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Evidence for allelism of leaner and tottering in the mouse

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SUMMARY

We found that *la* is located in linkage group XVIII. It is highly probable that *la* and *tg* are alleles, and closely linked to *Es*-1. Mice of the genotype *la*/*tg* are abnormal, with clinical signs similar to *tg*, although more severe. They develop earliest signs at about 15 days of age, similar to *la*, are runted but fertile and can live for months. Clinical signs are ataixa, stiffness, retarded motor activity and intermittent focal seizures. The pathological basis for these symptoms is still elusive. The three types of mice, *la*/*la*, *la*/*tg* and *tg*/*tg* are thus distinct clinically, *la*/*tg* resembling in some respect either of the other two.

1. INTRODUCTION

Tottering (gene symbol, *tg*) and leaner (*la*) are autosomal recessive mutations of the mouse. Both cause neuromuscular disorders but their clinical and pathological characteristics are different. Tottering is classified with a group of other mutations characterized by epileptiform seizures, and leaner is a so-called cerebellar mutant because of severe pathological lesions involving mainly the cerebellen (see Sidman, Green & Appel, 1965). The *tg* locus is in linkage group XVIII in close proximity to *Es*-1, a locus controlling esterase-1 isozymes (Green, 1966). The linkage group of *la*, prior to this study, has not been known.

In the course of studies on the genetic control of non-specific esterase isozymes in neuromuscular mutants we determined the *Es*-1 phenotypes of *tg*/*tg* and *la*/*la* mice. As a consequence of these studies we were interested in the possible genetic relationship of the two mutant types. Our findings indicate that *la* and *tg* are allelic. This paper presents the results of our breeding tests and isozyme determinations. Further, it describes the clinical and preliminary pathological findings in *la*/*tg* mice.

2. MATERIALS AND METHODS

Mice of strain C57BL/10JGn carrying the mutation *tg* were obtained from Dr Margaret C. Green, The Jackson Laboratory. We maintained *tg* linked with *Es*-1^b, an allele of *Es*-1 which controls esterase isozyme 1^b. This isozyme is revealed in agar gel zymograms as the cathodal portion of two broad, fast-migrating bands (Tsuji & Meier, 1969).

Leaner heterozygotes (*la*/+) were obtained from Miss Janice L. Southard, The Jackson Laboratory. These mice were found to be homozygous for *Es*-1^a, the

second allele at the *Es*-1 locus controlling the most anodal esterase band in agar-gel zymograms. Serum esterase types were determined by a combination of agar-gel electrophoresis and histochemical staining. We have previously described the details of our technique (Tsuji & Meier, 1969).

To test for allelism of *tg* and *la*, the following initial crosses were made:

(1) $\frac{Es\text{-}1^a\ la}{Es\text{-}1^a\ +}$ females were mated with $\frac{Es\text{-}1^b\ tg}{Es\text{-}1^b\ tg}$ males;

(2) $\frac{Es\text{-}1^a\ +}{Es\text{-}1^b\ tg}$ females mated to $\frac{Es\text{-}1^a\ la}{Es\text{-}1^a\ +}$ males.

Provided that all three genes, *Es*-1, *tg*, and *la*, are closely linked, such matings should produce triple heterozygote progeny as one-half chance in mating (1) and one-fourth in mating (2). Also, if *la* is an allele of *tg*, all triple heterozygote progeny should show behavioural abnormalities.

3. RESULTS

(i) *Breeding tests*

The results of matings are given in Table 1. The first cross yielded progeny of only one type with respect to their esterase isozyme pattern, *Es*-1^a/*Es*-1^b (Plate 1, fig. 1). Eighteen of 27 revealed abnormal behaviour, and 9 were clinically normal

Table 1. *Test for allelism of* la *and* tg *with reference to* Es-1 *phenotypes*

Type of mating	No. of matings	Progeny $\frac{Es\text{-}1^a}{Es\text{-}1^a}$ Normal	$\frac{Es\text{-}1^a}{Es\text{-}1^a}$ Affected	$\frac{Es\text{-}1^a}{Es\text{-}1^b}$ Normal	$\frac{Es\text{-}1^a}{Es\text{-}1^b}$ Affected	$\frac{Es\text{-}1^b}{Es\text{-}1^b}$ Normal	$\frac{Es\text{-}1^b}{Es\text{-}1^b}$ Affected	Total
(1) $\frac{Es\text{-}1^a +}{Es\text{-}1^a\ la} \times \frac{Es\text{-}1^b\ tg}{Es\text{-}1^b\ tg}$	3	0	0	9	18	0	0	27
(2) $\frac{Es\text{-}1^a +}{Es\text{-}1^a\ la} \times \frac{Es\text{-}1^a +}{Es\text{-}1^b\ tg}$	8	40	0	21	13	0	0	74
(3) F_1(A)* $\times \frac{Es\text{-}1^a +}{Es\text{-}1^b\ tg}$	5	7	1	8	7	1	9	33
(4) F_1(A) $\times \frac{Es\text{-}1^a +}{Es\text{-}1^a\ la}$	3	5	4	3	4	0	0	16

* Affected F_1 progeny from mating 1 and 2.

(mating 1). Thus, the observed frequencies of normal and abnormal progeny agreed satisfactorily with those expected if *tg* and *la* were allelic. In the second mating, two esterase phenotypes were found, *Es*-1^a/*Es*-1^a expressing only the anodal isozyme band, and *Es*-1^a/*Es*-1^b revealing both the anodal and cathodal band. None of the mice belonging to the homozygous *Es*-1^a phenotype were

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abnormal, but 13 of 34 heterozygotes showed clinical symptoms, in satisfactory agreement with the one-fourth expected if *tg* and *la* were allelic.

We then mated (matings 3 and 4) affected F_1 males from the two matings with both kinds of normal heterozygous parents:

(3) F_1 (A) mated to $\frac{Es\text{-}1^a\ +}{Es\text{-}1^b\ tg}$;

(4) F_1 (A) mated with $\frac{Es\text{-}1^a\ +}{Es\text{-}1^a\ la}$.

The results of these matings are shown in Table 1. We found two recombinants in the progeny of mating (3): one between *tg* and *Es*-1^a of an *la*/*tg* mutant and another between wild type (+) and *Es*-1^b of an *tg*/+ heterozygote.

From the frequencies of normal and abnormal progeny in the first two matings and the segregation ratios observed in the last two matings, we infer that (*a*) *tg* and *la* are alleles and (*b*) they are closely linked with *Es*-1 in linkage group XVIII.

(ii) *Description of* la/tg *mice*

Abnormal clinical signs occur between 15 and 17 days of age. They are ataxia, stiffness, and retarded motor activity. Usually within a day or two after the initial symptoms, the mice develop a wobbly gait and intermittent focal seizures. The complete seizure pattern is present in all mice by 4 weeks and persists throughout life. Seizure initiation is sudden. An attack may last an entire hour, although there are interphases in fits during which the mice attempt to walk. Their gait is always wobbly. Upon being lifted by their tail, they stiffen their hind legs and extend them sideways (Plate 1, fig. 2). Most mice surviving to weanling age cope very well with their affliction and may have slightly reduced lifespans compared to normal mice, although they are runted. At 1 month of age their weights (7·5 ± 0·1 g, mean and its standard error of 10 mice) are slightly greater than one half that of normal litter-mates (12·7 ± 0·2, 10 mice). Both sexes of *la*/*tg* mice are fertile, but females appear to lack sufficient milk for rearing their offspring.

A preliminary survey of serial frontal sections of the brains from one each of *la*/*tg* and *tg*/+ did not reveal any pathological changes. Sections were cut at 10 μ and alternately stained with Luxol fast blue, alcian-blue/periodic and acid–Schiff's Sudan black, and Palmgren's pyridine silver nitrate as described previously (Meier & MacPike, 1970).

4. DISCUSSION

We believe that our experimental data favour the idea that *tg* and *la* are indeed alleles: (1) matings between normal parents heterozygous for *tg* and *la* yielded adequate numbers of abnormal progeny (mating 2), and (2) matings 3 and 4 of abnormal F_1 males with both types of normal heterozygous females revealed segregation ratios which are in satisfactory agreement with those expected for

allelism of *tg* and *la*. Clearly the possibility of pseudoallelism is not ruled out entirely; neither do we have unequivocal disproof that the two genes represent closely linked non-alleles. To decide on the possibility of independent loci, a search for recombinants must be made among larger numbers of progeny from appropriate matings (Table 1). With two closely linked recessive genes having similar function, the *trans*-configuration is often of mutant-phenotype whereas the *cis*-configuration remains wild-type. Thus, closely linked genes may in part mask their normal expression. Unfortunately, *la/tg* mice are poor breeders, especially females, and we do not as yet have progeny from matings of abnormal F_1 mice in whom to search for recombinants if they occur at all. Similarly, it is difficult or impossible to obtain progeny from matings of F_1(A) and *tg/tg* mice, all of which should be affected.

We observed that clinical signs of *la/tg* mice appear as early in life as in *la/la* mice, i.e. at about 15 days. Although they are considerably more severe, they resemble qualitatively those of *tg/tg* mice (Green & Sidman, 1962). Tottering symptoms usually are first recognized at about 3–4 weeks and, except for the seizures, are relatively mild; they develop normally and their life-span is not reduced. In contrast, *la/tg* mice are runted, but they do not show any histopathological abnormalities, are fertile, and may live for many months. Leaner mice die at about 3 weeks of age; thus they are produced by mating known heterozygotes. Their cerebella are reduced in size and reveal severe cytoarchitectonic abnormalities with focal losses of Purkinje and granule cells, and proliferation of glia (Sidman *et al.* 1965).

The brief clinical and pathological comparison of the three different syndromes produced by the two mutant genes and their combination, *tg/tg*, *la/la*, and *tg/la*, suggests the possible role of complementation between them. Complementation relates to either a deletion or dysfunction of a specific portion in a linear sequence of nucleotides (Benzer, 1957). If, in accord with regulation of gene activity in bacteria, the *la* mutation occurred in an operon or cistron closer to the operator gene than that of *tg*, only a portion of the *la* region may be complemented (Jacob & Monod, 1961). Thus, the abnormalities occurring in *la/tg* mice may derive from that segment of a linear nucleotide sequence which overlaps the two deletions. Such a situation may also explain why *la/tg* mice have clinical and pathological features more like tottering than leaner.

The data from matings 3 and 4 indicate that no recombination occurred between *Es*-1 and *la*. There is no question, therefore, that *la* is located close to *Es*-1 in linkage group XVIII whether or not an allele of *tg*. Thus, by means of serum electrophoretic analysis of *Es*-1 phenotypes, *la/la* mutants can be identified preclinically without sacrificing them. *Es*-1 phenotypes are readily discernible during the first week of life (Tsuji & Meier, 1969). Yoon (1969) recently described the close linkage of *la* with *El* (esterase, liver), a locus consisting of two alleles that control *Ela* and *Elb* liver esterase isozymes. He determined the recombination frequency between *la* and *El* as $3{\cdot}84 \pm 1{\cdot}98\,\%$. Because he considered the *Ela* and *Elb* liver esterases to be the same as *Eela* and *Eelb* (esterase, erythrocytes) found in mouse

erythrocytes by Pelzer (1965), Yoon (1969) proposed the symbols, El^a and El^b for their new designation.

We have found in another study a recombination frequency between *tg* and *Es*-1 of $5{\cdot}83 \pm 2{\cdot}14$ (Tsuji & Meier, 1969). These values are similar to or identical with those between *la* and *El* determined by Yoon. Therefore, we conclude that *Es*-1, *El*, and *Eel* are the same. Because of priority in designation (Popp & Popp, 1962), the two isozyme bands should be symbolized by Es-1a and Es-1b, and the loci $Es\text{-}1^a$ and $Es1^b$.

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EXPLANATION OF PLATE

Fig. 1. An agar-gel zymogram of serum esterase; alpha-naphthyl butyrate was used as substrate.

$$(a)\ \frac{Es\text{-}1^{b}\ tg}{Es\text{-}1^{b}\ tg};\quad (b)\ \frac{Es\text{-}1^{a}\ +}{Es\text{-}1^{b}\ tg};\quad (c)\ \frac{Es\text{-}1^{a}\ la}{Es\text{-}1^{b}\ tg};\quad (d)\ \frac{Es\text{-}1^{a}\ la}{Es\text{-}1^{a}\ +}.$$

Channels (*a*), (*b*) and (*d*) are of the parental types, and channel (*c*) represents double heterozygote of *la* and *tg* with clinically abnormal behaviour.

Fig. 2. An *la*/*tg* mouse, age 10 weeks, assuming characteristic posture of stiffening of hind limbs and extending them sideways when lifted by the tail.

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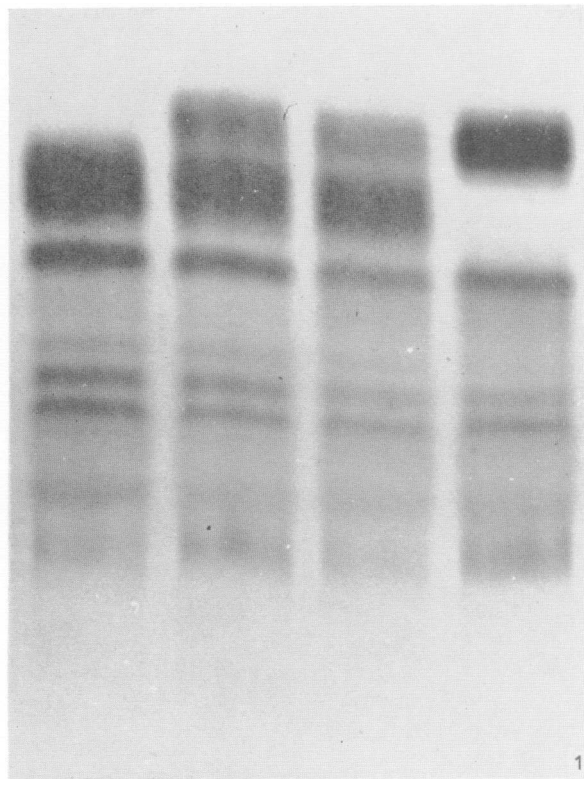
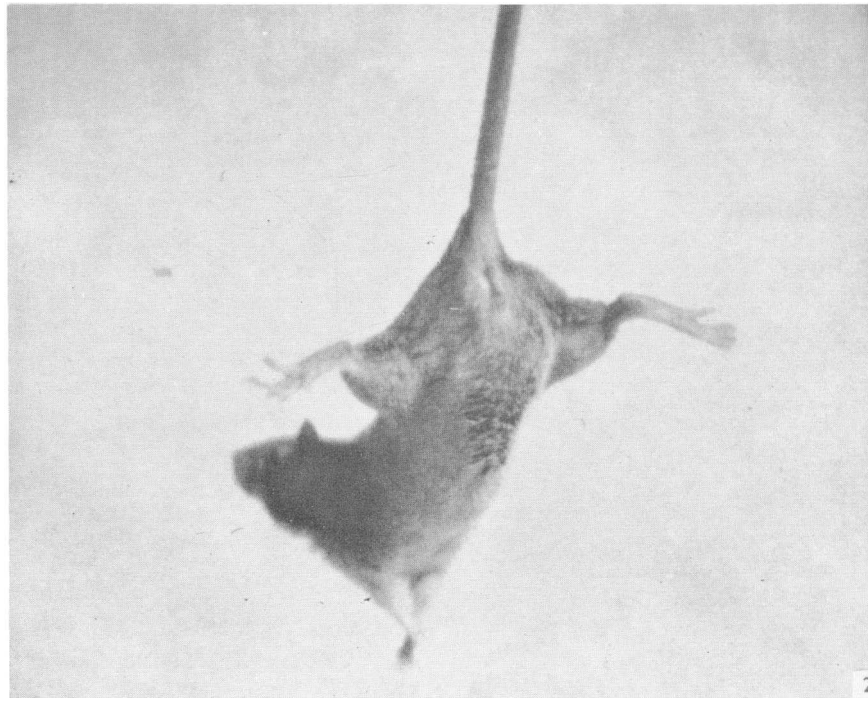


(a) (b) (c) (d)



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