SHORT PAPER

Lens opacity: a new gene for congenital cataract on chromosome 10 of the mouse

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1. INTRODUCTION

Mouse mutant genes which result in defects similar to those of medical importance in man may be of value as models for the study of the defect concerned. We report here a new gene causing congenital cataract in the mouse, which may be useful in the understanding of cataract in man.

A further point of interest is that Kratochvilova & Ehling (1979) have recently developed a new method of measuring increased mutation rates in the mouse, by examining offspring of animals treated with mutagens for the presence of cataracts due to mutant genes. For the purposes of this test it is valuable to have information on the number and map position of loci which can mutate to give cataracts.

2. ORIGIN AND GENETICS

The mutation arose spontaneously in a stock of mice homozygous for the Robertsonian translocation Rb (6.15)1Ald. The first affected animal found was a male, homozygous for albino (*cc*), whose pink eyes appeared to be opaque. When he was paired with a normal albino sister, 9 of their 28 offspring showed opaque eyes. Examination with an ophthalmoscope and dissection of the eyes revealed that the opacity was in the lens rather than in the cornea, and was present from the time that the eyes first opened. Further breeding showed the character to be inherited as an autosomal dominant (Table 1) and it was given the name and symbol *lens-opacity, Lop*.

The original male was next mated to a normal unrelated non-albino black-eyed female (C3H/HeH × 101/H, F₁ hybrid) in order to observe the effect of *Lop* on a pigmented eye. In the offspring no opacity was observable with the naked eye, but it could be detected by examination with an ophthalmoscope, 10 out of 15 offspring being scored as affected (Table 1, line A).

Affected offspring of the original male were crossed with normal animals, to give later generations on albino and coloured backgrounds, and good 1:1 segregations of normal:affected offspring were observed in each case (Table 1, line B).

Affected offspring were then mated together, in putative *Lop*+ × *Lop*+ matings. A new category of offspring was found, having very small eyes, again with an opaque lens. These offspring were presumed to represent the *LopLop* homozygous type, and the three categories of offspring occurred in a ratio of approximately 1:2:1 as expected from a normally segregating dominant gene. When putative *LopLop* animals were mated to

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unrelated normal mice, all offspring appeared $Lop+$ and when homozygotes were mated together, all offspring appeared $LopLop$ (Table 1). Thus Lop behaves as a semi-dominant autosomal gene, with good viability, fertility and penetrance. In general, the good segregation was maintained in extensive linkage tests described below. In a few crosses, however, there was a significant deficiency of $Lop+$ animals in backcrosses, indicating that reduced viability or penetrance may occur on certain genetic backgrounds.

Table 1. *Single-factor segregation of lens-opacity* (Lop) *on albino* (cc) *or coloured background*

	Offspring							
	cc background				Coloured background			
Mating	$LopLop$	$Lop+$	$++$	χ^2	$LopLop$	$Lop+$	$++$	χ^2
$++ \times Lop+$ A	—	9	19	3·57	—	10	5	1·67
B	—	62	72	0·75	—	33	49	3·12
$Lop+ \times Lop+$	17	30	25	3·72	36	70	41	0·67
$LopLop \times ++$	—	45	—	—	—	66	—	—
$LopLop \times LopLop$	24	—	—	—	139	—	—	—

A = matings of original male; B = later generations. χ^2 tests 1:1 or 3:1 segregation; d.f. = 1.

(i) *Description of eye defect*

The cataract appeared in most cases to be a total lens-opacity. In some heterozygous animals, however, the opacity appeared not to be total (Figs 1 and 2).

In all known or putative $LopLop$ homozygotes the eyes were small, and eye size could be used as a basis for classification of this category. The eyes were also slightly reduced in size in $Lop+$ heterozygotes (Fig. 2), but the effect was not sufficiently marked for animals to be classified on this basis. On dissection of $LopLop$ eyes the lenses were very much smaller than normal, and there was no regular arrangement of layers of lens fibres as found in normal animals.

No detailed investigations of the eye abnormality have been carried out.

(ii) *Linkage relations*

Linkage tests of Lop with markers on various chromosomes were carried out, with the dual aim of mapping the locus and of excluding allelism with other genes for cataract known to map at other positions.

Crosses were made of Lop to several standard linkage testing stocks, using visible marker genes. In addition, linkage was tested with several biochemical markers using electrophoretic polymorphisms. The tests were run on cellulose acetate plates using standard methods. Yet further crosses tested for linkage of Lop with Robertsonian translocations, using the latter as markers for centromeric regions of the chromosomes involved. Offspring were scored by examination of corneal mitoses by standard methods.

These tests revealed linkage of Lop with the marker grizzle-belly, Sl^{gbH}, on chromosome 10 (Table 2), and independence from all other markers tested. The recombination between Lop and Sl^{gbH} was $21·7 \pm 2·7\%$, and that between Lop and waltzer, v, also located on chr. 10 about 27 cm proximal to the Sl locus, was $45·5 \pm 3·4\%$. This suggested that Lop was located distally to Sl. In order to test this point and to map Lop in relation

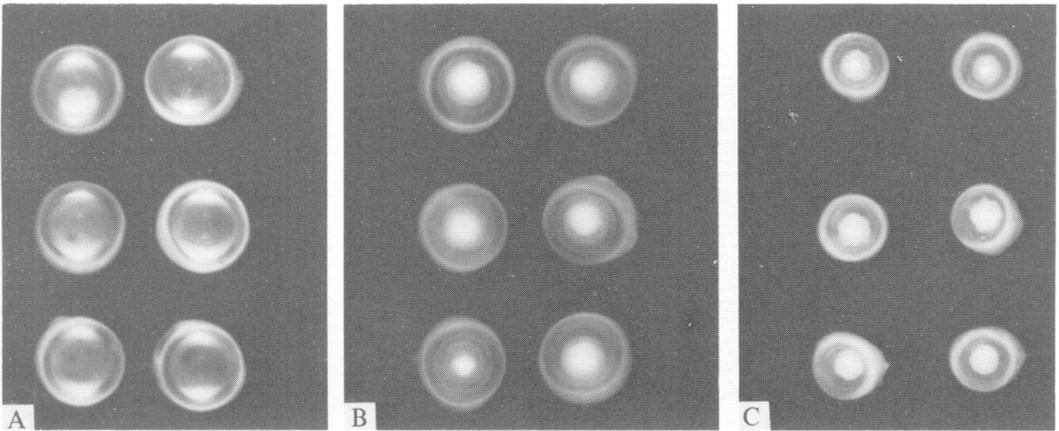Fig. 1. Head of albino *Lop*/+ heterozygote showing visible opacity in the pink eye.

Fig. 2. Eyes of albino animals showing reduced size and lens-opacity in *Lop*/+ and
Lop/*Lop*. (A) Normal; (B), *Lop*/+ ; (C) *Lop*/*Lop*.

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to other chr. 10 markers, crosses were made between crossover Sl^{gbH} $Lop/+ +$ animals and $v\,gr/v\,gr$ homozygotes so as to give in the following generation four-point backcrosses of type $+ + Sl^{gbH}Lop/v\,gr + + \times v\,gr + + /v\,gr + +$. The results of these crosses (Table 3) indicated that the order of the four loci was indeed as shown. With this order, all except

Table 2. *Results of backcross tests for linkage of* Lop *with various markers*

Dominant markers	Chromosome	Offspring					R.F. (%)	χ^2
		$Lop\,M$	$Lop\,+$	$+\,M$	$++$	Total		
Ahd-1	4	21	12	16	15	64	43·8	1·00
Gpi-1	7	12	21	17	14	64	59·4	2·25
Ldr-1	6	10	20	11	7	48	64·6	4·08
Pgm-1	5	11	22	16	15	64	59·4	2·25
Rb1Ald	6, 15	12	25	16	26	79	51·9	0·11
Rb163H	9, 19	23	18	30	19	90	46·7	0·40
Rb4Bnr	11, 13	14	27	25	24	90	42·2	2·18
Ca	15	15	12	11	17	55	58·2	1·47
E^{so}	8	6	11	16	17	50	46·0	0·32*
Os	8	9	8	15	18	50	54·0	0·32*
Ra	2	17	27	31	38	113	48·7	0·08
Re	11	37	44	56	43	180	44·4	2·22*
Sd	2	37	43	57	43	180	44·4	2·22
Sl^{gbH}	10	29	94	94	23	240	21·7	77·07
T	17	13	13	13	12	51	49·0	0·02
Tw	18	15	12	7	24	58	67·2	6·90
Va	3	17	8	16	9	50	52·0	0·08
W^v	5	14	8	13	22	57	63·2	3·95
Xt	13	13	14	13	14	54	50·0	0·00
Recessive markers	Chromosome	$Lop\,+$	$Lop\,m$	$++$	$+\,m$	Total	R.F. (%)	χ^2
a	2	15	16	13	26	70	41·4	2·06
b	4	24	11	13	7	55	43·6	0·89
c^{ch}	7	19	17	10	14	60	45·0	0·60
ep	19	13	10	14	14	51	47·1	0·18
fz	1	12	15	20	14	61	57·4	1·33
ln	1	9	14	12	16	51	50·9	0·02
ls	2	14	14	11	12	51	49·1	0·02
p	7	9	15	9	14	47	51·1	0·02
s	14	16	20	9	10	55	52·7	0·16
se	9	21	15	11	13	6	43·3	1·07
v	10	54	38	58	61	211	45·5	1·71
vt	11	15	13	7	16	51	39·2	2·37

* Significant deficiency of *Lop*.

χ^2 tests independent segregation of markers; d.f. = 1.

two of the observed offspring could be explained by the occurrence of only a single crossover. The remaining two (the 2 $Sl + v +$) would require a double crossover, and with any other order of loci the number of postulated double crossovers would be greater.

Among the offspring of this cross no animals were classified as being phenotypically *Sl gr*. However, since crossing-over between *Sl* and *gr* is expected, and since some of the reciprocal non-*Sl*, non-*gr* class were found, it seems likely that the *Sl gr* type occurred

but was not recognized, probably due to masking of gr by Sl^{gbH}. This does not alter the conclusion regarding order of loci, but means that recombination fractions involving gr can only validly be calculated using the non-Sl progeny.

There was good agreement between the linkage test data of Table 2 and the further data of Table 3. For recombination between v, gr and Sl the data of Table 3 showed concordance with published maps. The recombination percentages between Sl^{gbH} and

Table 3. *Offspring of four-point backcross of* Lop *with markers of chromosome 10*

Parental mating $+ + Sl^{gbH} Lop/vgr + + \times vgr + +/vgr + +$

Offspring

Sex of heterozygotes	Non-crossover				Crossovers				Total
	Sl Lop $+$ $+$	$+ +$ v gr	$+ Lop$ v gr	$Sl +$ $+ +$	Lop Sl $v +$	$+ +$ $+ gr$	$+ +$ $+ +$	$Sl +$ $v +$	
Female	26	13	3	7	3	4	6	2	64
Male	28	25	8	17	10	5	1	0	94

Recombination

	Female	R.F. $\pm$ S.E. (%)	Male	R.F. $\pm$ S.E. (%)
v-gr	4/26*	$15\cdot4 \pm 7\cdot1$	5/39*	$12\cdot8 \pm 5\cdot4$
gr-Sl^{gbH}	6/26*	$23\cdot1 \pm 8\cdot3$	1/39*	$2\cdot6 \pm 2\cdot5$
Sl^{gbH}-Lop	12/64	$18\cdot8 \pm 4\cdot9$	25/94	$26\cdot5 \pm 4\cdot6$
Sl^{gbH}-Lop†	7/35	$20\cdot0 \pm 6\cdot8$	45/205	$22\cdot0 \pm 2\cdot9$
v-Lop	23/64	$35\cdot9 \pm 6\cdot0$	41/94	$43\cdot6 \pm 5\cdot1$
v-Lop†	55/127	$43\cdot3 \pm 4\cdot4$	41/82	$50\cdot0 \pm 5\cdot5$

* Based on non-Sl offspring only (see text).

† Data of Table 2, analysed for sex of heterozygote.

Lop and between v and Lop were slightly greater in males than in females. The differences were not statistically significant but may nevertheless have been real, as a similar increased recombination in males is seen in the distal portion of mouse chromosome 15 (previously known as linkage group VI) (Dunn & Bennett, 1967; Robinson, 1972).

Thus the combined data give a recombination between Sl and Lop of 89/398 or $22\cdot4 \pm 2\cdot1 \%$ and the map order, based on earlier data for v, gr and Sl is

$$v-(13)-gr-(15)-Sl-(22)-Lop$$

The Lop locus must be very close to the loci of si, at and eb, but its position relative to them is unknown.

3. CONCLUSIONS

On the basis of its location Lop is clearly not allelic with several already mapped genes affecting the eye. These include aphakia (ak, chr. 19), blind (Bld, chr. 15), blind-sterile (bs, chr. 2), coloboma (Cm, chr. 2), Dickie's small eye (Dey, chr. 2), eye lens obsolescence (Elo, chr. 1), eye-ear reduction (Ie, chr. X), microphthalmia (mi, chr. 6), sightless (Sig, chr. 6) and vacuolated lens (vl, chr. 1). Although eye-blebs (eb) is located very close to Lop their effects seem dissimilar and allelism of these two genes seems unlikely. There remain several unlocated genes with effects on the eyes for which the possibility of allelism must be considered (Mouse News Letter, 1981).

These include several named genes for cataract, act, cac, Cad, Cts, Eo, nct and nuc, and

several unnamed cataracts found by Kratochvilova (1978) in a mutation experiment. There is a real possibility that *Lop* may later prove to be allelic with one or more of these genes. In particular some of Kratochvilova's mutants produced the type of total opacity seen here.

In any case the knowledge that there is a locus for a dominant cataract gene on chr. 10 may be valuable in mutation studies, and since *Lop* occupies almost an end position on the chromosome it may prove useful as a marker in linkage studies.

The mutant may further prove valuable in comparative studies of the causes of cataract in different species. Hereditary cataract, induced by X-rays, has been found in the rat (Leonard & Maisin, 1965). In the human McKusick (1975) lists numerous types of hereditary cataract and Kratochvilova & Ehling (1979) estimated that 3·1 % of dominant genetic diseases listed by McKusick involved cataract.

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