Genet. Res., Camb. (1991), **57**, *pp.* 135–138 *With 3 text-figures Printed in Great Britain*

Linkage between sperm abnormality level and major urinary protein phenotype in mice

JOZEFA STYRNA

Department of Genetics and Evolution, Institute of Zoology, Jagiellonian University, Karasia 6, 30–060 Krakow, Poland

(*Received 30 June 1988 and in revised form 27 March 1990*)

Summary

Segregation of sperm abnormality level and the pattern of major urinary proteins (MUPs) were investigated in F_2 and B_1 hybrid males obtained from crosses involving two contrasting inbred strains of mice: CBA/Kw (*Mup-1*^{a}*1*^{a}, 3·3% abnormal sperm) and C57BL/Kw (*Mup-1*^{b}*1*^{b}, 21·9% abnormal sperm). In the progeny of both crosses mean levels of abnormal spermatozoa were significantly higher for males typed as *Mup-1*^{b}*1*^{b} than for heterozygous *Mup-1*^{a}*1*^{b} males. Moreover, all F_2 hybrid males showing very high percentages of abnormal sperm were *Mup-1*^{b}*1*^{b} homozygotes. Similarly, among B_1 males with a high level of deformed spermatozoa, a statistically significant majority were *Mup-1*^{b}*1*^{b} genotypes. Our results suggest that at least two genes which influence sperm abnormality level are segregating in these crosses. Both appear to be recessive for high sperm abnormality level, and one shows weak linkage to *Mup-1* on chromosome 4.

1. Introduction

The study of spermatozoan head abnormalities in different strains of mice and their F_1 and F_2 crosses suggested that both autosomal and *Y*-linked factors are involved in the inheritance of this character (Krzanowska, 1969, 1972, 1976). However, no evidence was obtained connecting spermatozoan morphology with specific autosomal factors. Kiel-Metzger & Erickson (1984) using the method of DNA hybridization *in situ*, revealed a similarity between sex-related sequences of the *Y* chromosome and of segments of chromosome 17 close to the *H-2* locus, as well as to the mid-region of chromosome 4, suggesting that these sex-related regions may also influence the process of spermatogenesis and thus normality of sperm heads. To investigate this possibility in relation to chromosome 4, the *Mup-1* locus was chosen as a marker.

The major urinary proteins (MUPs) of the mouse are a group of low molecular weight proteins which can be clearly distinguished by acrylamide gel electrophoresis. In laboratory mice two phenotypic forms of MUPs have been identified, the differences being controlled by alleles at the *Mup-1* locus (*Mup-1*^{a} and *Mup-1*^{b}) located in the mid-region of chromosome 4. Dimorphism of the *Mup-1* locus has been previously reported by Hudson *et al.* (1967), Hainey & Bishop (1982), and Nikaido & Hayakawa (1984).

The aim of the present study was to investigate the possible relation between sperm abnormality level and MUP phenotype by testing segregation of these characters in F_2 and B_1 crosses involving the CBA/Kw and C57BL/Kw strains of mice. Only (CBA/Kw × C57BL/Kw)F_2 and (C57BL/Kw × F_1)B_1 crosses were analysed. The reciprocal crosses were not tested because the influence of the *Y* chromosome derived from the CBA/Kw strain strongly reduces the level of abnormal spermatozoa, so that the segregation for abnormalities is obscured.

2. Materials and methods

(i) *Inbred mouse strains and crosses*

The strains CBA/Kw and C57BL/Kw were maintained in the Department of Genetics and Evolution, Jagiellonian University. Twelve-week-old males from inbred strains and from (CBA/Kw × C57BL/Kw)F_1, F_2 and (C57BL/Kw × F_1)B_1 hybrid males were tested for MUP phenotype and for sperm abnormalities. When denoting crosses the female strain is written first.

(ii) *Analysis of MUP phenotypes*

Urine from each mouse was individually collected by gently massaging the ventral abdominal area. The

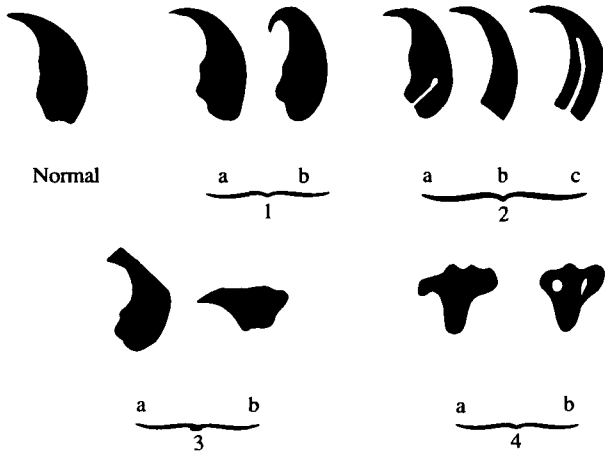

Fig. 1. Normal spermatozoan head and typical examples of abnormal heads (from Krzanowska, 1976)

urine, fresh or stored overnight at −20 °C, was diluted 2:1 with 40% sucrose solution, and 5 μl samples were applied to 7·5% acrylamide gels, which were run for 2 h with a constant voltage of 200 V per gel. The gels were stained with Coomassie Brilliant Blue R 250 and destained in methanol: acetic acid: water. Estimation of *Mup-1* alleles was based on the results of Hayakawa *et al.* (1983).

(iii) *Sperm analysis*

After collection of urine the males were killed by cervical dislocation and smears of sperm from the vasa deferentia were stained with eosin-Y and examined under 63 × objective. Spermatozoa numbering 200, from each male were counted and the percentages of abnormal heads were recorded. The spermatozoa were analysed according to the classification proposed by Krzanowska (1976) (Fig. 1). For statistical analysis, the percentages of abnormal spermatozoa from each individual male were transformed to angles, then the mean number of abnormal sperm and SD for each group were estimated (Snedecor, 1955). The significance was calculated by the Kruskal–Wallis *U* test (Zar, 1974).

3. Results

(i) *Relation between MUP phenotypes and sperm abnormalities*

CBA/Kw is of *Mup-1*^{a}*1*^{a} genotype and gave 3·3% sperm abnormalities; C57BL/Kw is *Mup-1*^{b}*1*^{b} and gave 21·9% sperm abnormalities (Figs 2, 3, Table 1). As was previously reported by Krzanowska (1976), both in CBA/Kw and C57BL/Kw strains the most frequent are types 3 and 4 of abnormalities. Basically, these two types of abnormality segregate in F_1, F_2 and B_1 generations and there was no relation between type of abnormality and *Mup-1* genotype.

F_1 males from CBA/Kw × C57BL/Kw gave the characteristic hybrid pattern of *Mup-1*^{a}*1*^{b}, which is

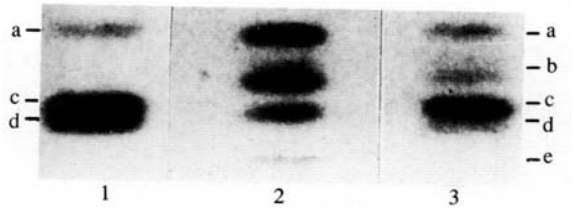

Fig. 2. Electrophoretic pattern of urine from: 1, CBA/Kw male (*Mup-1*^{a}*1*^{a}, bands a, c, d); 2, C57BL/Kw male (*Mup-1*^{b}*1*^{b}, bands a, b, c, e); 3, (CBA/Kw × C57BL/Kw)F_1 male (*Mup-1*^{a}*1*^{b}, bands a, b – middle, c, d – middle, band e is invisible).

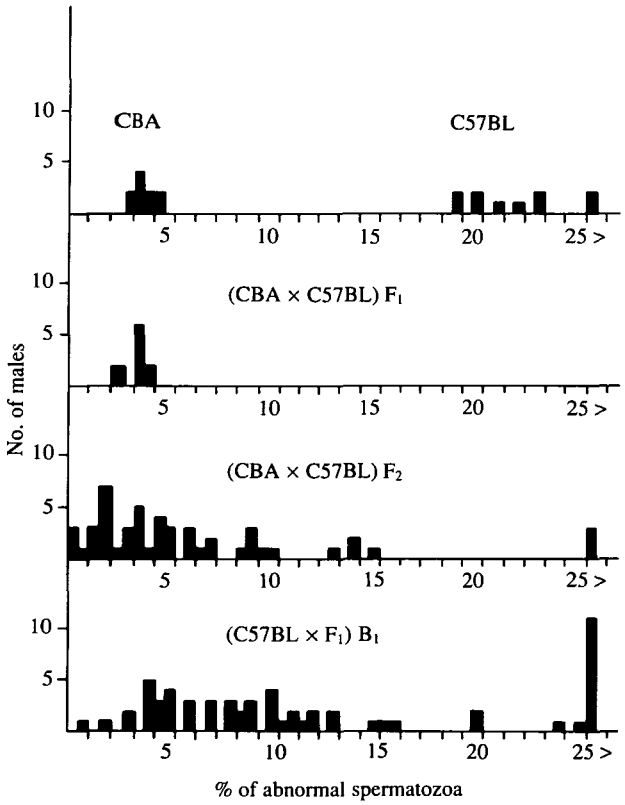

Fig. 3. Frequency distribution of CBA/Kw, F_1, F_2 and B_1 males possessing a given percentage of abnormal spermatozoa.

clearly distinguishable electrophoretically (Fig. 2). These males had the same frequency of sperm abnormalities as the CBA parent (3·5% compared with 3·3%, Table 1) suggesting that the gene or genes responsible for high sperm abnormality level in C57BL/Kw are recessive.

In F_2 and B_1 males an obvious segregation of genotypes has taken place (Table 1, Fig. 3). Segregation of *Mup-1* alleles appeared in the proportions expected for one locus difference (for F_2 $\chi^2 = 3{\cdot}24$, $P = 0{\cdot}2$; for B_1, $\chi^2 = 1{\cdot}06$, $P > 0{\cdot}2$) (Table 1).

In both crosses the mean levels of abnormal spermatozoa were highest in males typed as *Mup-1*^{b}*1*^{b} and lowest in heterozygous *Mup-1*^{a}*1*^{b} males. This suggests an association between the *Mup-1* genes and the sperm abnormality level (Table 1). To investigate this possibility, the following calculation was made. Because the males from the C57BL/Kw

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Table 1. *Parental phenotypes and segregation of total percentage of abnormal spermatozoa and* Mup-1 *marker in F_2 and B_1 crosses*

Type of cross and *Mup-1* genotypes	No. of males tested	Number of males with proportion of abnormal spermatozoa		Mean number of abnormal sperm	
		< 19%	> 19%	%	Angle ± S.D.
Inbred CBA/Kw	20	20	—	3·3	10·34 ± 1·72
Inbred C57BL/Kw	10	—	10	21·9	27·87 ± 1·79
(CBA × C57BL)F_1	10	10	—	3·5	10·60 ± 0·85
(CBA × C57BL)F_2					
Mup-1^{a}1^a	17	17	—	5·5	12·51 ± 5·51
Mup-1^{b}1^b	8	5	3	17·2	21·97 ± 12·70*
Mup-1^{a}1^b	25	25	—	4·1	11·25 ± 3·32
Total F_2	50	47	3†	6·7‡	13·39 ± 7·28
(C57BL × F_1)B_1					
Mup-1^{b}1^b	26	15	11	19·0	24·46 ± 10·44§
Mup-1^{a}1^b	34	30	4	9·8	17·18 ± 7·08
Total B_1	60	45	15 ‖	13·8‡	20·34 ± 9·36

* Significantly different from *Mup-1^{a}1^b* (Kruskal–Wallis *U* test, Zar, 1974), $P < 0{\cdot}05$.
† $\chi^2 = 0{\cdot}03$, $P > 0{\cdot}9$ to expected $(1/4)^2$ for segregation of two alleles in F_2 generation.
‡ Overall means for F_2 and B_1 generations respectively.
§ Significantly different from *Mup-1^{a}1^b* (Kruskal–Wallis *U* test, Zar, 1974), $P < 0{\cdot}01$.
‖ $\chi^2 = 0{\cdot}00$, $P > 0{\cdot}9$ to expected $(1/2)^2$ for segregation of two alleles in B_1 generation.

parental strain show variation in the proportion of abnormal spermatozoa ranging from 19 to 26%, (Fig. 3), 19% abnormalities was chosen as a border line separating the frequency distributions of the parental CBA/Kw and C57BL/Kw strains. Thus, F_2 and B_1 males possessing > 19% morphologically abnormal spermatozoa, were considered homozygous for alleles determining a high level of sperm abnormalities, inherited from the C57BL/Kw strain. In the F_2 generation all such males (3 individuals) were *Mup-1^{b}1^b* homozygotes, as in the C57BL/Kw strain (Table 1). Moreover, in the B_1 generation the proportion of males with > 19% of abnormal spermatozoa was significantly higher among *Mup-1^{b}1^b* homozygotes than among *Mup-1^{a}1^b* heterozygotes ($\chi^2 = 5{\cdot}79$, $P < 0{\cdot}02$, statistical significance calculated by test of independence adjusted for small samples, Snedecor, 1955).

These results suggest that at least one gene affecting sperm abnormality level is linked to *Mup-1* on chromosome 4.

(ii) *Probable number of autosomal genes involved in the inheritance of sperm head abnormalities*

In the F_2 generation 3 out of 50 mice, and in the backcross 15/60 mice, produced a high level of sperm abnormalities (> 19%). These ratios, close to 1/16 in the F_2 and 1/4 in the backcross, both fit the hypothesis that two independently segregating recessive genes, inherited from the C57BL/Kw parent, are segregating in these crosses.

4. Discussion

It is well known that the *Y* chromosome of the mouse plays a decisive role as a sex-determining factor, as well as being involved in sperm morphogenesis. The importance of the *Y* chromosome in determining the total percentage of sperm head abnormalities in mature males from different inbred strains has been clearly documented by Krzanowska (1969, 1972, 1976). Transferring the *Y* chromosome from the low percentage strain into the high percentage strain significantly reduced the level of sperm abnormalities in this strain.

On the other hand, the previous genetic study (Krzanowska, 1972) indicated an influence of autosomal genes in the inheritance of sperm abnormalities. The results presented here suggest that one of them is linked to the *Mup-1* locus on chromosome 4. As shown by Fig. 3, the majority of F_2 and B_1 individuals which are characterized by a high level of morphologically abnormal sperm are of *Mup-1^{b}1^b* genotype, as in the C57BL/Kw parental strain.

In the B_1 cross, 4 hybrid males out of 15 showing > 19% of sperm abnormality but, having the *Mup-1^{a}1^b* genotype, they can be considered as recombinants. This gives a recombination frequency of 4:15 = 0·27, suggesting weak linkage between *Mup-1* and a gene on chromosome 4 affecting sperm abnormality. No estimate of recombination frequency can be obtained from the F_2 segregation because all individuals with high sperm abnormality level (> 19%) were typed as *Mup-1^{b}1^b*.

In conclusion, the data presented here suggest that

https://doi.org/10.1017/S0016672300029207 Published online by Cambridge University Press

at least two recessive genes are involved in inheritance of sperm abnormality level in CBA/Kw × C57BL/Kw crosses, one of them being linked with the *Mup-1* locus on chromosome 4. The 2-gene hypothesis, however, is only provisional and does not explain all features of the distribution of *Mup-1* genotypes and levels of sperm abnormality.

I wish to thank Professor H. Krzanowska for critical review and helpful comments on the manuscript. This work was supported by the Polish Academy of Sciences within the project CPBP.05.05.

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