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Table 1. *Comparative development of control and experimental zygotes to the blastocyst stage*

Haplotype	Type of insemination*	Control		Experimental		
		No. of zygotes	No. of blastocysts (%)	No. of zygotes	No. of blastocysts (%)	*P*
t^{w73}	NM	262	242 (92)	251	237 (94)	$0{\cdot}90 > P > 0{\cdot}75$
	DM	257	254 (99)	266	258 (97)	$0{\cdot}25 > P > 0{\cdot}10$
	IVF	210	160 (76)	250	195 (78)	$0{\cdot}95 > P > 0{\cdot}90$
t^{h18}	NM	312	275 (88)	329	305 (93)	$0{\cdot}10 > P > 0{\cdot}05$
	DM	449	375 (84)	548	448 (82)	$0{\cdot}75 > P > 0{\cdot}50$
	IVF	281	135 (48)	258	130 (50)	$0{\cdot}75 > P > 0{\cdot}50$
t^{w18}	NM	458	342 (75)	742	509 (69)	$0{\cdot}10 > P > 0{\cdot}05$
	DM	306	236 (77)	808	615 (76)	$0{\cdot}75 > P > 0{\cdot}50$
	IVF	469	404 (86)	777	643 (83)	$0{\cdot}10 > P > 0{\cdot}05$

* NM, Normal mating; DM, Delayed mating; IVF, *in vitro* fertilization.

Table 2. *The* in vivo *and* in vitro *TRDs of the* t^{w73}, t^{h18}, *and* t^{w18} *haplotypes*

Haplotype	Type of insemination*	No. of blastocysts without Rb7 marker	Total no. of blastocysts	TRD
t^{w73}	NM	306	366	0·84
	DM	229	370	0·62
	IVF	358	423	0·85
t^{h18}	NM	205	309	0·66
	DM	215	305	0·70
	IVF	210	317	0·66
t^{w18}	NM	137	303	0·45
	DM	128	315	0·41
	IVF	169	314	0·54

* NM, Normal mating; DM, Delayed mating; IVF, *in vitro* fertilization.

higher than either the normal ($P < 0{\cdot}02$) or delayed ($P < 0{\cdot}02$) mating TRD. However, neither the IVF TRD nor the normal mating TRD is significantly different from the expected Mendelian ratio ($P > 0{\cdot}05$).

4. Discussion

The current model for TRD in $+/t$ males is that the *Tcd* loci intereact in *cis* or *trans* with the Tcr^t locus located on the *t*-haplotype bearing chromosome and in *cis* or *trans* with the Tcr^+ locus on the wild-type homologue to produce a deleterious effect so that a spermatozoon containing the wild-type homologue is dysfunctional and thus less likely to fertilize eggs than a spermatozoon containing the *t* haplotype bearing chromosome (Lyon, 1984, 1986; Silver & Remis, 1987). The extent of the dysfunction of the spermatozoon is dependent upon the number of *Tcd* loci. The *Tcd*s appear to have additive effects on TRD; that is, the TRD is highest when all are present in the *t* haplotype bearing chromosome, as in complete haplotypes, and decreases when one or more are missing as in partial *t* haplotypes. Also, Tcr^t must be present in order for the *Tcd*s to affect the TRD. If Tcr^t is absent, both chromosomes are transmitted at 0·50 regardless of the number of *Tcd*s present. Of the haplotypes currently examined, t^{w73} is complete (i.e. it contains all of the *Tcd*s and Tcr^t), t^{h18} contains only *Tcd-2* which is the most distal of the *Tcd*s and t^{w18} contains Tcr^t and all of the *Tcd* loci except *Tcd-2*.

Since t^{w73} is complete, it should be transmitted in a very high frequency, between 0·90 and 0·99. However, the data show that even in normal matings, the TRD of this haplotype is lower than 0·90. Bennett *et al.* (1983) have reported that modifier genes accumulate on the homologous 17th chromosome and reduce the TRD of the *t*-bearing chromosome in heterozygous animals which are maintained by *inter se* matings for many generations. Subsequent outcrossing, however, enhances the TRD of the *t* haplotype. Since the experimental males were obtained by outcrossing

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T/t^{w73} males with *Rb7/Rb7* females, there should be no accumulation of modifier genes which lower the TRD. Also, the only report of the *Rb7* chromosome affecting the TRD of a *t* haplotype shows that the translocation chromosome enhances TRD (Sánchez & Erickson, 1986). Consequently, the outcrossing of the T/t^{w73} males with homozygous *Rb7* females should therefore increase rather than reduce the TRD of t^{w73}. Neither the presence of the *Rb7* chromosome nor the presence of modifier genes on this chromosome should cause the reduced TRD noted in normal matings. The decrease could, however, result from the total genetic background of either the male or the female since both have been shown to effect TRD (Braden & Weiler, 1964; McGrath & Hillman, 1980*a*; Bennett *et al.* 1983; Gummere *et al.* 1986).

Although the TRD of the t^{w73} haplotype is the same in IVF as in normal matings, there is a significant decrease in the TRD of this haplotype in delayed matings. This observation suggests that the dysfunction of the wild-type spermatozoa must be not only time dependent (greater than two hours) but also dependent upon the environment in which insemination occurs. In IVF and in delayed matings the spermatozoa reside in capacitation medium or in the uterus for equivalent lengths of time (approximately 2 h) prior to fertilization. Consequently, the only variable between these two types of insemination is the environment in which fertilization occurs.

The pattern of the TRD of this complete haplotype in the various types of inseminations differs from those of other complete *t* haplotypes. For example, the TRD of the t^{w5} haplotype is high ($> 0{\cdot}90$) in all three types of inseminations whereas that of t^{12} is significantly higher in normal matings than in delayed matings and in IVF (Garside & Hillman, 1989*a*; McGrath & Hillman, 1980*b*).

The TRD of the t^{h18} haplotype is the same (0·66 to 0·70) in all types of inseminations showing that *Tcd-2* alone does not respond to differences in the types of inseminations. According to the model (Lyon, 1984, 1986), the transmission of this haplotype in normal matings should be fifty percent since the mutant chromosome lacks Tcr^t. In fact, in colony matings between $Tt^{h18}/++$ and BALB/c the transmission of this haplotype is Mendelian (1448 short tailed mice/2890 total mice). Although the presence of the *Rb7* translocation has not altered the TRD of other *t* haplotypes in previous normal mating insemination studies (Garside & Hillman, 1989*a*, *b*), or in the present normal mating t^{w73} and t^{w18} insemination studies, it is quite possible that this translocation influences the TRD of the t^{h18} haplotype in all types of insemination. Although Sánchez & Erickson (1986) suggest that the wild-derived *Rb7* chromosome contains *Tcd-1* which increases the transmission frequency of specific *t* haplotypes in a *trans* position, the fact that t^{h18} does not have a Tcr^t locus which is necessary for this *trans* effect to occur nullifies the possibility that the factor or factors on the *Rb7* chromosome influencing TRD are one or more of the *Tcd* loci.

The TRD of the t^{w18} haplotype in normal matings and in IVF fit the expected frequency based on Lyon's model (1984, 1986). These frequencies, although significantly different from each other, are not significantly different from Mendelian. The TRD of this haplotype is, however, significantly lower than expected in delayed matings.

Overall, the current studies show variances from the expected TRDs based on the genetic analyses of this region. The physiological explanation for these variances, however, will not be understood until the transcribed products of the *Tcd* and Tcr^t loci are characterized. Once characterized, these products can be monitored in the different types of insemination to determine their interaction and physiological effects on TRD.

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