

The influence of mutations on chromosome 17 upon the segregation of homologues in female mice heterozygous for Robertsonian translocations

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Summary

The influence of mutations in chromosome 17 upon the segregation of the metacentric and acrocentric homologues in the progeny of female mice heterozygous for Robertsonian translocations *Rb(8.17)IEm* and *Rb(16.17)7Bnr* was studied. Genetic analysis indicated that the portion of non-*Rb* (normal karyotype) progeny from mothers heterozygous for mutations *tf*, *qk*, *t¹²* was weakly different from the 50% Mendelian expected level (55–57%). Introduction of mutations *T*, *Fu^{Ki}*, *Fu*, *t⁶* into the female genotype caused a stronger segregation distortion and an increase in the portion of progeny with normal karyotype (63–67%). From the data on embryonic mortality and cytogenetic observations it is concluded that a distortion of equal transmission arises before M II of meiosis. Consequently, the preferential distribution of the metacentric chromosome to the polar body during the first meiotic division is relevant to the observed segregation distortion.

1. Introduction

Cytogenetic observations in natural populations and laboratory stocks of mice have led to the identification of more than 80 Robertsonian translocations involving various chromosomes of the normal karyotype (Gropp & Winking, 1981). The transmission ratio of the metacentric to the corresponding acrocentrics in the male progeny from heterozygous parents does not as a rule deviate from 1:1, which is what one would expect. In the female progeny, however, there occasionally occurs a significant increase in the number of progeny carrying the acrocentric chromosomes (Cattanach, 1978). This suggested that the metacentric chromosome may be preferentially distributed to the first polar body during the first meiotic division (Gropp & Winking, 1981).

In a previous paper we reported that the mutant genes located in the precentromeric region of chromosome 17 affect the segregation ratio of females heterozygous for *Rb(8.17)IEm* and *Rb(16.17)7Bnr* (Ruvinsky, Agulnik & Agulnik, 1984). This paper reports a further investigation of this observation.

2. Materials and methods

Mice heterozygous for *Rb(8.17)IEm* (*Rb1*) and *Rb(16.17)7Bnr* (*Rb7*) were produced for experimental

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purposes. The effects of the following mutations and haplotypes in the precentromeric region of chromosome 17 were studied: tufted (*tf*), quaking (*qk*), brachiury (*T*), fused (*Fu*), kinky (*Fu^{Ki}*), *t⁶* and *t¹²* (*t*-haplotypes) (Klein, Figueroa & Klein, 1982). The mutations were placed in *trans* to the Robertsonian translocations. The mutant mouse stocks used were *Ttf/t⁶*, *Fu/+(129RrDg)* and *tf/tf*, which was closely inbred for more than 40 generations. The chromosome *Ttf* was maintained during several generations by backcrossing to stock *tf/tf*. Also used were *qk/t¹²*, *Rb1/Rb1*, *Rb7/Rb7* mice supplied by Dr. V. S. Baranov, Institute for Experimental Medicine, Leningrad, USSR, and *Fu^{Ki}tf/+tf* (BTBR) mice provided by Dr. J.-L. Guènet, Pasteur Institute, Paris, France.

Chromosome analysis of bone marrow cells, blastocysts (on day 3–4 of development), and oocytes (at the M II stage) was performed by conventional methods (Dyban & Baranov, 1978). Pre-implantation and post-implantation embryonic mortality was estimated in some of the experiments.

3. Results

The results of two independently performed series of experiments are given in Table 1. In the first series the females were heterozygous for *Rb1*, in the second for *Rb7*. The mutations *tf*, *qk*, *Fu*, *Fu^{Ki}*, *T* and haplotypes

Table 1. Chromosome segregation in the progeny of female mice heterozygous for Rb(8.17)1Iem or Rb(16.17)7Bnr mated to *tf/tf* males

Mating	Female genotype	Sample size (n)		Percentage non-Rb		Mean percentage non-Rb
		<i>Rb1</i>	<i>Rb7</i>	<i>Rb1</i>	<i>Rb7</i>	
1	<i>Rb + / + tf</i>	245	350	57.1	57.4	57.3 ± 2.0
2	<i>Rb + / + qk</i>	51	111	54.9	58.6	57.4 ± 3.9
3	<i>Rb + / + Fu</i>	119	432	52.1	65.5	62.6 ^a
4	<i>Rb + + / + Fu^{Kt}tf</i>	242	265	62.8	63.0	62.9 ± 2.1
5	<i>Rb + / + t¹²</i>	185	115	51.4	58.5	54.7 ± 2.8
6	<i>Rb + / + t⁶</i>	118	170	66.9	66.5	66.7 ± 2.8
7	<i>Rb + + / + Ttf</i>	130	314	70.8	65.0	66.7 ± 2.2

^a The only case of a significant heterogeneity between *Rb1* and *Rb7*.

t⁶, *t¹²* were introduced in the acrocentric chromosome 17 in both cases. All the mutations and haplotypes studied showed similar, non-significantly differing segregation patterns for translocations *Rb1* and *Rb7*. The only exception was mutation *Fu*. The level of transmission of the chromosome carrying this mutation to the progeny was 52.1% from females *Rb1 + / + Fu* and 65.5% from females *Rb7 + / + Fu* ($\chi^2 = 6.60$, $P = 0.01$).

The similarity of segregation patterns for *Rb1* and *Rb7* allows us to analyse the obtained facts together (Table 1). Two groups of mutations can be specified. Recessive mutations *tf*, *qk* and *t¹²* haplotype constitute the first group. Their presence in the acrocentric homologue led to a weak (54.7–57.4% non-*Rb*), but significant increase of transmission of this chromosome to the progeny. The mutations belonging to the second group – *T*, *t⁶*, *Fu^{Kt}* – caused a sharp increase in transmission of acrocentric homologues to the progeny (62.9–66.7% non-*Rb*).

The observed phenomenon was most strongly expressed in case of the mutation *T* and *t⁶* haplotype. This segregation fits a 2:1, not the 1:1 ratio expected. It is necessary to emphasize that the difference in the level of transmission of acrocentric homologues, carrying the mutations *tf*, *qk* or *t¹²* haplotype was significant in comparison with that of mutation *T* and *t⁶* haplotype ($P < 0.05$).

An additional experiment was performed to test whether the observed phenomenon is mainly the effect of mutation on chromosome 17. For this purpose, female sibs from *+ Ttf / + + tf* × *Rb7 / Rb7*

mating were test-crossed. Their genotypes were *Rb7 + + / + + tf* and *Rb7 + + / + + Ttf*. The segregation ratio was 51*Rb*:94 non-*Rb* (i.e. 64.8% non-*Rb*) in the progeny of *Rb7 + + / + Ttf* females and *tf/tf* males. The mating of *tf/tf* males to *Rb7 + + / + + tf* females gave 61 *Rb*:67 non-*Rb* (i.e. 52.3% non-*Rb*). The female sibs progeny differ significantly ($P < 0.05$) in segregation, and the segregation data conform with those given in Table 1.

The question asked was 'what may be the cause of this segregation distortion?' In an attempt to answer this question, we estimated embryonic mortality in a series of crosses (Table 2). The estimates allowed us to discard it as a possible cause of the observed distortion. Some increase in post-implantation mortality in *Rb7* heterozygous females (crosses 3, 4, 5), can be accounted for by the death of trisomics, since the probability of occurrence of these among females heterozygous for *Rb7* is increased by about 5–12% (Dyban & Baranov, 1978).

It is reasonable to conclude that the ratio of progeny with *Rb* to those with normal karyotype has been established earlier than at the blastocyst stage. The tabulated results support this assumption (Table 3). Indeed, the percentage of blastocysts not carrying Robertsonian translocations in the *Rb7 + + / + + Fu* + ♀♀ × *+ + tf / + + tf* ♂♂ cross is 67.3%, not different from that in live-born progeny (65.5%, Table 1), thereby confirming the view that the ratio of *Rb* to non-*Rb* progeny has arisen during the enfoldment of meiotic events in females.

Additional support for this assumption is provided

Table 2. Embryonic mortality in different crosses: females mated to *tf/tf* males

Mating	Female genotype	No. of females	No. of corpora lutea	No. of implanted sites	No. of live embryos	Mortality pre-implantation (%)	Mortality post-implantation (%)	Total embryonic mortality (%)
1	<i>Fu + / + tf</i>	21	174	126	111	27.6	8.6	36.2
2	<i>Ttf / + tf</i>	20	189	146	123	22.8	12.1	34.9
3	<i>Rb7 + / + tf</i>	22	167	138	100	22.8	17.3	40.1
4	<i>Rb7 + / + Fu</i>	22	200	147	110	26.5	18.5	45.0
5	<i>Rb7 + + / + Ttf</i>	20	194	154	110	20.6	22.7	43.3

Table 3. The ratio of Rb to non-Rb blastocysts in the Rb7+/+Fu ♀♀ × +tf/+tf ♂♂ cross

No. of blastocysts examined	Rb (2n = 39)	Non-Rb (2n = 40)	Non-Rb (%)	Trisomy (2n = 40)
54	17	35	67.3	2

Table 4. Analysis of meiotic metaphase II in Rb7+/+/+Ttf ♀♀

No. of oocytes examined	Euploid		Non-Rb (%)	Aneuploid	
	Rb	Non-Rb		Non-Rb	Rb
60	15	34	69.4	5	6

by the results of analysis of meiosis at the M II stage in Rb7+/+/+Ttf females: the percentage of non-Rb oocytes among the euploid oocytes is 69.4%, i.e. not different from the percentage for Rb carriers among the blastocysts and live-born mice (Table 4). It is concluded that the segregation distortion results from the preferential distribution of the metacentric chromosome to the polar body during the first meiotic division.

4. Discussion

Previously it has been suggested that disturbance of the normal disjunction of the homologues in mice heterozygous for Robertsonian translocations depends, to some extent, on morphologically undetectable minor structural variations between metacentrics and corresponding acrocentrics (Cattanach & Moseley, 1973; Cattanach, 1978). The finding of this study supports this. It was shown that certain mutant genes located in the precentromeric region of chromosome 17 influence the segregation of the metacentric and acrocentric homologues in females heterozygous for Rb1 and Rb7. The experiments with the female sibs having the same genetic background except for chromosome 17 confirm the above statement. All the mutations considered here can be assigned to two groups. The first group consists of *tf*, *qk*, *t*¹²-haplotype which affect weakly the segregation ratio. There is an obvious similarity in the effects of these mutations on the two Robertsonian translocations. The second group comprises mutations *T*, *Fu*^{Ki} and *t*⁶-haplotype. Members of this group more strongly alter the transmission of acrocentric chromosome 17: 63–70% of the progeny bear the acrocentric chromosome. Mutation *Fu*, in contrast, is without effect on the segregation ratio in Rb1 female heterozygotes (52.1%) but strongly affects it in Rb7 female heterozygotes (65.5%). No straightforward explanation can be offered for the discrepancy. We are inclined to assign

Fu to the second group. If so, recessive and dominant mutations would affect segregation differently.

The different behaviour of *t*⁶ and *t*¹²-haplotypes is unexpected. True, *t*⁶ and *t*¹² are members of different complementation groups, and they differ in phenogenetics (Lyon, 1981). Nevertheless, they have features in common, and abnormal chromatin stretches over large proximal areas of chromosome 17 in both haplotypes (Silver, 1981; Silver & Remis, 1987). The difference in the effect of *t*⁶ and *t*¹² upon the segregation of the homologues in Rb female heterozygotes possibly reveals another difference between the two.

Search for the step when aberrant segregation may have first developed led us to the belief that it arose some time before M II of meiosis in females. The phenomenon owes its existence to the preferential distribution of the metacentric chromosome to the polar body. Consequently, *T*, *Fu*, *Fu*^{Ki} and *t*⁶-haplotype accomplish their action also during oogenesis, but not later than after the first meiotic division. The possible mechanism of this phenomenon will be the subject of the next paper. Is the phenomenon universal? It may not be restricted to chromosome 17. There is, as yet, no evidence to the contrary. The fact that many Robertsonian translocations segregate aberrantly justifies further investigations along these lines.

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