Retardation of ovarian growth in male-sterile mice carrying an autosomal translocation

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SUMMARY Ovarian volumes in 3- and 5-day-old mice heterozygous for the t(11;19)42H translocation were found to be smaller than those of normal controls. Comparisons between litter mates suggested a slight increase in body weight of the translocation bearers and this effect increased the difference in relative ovarian volumes between t42H/+ mice and controls. It is suggested that the differential effect of the translocation on fertility in males and females might be the result of the higher meiotic requirements inherent in spermatogenesis.

Autosomal translocations are a cause of male sterility in mice as well as men. Lyon and Meredith¹ were the first to show that certain translocations that did not involve sex chromosomes caused sterility in male mice, while females were fertile, albeit with reduced litter size. Searle *et al*² described eight autosomal translocations that are maintained through female carriers, the males being sterile. Testis weight is low and spermatogenesis is arrested at different stages characteristic for any particular translocation.

An apparently similar situation has been described in man.³ Three brothers, carriers of a balanced reciprocal translocation between chromosomes 9 and 22, were sterile. A testicular biopsy in one of them revealed spermatogenesis until metaphase I but few spermatids and no spermatozoa. Chain quadrivalents were present at diakinesis. The translocation had been inherited from the mother in whom oogenesis appeared to proceed normally.

The question arises of why the same translocation causes meiotic arrest in males but not in females. Does the altered chromosome constitution affect testicular but not ovarian development or could the primary effect be similar in both sexes with females showing reduced sensitivity?

We have measured ovarian volumes in immature mice carrying a balanced autosomal translocation, t(11;19)42H/+,² and found them to have reduced ovary size and a reduced ratio of ovarian volume to body weight.

Materials and methods

Female mice heterozygous for the t42H translocation
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were mated to (C3H×101)F1 males. Young mice, aged either 3 or 5 days, were weighed before being killed by decapitation.

Presence or absence of the translocation was assessed in one of two ways. The mothers of five females were heterozygous for the wavy-coat allele of *rex* (*Re^{wc}*), which is located on chromosome 11 and gives a recombination frequency of about 9% with the breakpoint of the translocation.⁴ Mice carrying this allele can be identified shortly after birth by their abnormally bent whiskers.⁵ The remaining litters totalling 19 mice did not segregate for *Re^{wc}* and chromosomes were analysed from liver preparations by a modification of the method of Eicher and Washburn⁶ (Evans, 1980, personal communication). The t42H translocation has two markers: chromosome 11¹⁹ has a conspicuous constriction and chromosome 19¹¹ is shorter than any normal chromosome (fig 1). At least two cells were analysed from each mouse.

In order to measure ovarian volumes, the two ovaries were dissected out, fixed in Bouin's solution, and prepared for serial sectioning at 7 µm. Haematoxylin and eosin were used for staining. Areas of sectioned ovaries were measured by a counting method using a modified Whipple eyepiece micrometer (Graticules Ltd) as described previously.⁷ The method involves counting squares overlying the sectioned ovaries, taking care to randomise partially overlying squares. Absolute volumes were calculated using a stage micrometer.

Results

Values of mean ovarian volumes of right and left ovaries in relation to body weight in t42H/+ and

FIG 1 Chromosomes of *t42H/+* female mouse showing two markers. Arrows point to chromosome 11^{19} (constriction) and 19^{11} (small). Length of bar = 0.01 mm.

FIG 2 Ovarian volumes and body weight in normal and *t42H/+* mice. ○ normal, ● *t42H/+*.

control $+/+$ mice are shown in fig 2. Analysis of covariance resulted in $t=4.14$, which has a probability of less than 0.001, thus confirming the visual impression that the ovaries of *t42H/+* mice are smaller in relation to body weight than those of $+/+$ mice.

Additional information on the effect of the translocation on ovarian development and body weight is obtained by comparisons between litter mates. Fourteen of the mice came from litters containing at least one female with and one without the translocation (table).

The significance of the differences between translocation mice and controls was tested on the null hypothesis that the values for *t42H* mice did not differ from the means of their respective litters, using the formula

$$\frac{\sum Sx - \sum E}{\sqrt{\sum V_{Sx}}}$$

where Sx = sum of body weights of *t42H* females in each litter

Nx = number of *t42H* females in each litter

S = sum of body weights of all females in each litter

N = number of all females in each litter

E = expected sum of body weights of *t42H* females in each litter

$= SNx/N$

V = variance of body weights in each litter

V_{Sx} = variance of Sx

$= VNx(N - Nx)/N$

and similarly for the other variables shown in the table.

The results indicate that the differences are significantly larger than their standard errors and suggest that the *t42H/+* mice differ from their normal litter mates in having slightly increased body weights and smaller ovarian volumes.

Discussion

In the male, the *t42H* translocation results in breakdown of meiosis: *t42H/+* spermatocytes start to die in stage IV, and finally disappear in stage IX (mid-pachytene) of the cycle of the seminiferous epithelium.⁸ Adult testis weight is about a quarter of the normal.²

Forejt and Gregorová⁹ studied meiotic prophase in three autosomal translocations and observed non-random contact of the translocation configuration with the C bands of the X chromosome. The authors postulated interference with the inactivation of the X chromosome as a cause for spermatogenic arrest. A similar chromosome association in female

TABLE Body weights and ovarian volumes in female mice from litters segregating for t42H

Litter No	Mouse	Chromosomes	Body weight (g)	Mean ovarian volume (mm ³)	Ovarian volume/body weight (mm ³ /g)
4	15	t42H	4.3	0.0494	0.0115
	16	+	4.0	0.0609	0.0152
7	25	t42H	4.4	0.0544	0.0124
	26	+	3.8	0.0635	0.0167
10	32	t42H	4.73	0.0669	0.0141
	33	+	3.94	0.0862	0.0219
	34	+	4.23	0.1154	0.0273
12	38	+	5.41	0.1379	0.0255
	39	t42H	5.13	0.0722	0.0141
13	40	t42H	3.17	0.0373	0.0118
	41	+	2.74	0.0558	0.0204
	43	t42H	3.25	0.0500	0.0154
24	72	+	2.36	0.0573	0.0243
	73	t42H	2.60	0.0512	0.0197
$\Sigma Sx - \Sigma E^*$			2.12	-1.92	-2.51
$\sqrt{\Sigma VSx}$					
P			0.05	0.05	0.01

*See Results section.

meiosis would not be expected to have deleterious effects since both X chromosomes are active during oogenesis.⁹ Our finding that the translocation is associated with retardation of ovarian development weakens this hypothesis, although it does not disprove it.

The other suggestion implicit in the data, that the t42H/+ karyotype causes a small increase in the body weight of females, although clearly in need of confirmation, raises the possibility of a systemic effect of the translocation on processes of growth and development. Theoretically this could be the result of either position effect or a possible loss of a small amount of chromosomal material resulting from the translocation. It may be relevant that Russell and Montgomery¹⁰ observed decreased weights in female mice heterozygous for various X;autosome translocations.

The adverse effect on ovarian growth suggests that the t42H translocation might exert similar effects on spermatogenesis and oogenesis which result in sterility in males while females are fertile. The reason could perhaps be sought in the disparate numbers of male and female gametes required for fertilisation, when many millions of spermatocytes need to complete meiosis in order to fertilise a single egg. Also, the time available for meiosis is greater in females than in males. The data do not support the argument by Dawkins¹¹ that because the egg is larger than the sperm a mother's investment in a child is greater than the father's. On the basis of simple arithmetical considerations regarding relative gamete sizes and numbers in mammals, one would

conclude that the investment in spermatozoa is greater than that in eggs. This conclusion is borne out by numerous chromosome abnormalities in mammals that result in breakdown of spermatogenesis while being compatible with reproduction in the female.

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diseases and to make these cells available at a nominal charge to all legitimate investigators. We welcome any appropriately characterised cell cultures for repository inclusion. For further information and cell strain contribution write to: Drs

H Goldman or Sergio Pena, The Cell Repository of Neuromuscular Diseases, deBelle Laboratory for Biochemical Genetics, Montreal Children's Hospital, 2300 Tupper Street, Montreal, Quebec, Canada H3H 1P3.

Correction

In the article 'Retardation of ovarian growth in male-sterile mice carrying an autosomal translocation' by Mittwoch *et al* (*J Med Genet* 1981;18:414-7), the mouse translocations were printed throughout using human instead of mouse cytogenetics nomenclature, that is, t instead of T. We apologise for this error.