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An increased level of sperm abnormalities in mice with a partial deletion of the Y chromosome

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Summary

Two congenic lines of mice, one with a partial deletion of the Y chromosome, differ in the percentage of spermatozoa with abnormal heads: B10.BR/SgSn males give 22·6% and B10.BR-Y^{del}/Ms males give 64·2% abnormal sperm. The F_1s resulting from crosses of B10.BR/SgSn males with females of five common inbred strains exhibited significantly lower levels of abnormal sperm than the parental strains, as opposed to F_1 hybrids sired by B10.BR-Y^{del}/Ms mutant males, where very high levels of abnormal spermatozoa were found. About 30% of abnormal spermatozoa, produced by males with deletion on the Y chromosome, were characterized by a flat acrosomal cap. This class of abnormality was never observed in non-mutant males, suggesting a mutant-specific defect. These results demonstrate the important role of the Y chromosome in spermatogenesis.

1. Introduction

The proportion of morphologically abnormal sperm heads in adult males is largely under genetic control and differs considerably among inbred strains of mice (Bruce *et al.* 1974; Wyrobek & Bruce, 1975; Wyrobek, 1979; Krzanowska, 1981; Ballachey *et al.* 1986). These differences depend on a small number of autosomal genes and Y-linked factors. Several lines of evidence suggest that the Y chromosome plays an important role in determining the total proportion of sperm head abnormalities, but it does not seem to affect specific abnormality types. Introduction of the Y chromosome from certain inbred strains with low proportions of abnormal sperm heads into the genetic background of high percentage strains causes a significant reduction of sperm abnormalities, although the level characteristic for the low percentage strain is not reached. These observations suggest that the effects of Y-linked genes on percentage of abnormal sperm heads are modified by the autosomal genome with which the Y chromosome is associated (Krzanowska, 1972; 1976). A lower proportion of abnormal sperm heads is also observed in all types of F_1 crosses, relative to the parental strains (Brozek, 1970; Krzanowska, 1976; Wyrobek, 1979), indicating the existence of heterosis for that trait.

In this paper we describe the effect on the frequency of sperm-head abnormalities of a new mutation Y^{del} caused by a partial deletion of the Y chromosome. The normal and the reduced size of the Y chromosome are shown in Fig. 1. The mutation was found during routine chromosomal analysis of B10.BR/SgSn males originally received from the Jackson Laboratory (Bar Harbor, ME). From the mutant male the congenic line to B10.BR/SgSn was developed, designated as B10.BR-Y^{del}/Ms and maintained since 1977 in the National Institute of Genetics, Mishima, Japan.

The aim of this paper was to analyse the effects of the Y^{del} mutation on sperm head abnormalities.

2. Materials and methods

(i) *Mice*

Strain B10.BR/SgSn was obtained from Shizuoka Laboratory Animal Centre, and other mouse strains were maintained in colonies of the National Institute of Genetics, Mishima. To avoid the possibility of divergence between B10.BR/SgSn and B10.BR-Y^{del}/Ms congenic lines, the mutant males were produced by backcrossing B10.BR-Y^{del}/Ms males with B10.BR/SgSn females.

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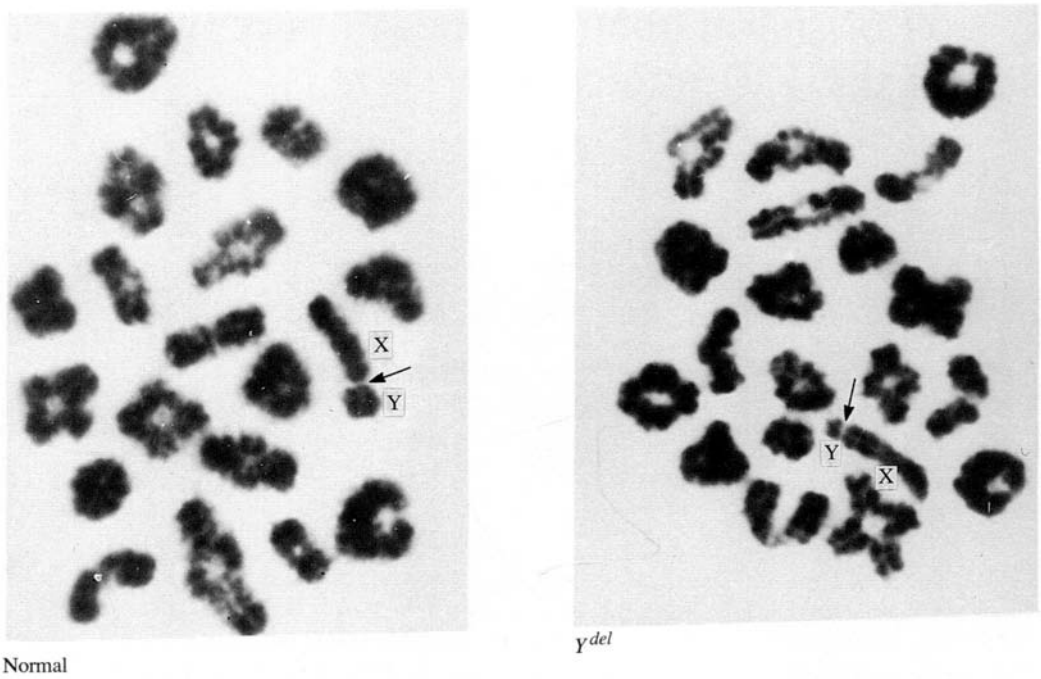

Fig. 1. Meiotic chromosomal plates of B10.BR (normal) and B10.BR-Y^{del} (Y^{del}) males. The mutant Y chromosome is clearly shorter than the normal one, but conjugation between the sex chromosomes occurs normally. The boundaries between X and Y chromosomes are marked by arrows.

(ii) *Sperm analysis*

In the first series of experiments 25 B10.BR/SgSn males and 15 B10.BR-Y^{del}/Ms mutant males (referred to below as B10.BR and B10.BR-Y^{del}) were used for comparative analysis of morphologically abnormal sperm heads.

To test whether the effect on sperm morphology attributed to the Y^{del} mutation could be caused by autosomal factors present in the male parent, sperm of 10 males generated from crosses between B10.BR-Y^{del} females (i.e. females sired by mutant males) and B10.BR males were also analysed.

The second series involved F_1 hybrid males obtained from crosses of females of five inbred strains: CBA/J, DBA/2J, AKR/J, C3H/HeJ and BALB/cAnN (referred to later on as CBA, DBA/2, AKR, C3H and BALB/c, respectively) with B10.BR and B10.BR-Y^{del} males. In all crosses when denoting the parentage of hybrids the strain of the mother is written first.

Only adult 10- to 12-week-old males were used for testing and were killed by cervical dislocation. After pressing the cauda epididymis, allowing sperm to pass to the vas deferens, the content of the latter was expressed into a small drop of 0·95% NaCl. A few minutes later a smear was prepared, air-dried and fixed for 20 min. in acetic alcohol. The smears were stained with 1% aqueous solution of eosin Y. Stained smears were dehydrated in alcohol and cleared with xylene. Preparations were examined under 60× objective. 200 spermatozoa from each male were counted and the percentages of abnormal heads were calculated. The classification of abnormal forms was based on Krzanowska (1974) with slight modification. The first class was divided into two subclasses: 1 and 1a (Fig. 2), the 1a class being characterized by a flattening of the acrosomal part of the head.

Fig. 2. Normal spermatozoan head and typical examples of abnormal heads divided into 5 classes (from Krzanowska, 1974) Class 1a is mutant specific for Y^{del} males.

For statistical treatment the percentages of abnormal spermatozoa were transformed to angles (Snedecor, 1955), and compared by a Kruskal–Wallis U test (Zar, 1974).

3. Results

(i) *Congenic lines*

B10.BR and B10.BR-Y^{del} males produce, respectively, 22·6 and 64·2% abnormal spermatozoa (Table 1). Predominant in mutant males is class 1a of abnormality, with flat acrosomal cap, which was never observed in the B10.BR line. The proportion of other abnormality classes was also elevated in mutant males (Fig. 3, Table 1).

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Table 1. *Sperm-head abnormalities in inbred strains of mice and their F_1 hybrids*

Genotype of males	No. of males	Sperm-head abnormality: in %	Sperm-head abnormality: in angular transformation $\bar{x} \pm$ S.D.
Inbred strains			
B10.BR × B10.BR	15	22·6	28·33 ± 1·68
B10.BR × B10.BR-Y^{del}	15	64·2 (28·5)*	53·33 ± 4·87†
B10.BR-Y^{del} × B10.BR	10	21·5	27·62 ± 1·54
CBA	7	3·2	10·17 ± 2·00
DBA/2	7	3·4	10·45 ± 1·69
AKR	10	3·6	11·05 ± 1·74
C3H	4	12·5	21·13
BALB/c	8	44·3	44·38 ± 3·73
F_1 hybrids			
CBA × B10.BR	7	1·5	6·95 ± 1·19
CBA × B10.BR-Y^{del}	8	54·9 (32·7)*	47·83 ± 0·84†
DBA/2 × B10.BR	9	2·2	8·38 ± 1·26
DBA/2 × B10.BR-Y^{del}	9	58·7 (39·3)*	49·99 ± 2·37†
AKR × B10.BR	7	1·4	5·63 ± 1·22
AKR × B10.BR-Y^{del}	9	61·0 (37·9)*	51·89 ± 9·61†
C3H × B10.BR	7	1·3	6·55 ± 1·47
C3H × B10.BR-Y^{del}	9	54·6 (34·8)*	47·67 ± 4·49†
BALB × B10.BR	9	5·4	13·00 ± 4·00
BALB × B10.BR-Y^{del}	9	67·9 (29·1)*	55·91 ± 8·43†

* The percentage of class 1a of abnormalities is given in parentheses.
† Significant difference ($P < 0·001$), between progeny from crosses of females belonging to the same inbred strain and B10.BR or B10.BR-Y^{del} males.

Males derived from crosses between B10.BR-Y^{del} females and B10.BR males have almost the same level of abnormal sperm (Table 1) and the same classes of abnormalities as B10.BR males. These results show that the higher level of abnormalities in the mutant line is attributable to deletion of the Y chromosome, and not to autosomal genes.

(ii) *F_1 hybrids with inbred strains*

The percentages of abnormal sperm heads from F_1 hybrid males generated from crosses between common inbred strains with B10.BR and B10.BR-Y^{del} males are summarized in Table 1. Data referring to the pure strains are also included for comparison. The percentages of sperm with abnormal head morphology in these five common inbred strains are close to the previous estimates obtained by Buda & Krzanowska (1975) and Wyrobek (1979). In the F_1 hybrid males sired by B10.BR fathers, the level of abnormal sperm heads was always lower than in either parental strain. Regular shape of most spermatozoa is illustrated for appropriate crosses in Fig. 3 (left row). In contrast, the F_1 hybrids derived from the crosses with mutant males are characterized by a very high percentage of abnormal spermatozoa (Table 1). Similarly as in the pure mutant strain, subclass 1a of abnormalities is predominant in all types of crosses (Fig. 3, right row), although other classes of sperm head abnormalities exhibit also higher levels in comparison to F_1 progeny from matings with B10.BR males. The differences between the corresponding crosses where females belonged to the same inbred strain and males were either B10.BR or B10.BR-Y^{del}, must have been caused by the deletion on the Y chromosome in mutant males.

4. Discussion

The data obtained in this study support the earlier findings by Krzanowska (1976) that Y-linked factors are involved in determining the total percentage of morphologically abnormal sperm heads. Using mutant males with a partial deletion of the Y chromosome, we clearly demonstrate that the absence of a segment of the Y chromosome leads to a significant increase in the frequency of deformed sperm heads, regardless of the genetic background in which the deleted Y chromosome is operating.

It was also interesting to find out a new, *Y^{del}* mutant-specific class of abnormality (1a), which was characterized by a flat acrosomal cap. Preliminary study showed absence of some acrosomal enzymes which possibly causes a distortion of acrosomal shape.

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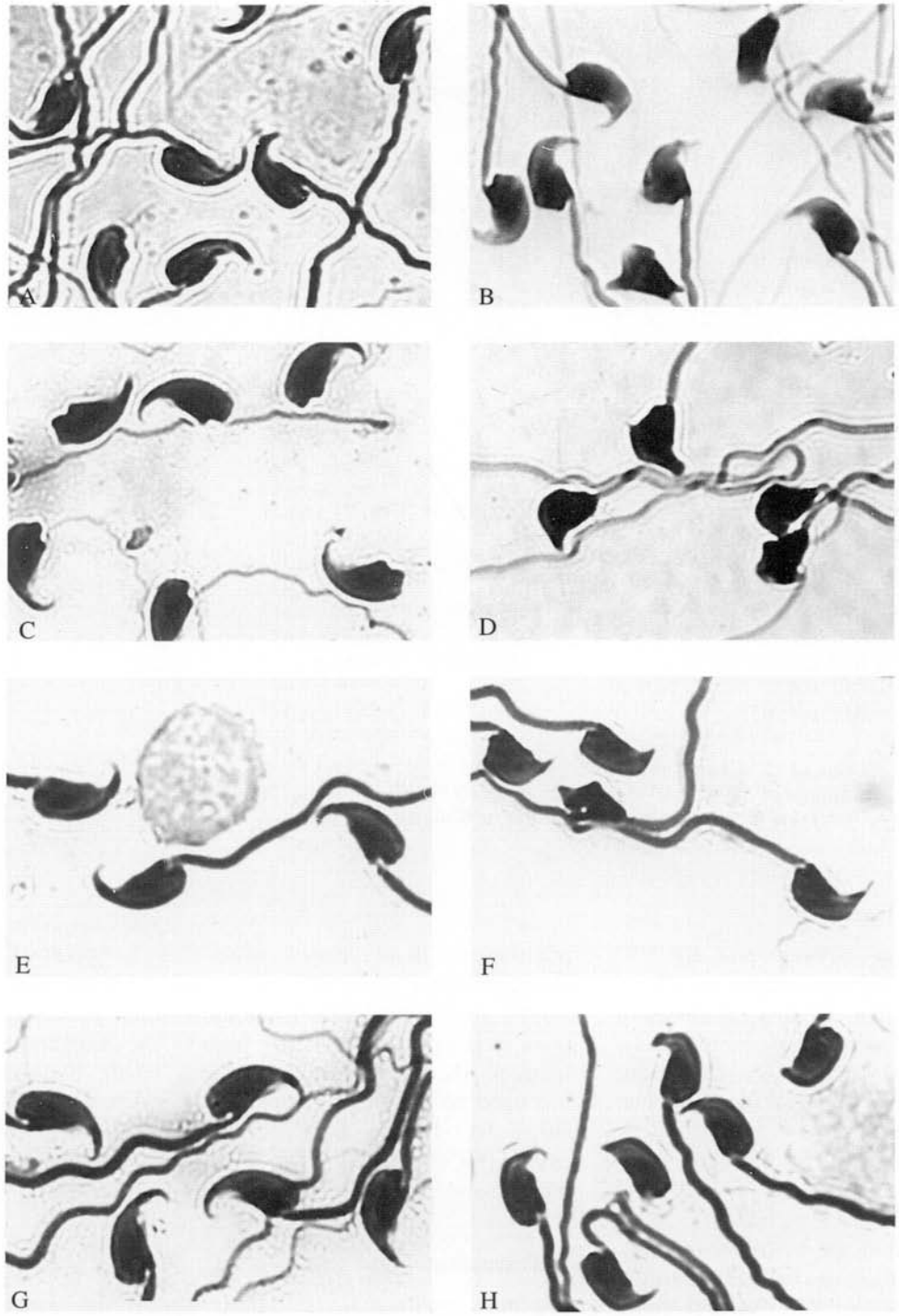

Fig. 3. Light microscope views of spermatozoa from congenic B10.BR and B10.BR-Y^{del} mice and their F_1 hybrids: A, B10.BR; B, B10.BR-Y^{del}; C, (BALB/c × B10.BR); D, (BALB/c × B10.BR-Y^{del}); E, (CBA × B10.BR); F, (CBA × B10.BR-Y^{del}); G, (AKR × B10.BR); H, (AKR × B10.BR-Y^{del}); (× 2200 magnification).

Further analysis is needed to test whether the same gene(s) is controlling the proportion of 1a and non-1a classes of abnormalities.

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