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Genetic background affects expression of *t* haplotype in mouse sperm

PATRICIA OLDS-CLARKE AND SUSAN McCABE

Department of Anatomy, Temple University School of Medicine, Philadelphia, PA 19140, U.S.A.

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SUMMARY

Two aspects of sperm phenotype were examined for $t^{w32}/+$ males of the inbred strains C3H and C57BL/6 (B6). Sperm from fertile C3H-$t^{w32}/+$ males very rarely achieved fertilization in vitro, while sperm from congenic C3H-$+/+$ males had no such difficulties. The presence of t^{w32} had no effect on ability of B6 sperm to undergo fertilization in vitro. In fertile hybrid males produced from crosses of B6 and C3H strains, t^{w32} significantly reduced, but did not inhibit completely, the sperms' ability to fertilize in vitro. The presence of t^{w32} decreased by a factor of two the frequency of abnormal sperm heads in males of the B6 strain, but doubled the frequency in the C3H strain. Hybrid males resembled the C3H strain in this respect. The presence of T or T^{2J} had no effect on the level of sperm abnormalities in any strain. These results emphasize the importance of genetic background in expression of *t* haplotypes in sperm, and suggest that t^{w32} can influence a range of sperm characteristics, by interacting with products of other loci.

INTRODUCTION

The T/t locus in the mouse is a region of chromatin which probably includes a number of cistrons (Lyon *et al.* 1979; Silver, White & Artzt, 1980). Mutations in this region have severe effects on development and reproduction (for reviews, see Bennett, 1975; Klein & Hammerberg, 1977; and Sherman & Wudl, 1977). It has been known for a long time that expression of dominant mutations are subject to modifying factors elsewhere in the genome (Bennett, 1964; Micková & Iványi, 1974). Bennett (1964) also found that by selecting for longer tail length in T/t^{w18} mice, she could postpone the lethal period of t^{w18}/t^{w18} embryos by about one day, although the types of abnormalities observed in these embryos were unchanged. From this she concluded that there were 'modifier genes' for recessive mutations at the T/t locus as well, but their effects were modest.

Little attention has been given to the influence of other genes on the expression of *t* mutations, perhaps because their cumulative effect on the lethality of the

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homozygote seems slight. Likewise, it has often been assumed that genetic background is unimportant for expression of *t* haplotypes in sperm. Yet it is generally agreed that the distorted transmission of *t* mutations through the male is caused by some peculiarity of spermatogenesis or sperm function which gives *t*-bearing sperm an advantage in fertilization (Braden, 1972; Hammerberg & Klein, 1975). This would imply the expression of the *t* haplotype at one or more stages in spermatogenesis, a complex process involving expression of many genes (Beatty, 1970). With the advent of several different congenic mouse strains carrying *t* mutations, it has become possible to determine the influence of genetic background on expression of *t* haplotypes in sperm. In this paper we report the effect of C3H and C57BL/6 (B6) genes on expression of t^{w32} in sperm. We find that t^{w32} affects the ability of the sperm to undergo fertilization in vitro, and that t^{w32} also affects the level of sperm abnormalities; these examples of t^{w32} expression in sperm, however, are not consistent from strain to strain.

MATERIALS AND METHODS

(i) *Mice*

The C57BL/6-t^{w32}/+ (B6-t^{w32}/+) males used were part of a colony derived from nine consecutive backcrosses of T/t^{w32} mice (a gift of Dr Dorothea Bennett, Sloan-Kettering Institute for Cancer Research, New York) to C57BL/6J-+/+ (B6-+/+) mice (The Jackson Laboratory, Bar Harbor, Maine), followed by intercrosses interspersed with additional backcrosses to B6/J-+/+ mice to prevent the occurrence of a separate substrain of B6. B6-T^{2J}/+ males were derived from mating pairs (a gift of Dr Don Bailey, The Jackson Laboratory). T^{2J} is probably a recurrence of Brachyury (*T*), because the time of death and the spectrum of abnormalities seen in embryos from $T^{2J}/+ \times T^{2J}/+$ crosses are similar to those of embryos from $T/+ \times T/+$ crosses (unpublished observations).

The C3H-t^{w32}/+ and C3H-*T*/+ males used were part of colonies derived from mating pairs of C3H-t^{w32}/+ and C3H-*T*/+ mice (gifts of Dr Hugh McDevitt, Stanford University). The C3H-t^{w32}/+ mice were derived from 13 consecutive backcrosses of T/t^{w32} (from Dr Bennett's colony) to C3H/DiSn-+/+ mice. Since receiving these mice, both C3H-t^{w32}/+ and C3H-*T*/+ mice have been backcrossed several times to C3H/HeJ-+/+ mice (The Jackson Laboratory). All C3H-+/+ males used in these experiments were sibs of C3H-t^{w32}/+ and C3H-*T*/+ males.

For assurance that the t^{w32} haplotype was the same in both B6 and C3H strains, T/t^{w32} (ot) mice carrying t^{w32} from a B6 parent were crossed with T/t^{w32} mice carrying t^{w32} from a C3H parent, and the tail length of their offspring assessed. In eight litters from this type of cross, all 27 offspring were tailless. This data confirms that the t^{w32} haplotypes in both strains are in the same complementation group.

Reciprocal F_1 males were produced by crosses between B6 and C3H strains. Although (C3H × B6) F_1 and (B6 × C3H) F_1 males differed in Y chromosomes, this

did not seem to affect the aspects of sperm phenotype measured in these studies, so both types were pooled.

Attempts were made to produce T/t^{w32} males of all strains, but those of the B6 and C3H strains were few in number and usually sterile. All males used were at least 3 months old, and fertile.

Transmission ratios of $t^{w32}/+$ males were estimated from the proportion of tailless mice in litters sired by these males when crossed to Brachyury ($T/+$ or $T^{2J}/+$) females of various genotypes (C3H, B6, F_1 or 'other'). At least two litters were obtained from each male. The estimated frequency of transmission of t^{w32} in each strain was: B6-$t^{w32}/+$, 91 %; C3H-$t^{w32}/+$, 94 %; F_1-$t^{w32}/+$, 90 % (at least 38 litters and 290 offspring assessed for each genotype). F_1-T/t^{w32} males' transmission of t^{w32} was 96 % (estimated by the proportion of short-tailed ($T/+$) mice in litters sired by these males when crossed to $+/+$ females; six litters, 54 offspring).

(ii) *Fertilization in vitro*

Procedures used to determine ability of sperm to fertilize normal eggs in vitro have been described elsewhere (Olds-Clarke & Carey, 1978). Females used as the source of eggs were B6D2F_1/J-$+/+$ females (The Jackson Laboratory), and ranged in age between 6 and 12 weeks.

(iii) *Sperm head abnormalities*

Samples of cauda epididymal sperm from each male were stained with Eosin Y, placed on slides and air-dried (Hillman & Nadijcka, 1978). Sperm heads were viewed at 200×, and classified using Krzanowska's (1974) head types. Only those in classes 3–5 were considered abnormal. This may have resulted in an underestimate, since small variations in head shape were considered normal. Class 3 was the shape most frequently seen. This class is similar to that labelled 'Type II' by Bryson (1944) and to those called 'spatulate', 'angular', or 'blunted' by Dooher & Bennett (1977).

RESULTS

(i) *Sperm fertilizing ability in vitro*

In the B6 strain, the presence of t^{w32} had no effect on ability of sperm to undergo in vitro fertilization (IVF). In the C3H strain, however, the presence of t^{w32} significantly impaired the ability of sperm to fertilize eggs in vitro (Table 1). The presence of a dominant T factor, Brachyury, had no apparent effect on the C3H sperms' ability to complete IVF. In the hybrid strain resulting from crosses of these two inbred strains, the presence of t^{w32} permitted some IVF to occur, but the level was significantly lower than that of the control, F_1-$+/+$ (Table 1).

Table 1. *Effect of genetic background on ability of sperm from* $t^{w32}/+$ *males to undergo fertilization in vitro*

Male genotype	No. of males tested	Mean % ±S.D. fertilization in vitro*	Range
B6-+/+	13	60±23	19–100%
B6-t^{w32}/+	10	65±23	8–96%
C3H-+/+	14	53±17	36–96%
C3H-T/+	14	58±23	22–92%
C3H-t^{w32}/+	10	16±15†	0–41%
F_1-+/+	3	67±15	50–78%
F_1-t^{w32}/+	8	43±5‡	38–53%

* Defined as the per cent 2-celled embryos after overnight culture of eggs inseminated with epididymal sperm for 5 hours.

† Significantly different than C3H-+/+ and C3H-T/+ by the Mann-Whitney-U test ($P < 0.001$).

‡ Significantly different than F_1-+/+ by the Mann-Whitney-U test ($P < 0.025$).

Table 2. *Effect of genetic background on frequency of abnormal sperm heads in epididymal sperm populations from* $t^{w32}/+$ *males*

Male genotype	No. of males tested	Mean age ±S.D. of males (in mo.)	Mean % ±S.D. abnormal sperm heads
B6-+/+	20	5·8±1·4	7·9±2·4
B6-T^{2J}/+	13	6·8±1·5	7·7±1·4
B6-t^{w32}/+	19	7·6±1·9	4·0±1·4*
C3H-+/+	25	5·9±1·3	2·7±1·0
C3H-T/+	17	7·1±2·0	3·2±0·9
C3H-t^{w32}/+	11	7·3±2·5	7·2±3·1*
F_1-+/+	8	4·6±1·2	1·2±0·5
F_1-$T(T^{2J})$/+	5	4·3±0·6	1·1±0·4
F_1-t^{w32}/+	18	4·1±0·7	6·7±1·4*
F_1-T/t^{w32}	6	4·9±1·4	8·8±3·2*

* Significantly different than +/+ and $T(T^{2J})$/+ of the same strain, by the Mann-Whitney-U test ($P < 0.001$).

(ii) *Frequency of abnormal sperm heads*

In the B6 strain, the presence of t^{w32} significantly decreased the frequency of abnormal sperm heads, while the same factor had the reverse effect on sperm of the C3H and hybrid strains (Table 2). These strains also differed in the frequency of sperm head abnormalities in +/+ males, the B6 strain having twice the frequency of the C3H strain. Neither T nor T^{2J} had any effect on the frequency of abnormalities, either in combination with the + or the t^{w32} haplotype (Table 2).

DISCUSSION

In the search for the physiological basis of the distorted transmission of t haplotypes through the male, there have been many studies demonstrating differences from the normal in sperm phenotype (e.g. Yanagisawa, 1965; Ginsberg

& Hillman, 1974; Katz, Erikson & Nathanson, 1979; and Nadijcka & Hillman, 1980). In none of these studies were the controls ($T/+$ or $+/+$) of strains congenic to the $t/+$. The results described in this paper demonstrate that the phenotype of sperm from $t/+$ males is modified by other genes. Thus, not all differences between sperm of $t/+$ and $+/+$ males can be assumed to relate to the distorted transmission of t haplotypes. For example, neither of the effects of t^{w32} observed in the congenic strains used in the present study could be the cause of the distorted transmission frequency, because both effects vary between strains, while the transmission frequency is uniform (see Materials and Methods). It is also possible that different alleles at loci other than T/t could explain the extreme heterogeneity between individuals in levels of abnormal sperm from $t^1/+$ males (Bryson, 1944), and the different conclusions of studies by Dooher & Bennett (1977) and Hillman & Nadijcka (1978) as to whether or not the presence of a t haplotype changes the level of abnormalities in sperm.

That t^{w32} can influence such different aspects of sperm phenotype as in vitro fertilizing ability and level of morphological abnormalities suggests a highly pervasive effect of the t haplotype on sperm development. It is possible that the region of chromatin covered by the t haplotypes include several factors whose expression is necessary for normal spermatogenesis. One of these may be the surface protein found in great abundance on testicular germ cells (Silver, Artzt & Bennett, 1979). Another could be the enzyme phosphoglycerate kinase B, which is sperm-specific and thought to be located close enough to the *H-2* complex to be included in t haplotypes (Eicher, Cherry & Flaherty, 1978; Vandeberg & Klein, 1978).

There is probably little physiological significance to the impaired fertilizing ability in vitro of sperm from C3H-$t^{w32}/+$ males, since these animals are fertile. Sperm fertility in vitro does not necessarily reflect sperm fertility in vivo (Carey & Olds-Clarke, 1980). In a different environment, sperm from C3H-$t^{w32}/+$ might be capable of in vitro fertilization. Similarly, the level of morphological abnormalities in these sperm populations may not be crucial to fertility. Abnormal sperm may have more difficulty than normal sperm in reaching the site of fertilization in vivo (Krzanowska, 1974); there is also evidence that what is usually considered abnormal morphology may not prevent such sperm from penetrating eggs (Smith, Oura & Zamboni, 1970; Olds-Clarke & Becker, 1978). The strikingly different effect of t^{w32} on B6 and C3H sperm morphology may be of interest to those studying sperm development, but there is no certainty that t^{w32} had its effect on morphology by affecting the same process in both cases.

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