Genet. Res., Camb. (1993), **61**, *pp.* 97–100 Copyright © 1993 Cambridge University Press

Effect of sperm genotype on chromatid segregation in female mice heterozygous for aberrant chromosome 1

SERGEI I. AGULNIK, IGOR D. SABANTSEV AND ANATOLY O. RUVINSKY*
Institute of Cytology and Genetics, Siberian Department of Academy of Sciences of Russia, Novosibirsk 630090 Russia

(*Received 30 January 1992 and in revised form 8 July 1992*)

Summary

An aberrant chromosome 1 with two large homogeneously staining insertions was isolated from wild populations of *Mus musculus musculus*. The specific features of the aberrant chromosome have been described elsewhere (Agulnik *et al.* 1990). These include its preferential entry into the oocyte of heterozygous females, increased mortality of homozygotes and decreased fertility of homozygous females. Here we present data indicating that chromatid segregation in heterozygous females depends upon which sperm enters the oocyte before the second meiotic division: meiotic drive is powerful when it is sperm bearing normal chromosome 1, and segregation normalizes during MII when it is sperm bearing chromosome 1 with the extra segment. Experimental data are adduced and explanations offered for the observed phenomenon.

1. Introduction

An aberrant chromosome 1 with a large fragment of amplified DNA has been identified in several populations of wild mice (Traut *et al.* 1984; Agulnik *et al.* 1988; Weith *et al.* 1987). When in a certain genetic background the aberrant chromosome shows preferential segregation in heterozygous females (Agulnik *et al.* 1990*a*; Agulnik *et al.* 1993). The meiotic drive observed is manifested as preferential entry of the aberrant homologue into the oocyte and the normal into the polar body during meiotic division. In this study of the inheritance of the aberrant chromosome 1 generated by different matings, we demonstrate low viability and fertility in homozygotes for the aberrant chromosome and the effect of sperm genotype upon transmission of the aberrant chromosome to offspring by heterozygous females.

2. Material and methods

The aberrant chromosome 1 carrying two linked homogeneously staining insertions previously referred to as Is(HSR; 1C5)1Icg and Is(HSR; 1E3)2Icg, will be henceforth designated as an inversion of amplified sequence In(1D HSR,E3)1Lub (Nomenclature Committee, Lunteren, November 1991), designated In in this paper. Mice bearing aberrant chromosome 1 were isolated from populations of house mice inhabiting Eastern Siberia (Agulnik *et al.* 1990*b*). The aberrant chromosome was maintained by backcrossing to strain CBA. In/In homozygotes were generated by intercrossing heterozygotes. CBA mice were taken as normal +/+. Estimation of embryonic mortality was based on comparisons of the number of *corpora lutea*, implantation sites and live embryos on days 18–19 of development. The standard method was used in cytogenetic analysis of embryos and adults (Dyban & Baranov, 1978).

3. Results

(i) Genotype ratio in adult offspring

The data of Table 1 (mating 1) provide further evidence for the powerful meiotic drive exerted by chromosome 1 with an inversion in heterozygous females in crosses with males homozygous for the normal chromosome (Agulnik *et al.* 1990*a*). The meiotic drive coefficient was estimated as 0·85 and, accordingly, the proportion of heterozygous offspring was 85%. This is in contrast with the data for crosses of heterozygous females with males homozygous for the inversion (Table 1, mating 2): the number of homozygous offspring is not only much smaller than that expected at a meiotic drive coefficient of 0·85, it is also significantly smaller than the one expected at an equal segregation of homologues, being only 35·2% ($\chi^2 = 22{\cdot}7$, $P < 0{\cdot}01$). Hence, in this mating there was a strong lack of mice receiving the aberrant chromosome from the mother. This suggested that the

* Corresponding author.

https://doi.org/10.1017/S0016672300031190 Published online by Cambridge University Press

Table 1. *Results of matings of In/+ females with homozygous males +/+ and In/In*

No.	Parental genotype ♀♀	Parental genotype ♂♂	Total Number of offspring	Offspring genotype[a] Observed In/In	In/+	+/+	Expected at $m = 0.85$[b] In/In	In/+	+/+	χ^2	Expected at $m = 0.5$ In/In	In/+	+/+	χ^2
1	In/+	+/+	473	0	406	67	0	402	71	0·56	0	236·5	236·5	243*
2	In/+	In/In	261	92	169	0	221·9	39·1	0	498*	130·5	130·5	0	22*

[a] At the age of 2 months.
[b] m, coefficient of meiotic drive in heterozygous female.
* $P < 0.01$.

Table 2. *Viability and genotype ratios in ♀♀ In/+ × ♂♂ In/In mating*

	Observed *Corpora lutea*	Number of implantation sites	Live embryos	Embryonic mortality (%)	Number of cytogenetically tested embryos	Genotype of embryo[a] Observed In/In	In/+	Expected at $m = 0.5$ In/In	In/+	χ^2
Embryos	165	153	142	14	91	43	48	45·5	45·5	0·3
	Observed New born	**Died during 2 months**	**Tested mice**	**Postnatal mortality (%)**	**Number of cytogenetically tested mice**	**Genotype of adult mice[b] Observed In/In**	**In/+**	**Expected at $m = 0.5$ In/In**	**In/+**	**χ^2**
Adults	124	46	78	37	78	30	48	39	39	4·15*

[a] On days 18–19 of development.
[b] 2 months after birth.
* $P < 0.05$.

Table 3. *Expected genotype frequencies in ♀♀ In/+ × ♂♂ In/+*

♀ \ ♂		In 0·5	+ 0·5
Hypothesis I			
In	0·85	0·425	0·425
+	0·15	0·075	0·075
Hypothesis II			
In	0·85	—	0·425
	0·5	0·25	—
+	0·15	—	0·075
	0·5	0·25	—

Hypothesis I: 0·425 In/In:0·5 In/+:0·075 +/+.
Hypothesis II: 0·25 In/In:0·675 In/+:0·075 +/+.

mortality of homozygotes for chromosome 1 with the inversion may be very high.

(ii) *Embryonic and early postnatal mortality, genotype ratio*

As the data of Table 2 show, total embryonic mortality in ♀♀ In/+ × ♂♂ In/In does not differ from normal, being just 14%. The ratio of homo- to heterozygous embryos is close to 1:1 on days 18–19 of development. This indicates that there is significant death of In/In offspring and a great deviation from the segregation expected in the case of a meiotic drive acting on heterozygous females. If there were a meiotic drive, the expected segregation ratio would be 77·35 In/In:13·65 In/+ instead of 43 In/In:48 In/+, which is very much different from the observed values ($\chi^2 = 102$, $P = 0.001$). Analysis of postnatal mortality of offspring from the above mating demonstrates that about 37% of newborn die during the first two months of life. The segregation ratio for 78 cytogenetically studied adults was 30 In/In:48 In/+, deviating from the 1:1 observed for embryos on days 18–19 of development due to lack of In/In homozygotes. This deviation from the expected is obviously still greater in the case of a meiotic drive influence. Comparisons of the expected (1:1) and observed (92:169) ratios of homo- and heterozygous offspring from ♀♀ In/+ × ♂♂ In/In (Table 2, no. 2) in adulthood allowed us to estimate embryonic and postnatal losses as about 45%. This percentage for deaths of homozygotes is too low to account for the great differences between the observed segregation ratio

https://doi.org/10.1017/S0016672300031190 Published online by Cambridge University Press

Table 4. *Comparison of observed and expected segregation ratios of embryos and adult offspring from ♀♀ In/+ × ♂♂ In/+*

	Genotype of embryos[a]					Offspring genotype				
	Total	In/In	In/+	+/+	χ^2	Total	In/In	In/+	+/+	χ^2
Observed	85	26	56	3	—	292	47	215	30	—
Expected under										
Hypothesis I	85	36·1	42·5	6·4	8·9*	292**	78·9	185·4	27·7	17·8***
Hypothesis II	85	21·3	57·3	6·4	3·2	292	45·2	222·1	24·7	1·4

[a] On days 18–19 of development.
[b] For designations of Hypotheses I and II see Table 3.
* $P < 0{\cdot}05$; ** expected with 45% mortality of In/In homozygous taken into account; *** $P < 0{\cdot}001$.

and the one expected in the case a meiotic drive would exert its effect on heterozygous females.

(iii) *Comparison of hypotheses*

The discrepancy can be explained only under the assumption that meiotic drive is abolished in the mating between In/In homozygous males and In/+ heterozygous females. This would mean that the male genotype and, consequently, the produced sperm, after the entrance into the oocyte, can significantly affect the second meiotic division and chromatid segregation in In/+ females, thereby producing the normalization of segregation. Two hypotheses are compared in Table 3. According to the first hypothesis the genotype of the sperm does not affect segregation in heterozygous females, and the segregation ratio for the mating between heterozygotes would be 0·425 In/In:0·5 In/+:0·075 +/+; with this hypothesis, selective mortality of all classes is discounted, and meiotic drive in females is 0·85. According to the second hypothesis the sperm bearing In during fertilization of the oocyte would normalize chromatid segregation to equal probability. Normal sperm is without such effect. The expected segregation in the mating between heterozygotes, with the above indicated parameters, would then be 0·25 In/In:0·675 In/+:0·075 +/+. Based on the data of Table 4, comparisons can be made for genotype ratios observed in embryos on days 18–19 of development and adults from the mating between heterozygotes with those expected according to the two hypotheses. The first hypothesis is clearly refuted, and the second reasonably well agrees with the observed data.

4. Discussion

We have previously inferred that meiotic drive in In/+ heterozygous females exerts its effect mainly during the second meiotic division. This inference was based on the observation that because of the great recombination distance between the centromere and the double insertion block heteromorphic chromosomes arose in 80% of cases; one chromatid carried an inversion, and the other did not. The entrance of the spermatozoid into the oocyte after the first meiotic division initiates the second, and this justified the assumption that male genotype, its product, the spermatozoon, affects chromatid segregation during oogenesis.

In this study we disclosed a phenomenon: sperm carrying chromosome 1 with an inserted amplified segment normalizes the disjunction of chromatids in the oocyte during the second meiotic division, and, as a result, meiotic drive, a feature of females heterozygous for aberrant chromosome 1, is abolished. Normal sperm does not have this property. The question then is how the spermatozoon can affect the disjunction of chromatids during the second meiotic division of the oocytes: either directly, through the participation of sperm structures in division, or indirectly, through a signal the spermatozoon emits?

In mice, the formation of the spindle in the oocyte is complete before the sperm enters it, initiating thereby the beginning of anaphase and the termination of MII (Maro *et al.* 1986). Thus, in the case of the entry of sperm with aberrant chromosome 1 into the oocyte, for homologue disjunction to normalize, one has to assume that this sperm may emit a biochemical signal with specific effect on segregation. The molecular–cytological basis of this assumption needs proof.

The aberrant chromosome presumably exerts its influence on the sperm properties during the span of time from the end of the second meiotic division during spermatogenesis to the beginning of the second meiotic division during oogenesis. This inference is reached through survey of Table 4. Indeed, the data of these tables indicate that the two sperm types formed in heterozygous males significantly differ in their effect on the segregation process of chromatids during the second meiotic division in females. In case if the aberrant chromosome exerts its influence at a stage preceding the segregation of chromatids during the second meiotic division of spermatogenesis, differences in the two types of sperm would hardly be expected.

Thus, the phenomenon in question may be regarded as a demonstration of the pleiotropic effect of a block

https://doi.org/10.1017/S0016672300031190 Published online by Cambridge University Press

of amplified material of chromosome 1. Other effects of this amplified material have been observed: meiotic drive in heterozygous females, a sharp decrease in fertility in homozygous females and high postnatal mortality of homozygotes of both sexes during the first two months of life (Agulnik *et al.* 1990*a*). It may, therefore, be suggested that amplification of genetic material and the associated rearrangement(s) in chromosome 1 (Agulnik *et al.* 1990*b*) might have affected hereditary structures of vital importance.

Genetic studies of meiosis, like those of any other biological process, proceed from revealed variability. A good number of mutations affecting the major step of meiosis has been identified in maize, *Drosophila* and other species (Golubovskaya, 1979; Baker *et al.* 1976), the number reported for mammals is small. It is hoped that the facts presented here would provide clues for studying meiotic processes.

The authors wish to thank Ms A. Fadeeva for translation of the paper from Russian into English.

References

Agulnik, S., Gorlov, I. & Agulnik, A. (1988). New variant of chromosome 1 in the house mouse *Mus musculus*. *Citologija* **30**, 773–776 (in Russian).

Agulnik, S. I., Agulnik, A. I. & Ruvinsky, A. O. (1990*a*). Meiotic drive in female mice heterozygous for the HSR insertions on chromosome 1. *Genetical Research* **55**, 97–100.

Agulnik, S. I., Borodin, P. M., Gorlov, I. P., Ladygina, T. Yu. & Pak, S. D. (1990*b*). The origin of a double insertion of homogeneously staining regions in the house mouse (*Mus musculus musculus*). *Heredity* **65**, 265–267.

Agulnik, S., Sabantsev, I. D., Orlova, G. V. & Ruvinsky, A. O. (1993). Meiotic drive on aberrant chromosome 1 in the mouse is determinated by a linked distorter. *Genetical Research* **61**, 91–96.

Baker, B. S., Carpenter, A. T. C., Esposito, M. S. *et al.* (1976). The genetic control of meiosis. *Annual Review of Genetics* **10**, 53–134.

Dyban, A. P. & Baranov, V. S. (1978). Methods. In *Cytogenetics of Mammalian Development*, pp. 216–218. Moscow: Nauka (in Russian).

Golubovskaya, I. N. (1979). Genetic control of meiosis. *International Review of Cytology* **58**, 247–290.

Maro, B., Howlett, S. K., Johnson, M. H. (1986). Cellular and molecular interpretation of early development. The first cell cycle. In *Gametogenesis and the Early Embryo*, pp. 389–407. Alan R. Liss Inc.

Traut, W., Winking, H. & Adolph, S. (1984). An extra segment in chromosome 1 of wild *Mus musculus*: a C-band positive homogeneously staining region. *Cytogenetics and Cell Genetics* **38**, 290–297.

Weith, A., Winking, H., Brackmann, B., Boldyreff, B. & Traut, W. (1987). Microclones from a germ line HSR detect amplification and complex rearrangements of DNA sequences. *EMBO Journal* **6**, 1295–1300.

https://doi.org/10.1017/S0016672300031190 Published online by Cambridge University Press