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A gene location for the inheritance of the Cataract Fraser (Cat^{Fr}) mouse congenital cataract

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Summary

Animal models which emulate defects similar to those in man are required for medical research. Many investigations on the cellular, developmental and molecular aspects of cataractogenesis use the cataract Fraser (Cat^{Fr}) mouse. This report shows that the Cat^{Fr} and *Lop* lens abnormalities are linked, and are probably allelic genes on chromosome 10. It also shows that the Cat^{Fr} gene is maintained on an inbred genetic background which differs from 79 other strains; it is proposed that this strain be named CAT.

1. Introduction

The Cataract Fraser (Cat^{Fr}) mouse develops a cataract prenatally, and the mutant gene was first described in strain A/J mice as an autosomal mutation known as 'shrivelled', showing intermediate dominance and high penetrance (Verrusio & Fraser, 1966). The lens abnormality begins to form before 14 days of intrauterine life. Initially the cell nuclei in the deep cortex become abnormally pyknotic; degeneration of cytoplasm and destruction of the lenticular nucleus follows. Two weeks post-natally, the anterior epithelia show unusual mitotic activity, with the formation of multiple cell layers that infiltrate into the abnormal fibres of the anterior cortex. Cells at the equatorial region retain their ability to differentiate until complete hydration of the lens occurs after one year of age. Inhibition of elongated older cells takes place by the breakdown of the nuclear membrane (Gellatt & Das, 1984). Specialized proteins called crystallins are associated with the lens structure (Papaconstantinou, 1967); the synthesis of these proteins in the lens is well documented (Harding & Dilley, 1976; Piatigorsky, 1981). Selective loss of a family of gene transcripts for specific crystallin synthesis in this mutant has been reported (Garber *et al.* 1985), but the chromosome carrying the gene responsible for the inheritance of the lens abnormality has not been identified. Earlier studies had failed to show any evidence of linkage with loci *a*, *b*, *c* or *N*, on chromosomes 2, 4, 7 and 15 respectively (Fraser & Schabtech, 1962).

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2. Materials and methods

The strain of mouse carrying the Cat^{Fr} mutation has been maintained for ?+26 generations by brother × sister inbreeding. Initial attempts to map the position of the Cat^{Fr} mutation were undertaken using inbred strains and mutant stocks with biochemical and morphological markers. F_1 progeny from a cross between the homozygous Cat^{Fr} mouse and marker stocks were backcrossed to the marker stock. Between 40 and 80 backcross progeny were typed for evidence of linkage. Details of the markers used and their appropriate chromosome are shown in Table 1. The biochemical phenotypes were determined by cellulose acetate electrophoresis using methods described by Hoffman (1984). In addition 332 outcrosses (Cat^{Fr}/Cat^{Fr} × Lop/Lop) F_1 × +/+) and 193 F_2 progeny from a cross between Cat^{Fr}/Cat^{Fr} and the mutant stock *Lop/Lop* were scored for the presence of a cataract. The *Lop* homozygous mouse develops a congenital cataract and *Lop* has been mapped to chromosome 10, approximately 22·4 cM distal to Sl^{gbH} (Lyon *et al.* 1981). Breeding pairs of *Lop* mice were kindly supplied by Dr Lyon (MRC Radiobiology Unit, Harwell, Oxon, U.K.).

In both backcrossed and F_2 animals, the progeny were scored for cataracts at 30–90 days of age. The mice were killed by cervical dislocation, the eyes removed and placed in cold isotonic saline. The lenses were removed and placed in individual wells of multiwell plastic plates containing isotonic saline. Lenses from non-cataractous MF1 mice of similar age were used as controls. The lenses were scored visually using a binocular microscope with direct overhead

Table 1. *Genotype of the* Cat^{Fr} *inbred strain, and loci used in the linkage studies*

Locus	Genotype[1]	Chromosome
A*	A	2
Adh-3e*	a	3
B*	B	4
C*	c	7
Car-1	a	3
Car-2*	b	3
Ce-2	a	17
D*	D	9
Es-1*	b	8
Es-3*	c	11
Got-1	a	19
Gpd-1*	a	4
Gpi-1*	b	7
Gpt-1	a	15
Hbb*	d	7
Idh-1	b	1
Ldr-1*	b	6
Mod-1*	b	9
Mod-2	b	7
Pgm-1*	b	5
Sep-1*	b	9
Sdh-1	a	2
Trf*	b	9

[1] All loci were homozygous.

* These loci together with *Mi*^{wh}, *Re*, *Xt* and *Lop* on chromosomes 6, 11, 13 and 10 respectively were tested for linkage with *Cat*^{Fr}.

illumination. As both *Cat*^{Fr} and *Lop* mutants are dominant and phenotypically similar, only 1/16 of the F_2 and 1/4 of the outcrossed ($Cat^{Fr}/Cat^{Fr} \times Lop/Lop$) $F_1 \times +/+$ progeny would be expected to be non-cataractous if the loci are unlinked. Samples of test and control eyes were coded and scored in random order to avoid bias.

Control, *Cat*^{Fr}/*Cat*^{Fr} and *Lop/Lop* lenses were examined histologically. The lenses were fixed in Carnoys fluid (3:1 ethanol/acetic acid), paraffin-embedded, serially sectioned, and stained with haematoxylin and eosin. Photographs of appropriate sections were taken with a Zeiss photomicroscope and Pan F film.

3. Results

None of the backcross progeny involving the markers shown in Table 1 showed a statistically significant deviation from that expected assuming independent segregation of the cataractous and marker loci. However, no-cataractous mice were observed among the 332 double backcrosses involving *Lop* and *Cat*^{Fr}, whereas 83 would have been expected had the two loci been unlinked. Nor were there any among the 193 F_2 progeny, where 12 would have been expected. A formula for calculating the 95% confidence interval for a binomial parameter p when the observed number x is 0, has been given by Green (1981). When account

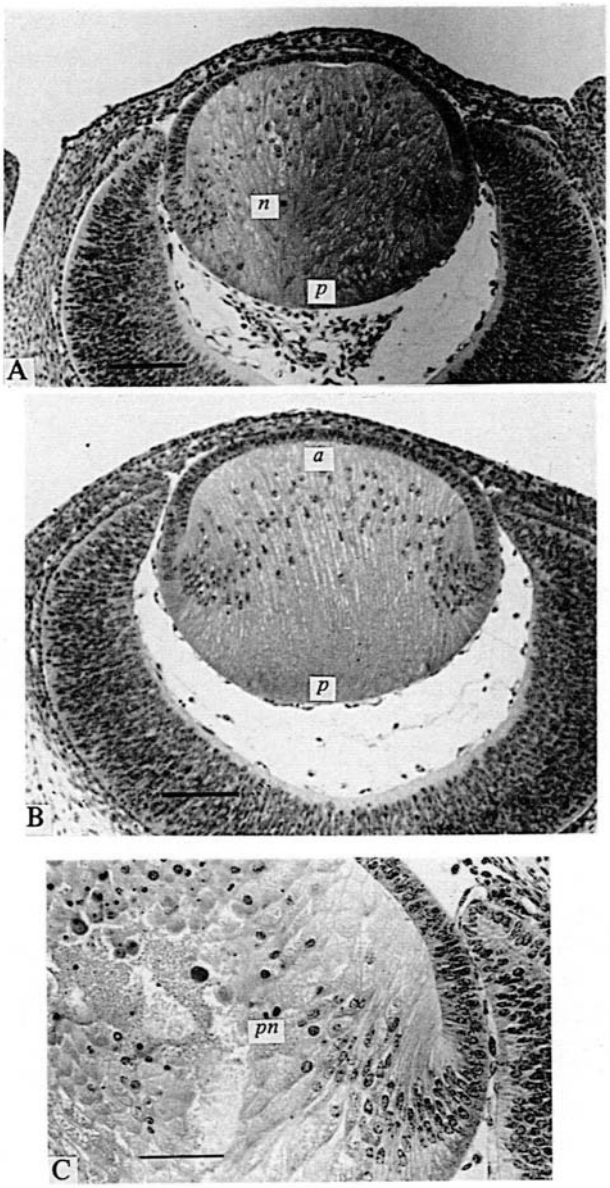

Fig. 1. (A) A section through a 15-day-gestation congenital cataractous (CAT) lens, with swollen club-shaped cortex fibre cells (*sfc*), with vacuoles (*v*) forming between the fibre cells. The mitotic bow area is disturbed and nuclei (*n*) are found in the anterior (*a*) and posterior (*p*) region of the lens. Bar graph = 80 μm. (B) A section through a 15-day-gestation non-cataractous DBA/2 lens. The fibres run the length of the lens from the anterior (*a*) to the posterior region. The germinative zone nuclei form a regular pattern. Bar graph = 80 μm. (C) A section through a 15-day-gestation homozygous *Lop* congenital cataractous lens. The cortex fibre cells are misshapen, the vacuolation and pyknotic nuclei (*pn*) are extremely obvious at this stage of embryonic development. Bar graph = 32 μm.

is taken of the impossibility of distinguishing *Lop*/*Cat*^{Fr} from *Lop*/+ or *Cat*^{Fr}/+ mice, it is concluded that there is a 95% probability that the interval from 0 to 0·0179 contains the true linkage parameter. In other words, there is a 95% probability that *Cat*^{Fr} and *Lop* are less than 1·79 cM apart on chromosome 10.

In view of their phenotypic similarities with regard

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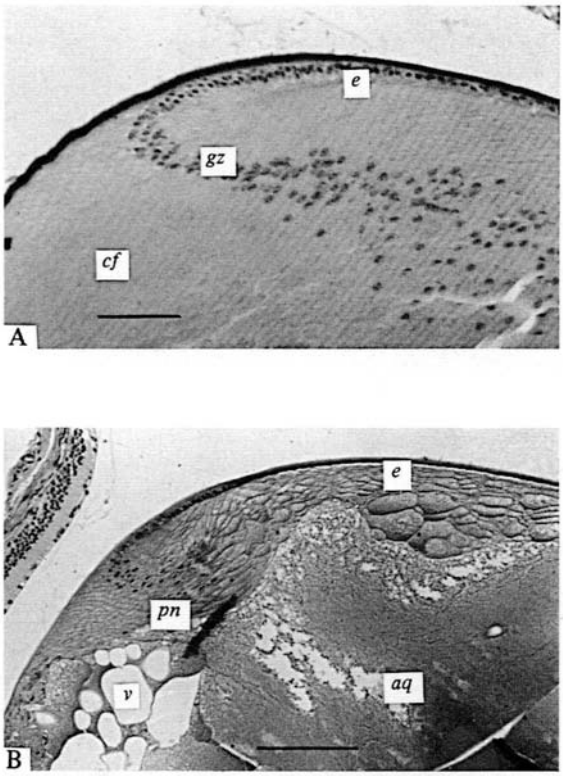

Fig. 2. A section of the lens of non-cataractous DBA/2 adult mouse. The mitotic bow region follows the normal pattern, the lens fibres running the length of the lens. The cortex fibres (*cf*) are aligned, with no vacuole disturbing them. The epithelial cells (*e*) form a monolayer. Bar graph = 50 μm. (B) A section through the lens of a congenital cataractous (CAT) adult mouse lens. The mitotic bow is disorganized, pyknotic nuclei (*pn*) and vacuoles (*v*) are evident. Large aqueous areas have formed following the degeneration of the cortex fibre cells. The lens epithelial cells (*e*) are still in a monolayer in the anterior area of the lens. Bar graph = 50 μm.

to the lens anomalies the data also suggest that *Cat*^{Fr} and *Lop* genes are allelic. Fig. 1A shows a section of the *Cat*^{Fr} embryonic lens at 15 days gestation. Swollen club-shaped cortex fibre cells (*sfc*) with vacuoles (*v*) forming between the cells are evident. The nuclei (*n*) of the fibre cells are disturbed and can be found both in the anterior (*a*) and posterior (*p*) region. Fig 1B shows the well-regulated pattern of the nuclei in the non-cataractous DBA/2 embryonic lens. There are no nuclei in the posterior region, the fibres running the length of the lens from the anterior (*a*) to the posterior (*p*). We have noted that the lens anomalies of the homozygous *Lop* embryonic lens at this same stage of development appear even more severe than those in the homozygous *Cat*^{Fr} lens. Fig. 1C shows that the nuclei of the displaced fibre cells are pyknotic (*pn*), and that the vacuolation and degeneration of the cortex fibres is already well advanced. Fig. 2A shows the mitotic bow region of a non-cataractous DBA/2 mouse lens at 60 days of age; the lens fibres are tightly regulated and run the length of the lens. The cortex fibres (*cf*) have no vacuoles disturbing them. The epithelial cells form a monolayer (*e*). The nuclei follow the normal pattern in the germinative zone (*gz*). Fig. 2B shows a similar regions in the homozygous *Cat*^{Fr} lens. The nuclei of the cortex fibres are pyknotic (*pn*) and vacuoles are evident (*v*). The swollen and misshaped cortex fibre cells are evident close to the germinative zone area. The centre of the lens has large aqueous areas (*aq*) and the monolayer of lens epithelial cells (*e*) can be seen in the anterior of the lens. In many adult congenital cataractous lenses there are areas where the epithelial cells form a multilayer, and the epithelial cells infiltrate into the cortex fibre area.

Studies to map the gene involved characterizing the mouse strain carrying the *Cat*^{Fr} gene with respect to 19 biochemical loci. Details of the genotype are given in Table 1. No segregation was observed at any of these loci, indicating that the strain carrying the *Cat*^{Fr} gene is fully inbred. However, the strain differs from A/J at *Gpd-1*, *Gpi-1*, *Idh-1*, *1Ldr-1* and *Pgm-1* loci, suggesting that it has been outcrossed at some time in its past history. It also differed from the strain-distribution pattern of 78 other inbred strains using data of Roderick, Staats & Womack (1979).

4. Discussion

Congenital and early developmental cataracts are common ocular abnormalities and represent an important visual impairment in childhood. Between 10 and 38% of all blindness in children is caused by developmental cataract. In a child with cataracts who is otherwise healthy, between 8·3 and 23% of cataracts are familial, autosomal dominance being the most frequent mode of inheritance (Nelson, 1984). The limited data on the causative mechanisms of human congenital cataract relate to the medical and ethical difficulties of work with human material. Therefore, mouse models are especially suited for genetic analysis of cataractogenesis because the litter size is large, the genetics well documented, and the lifespan relatively short. Recent publications list mouse mutants and the chromosomes associated with the lens defects which are presently available to study congenital and inherited cataracts (West & Fisher, 1985; Muggleton-Harris, 1986).

In this report we have shown that the *Cat*^{Fr} gene responsible for the inheritance of the cataract is located on chromosome 10 and is probably allelic with the *Lop* gene which was mapped to 22·4 cM distal to *Sl*^{gbH} (Lyon *et al.* 1981). The expression of the cataract was more severe in the embryonic *Lop* lens than in *Cat*^{Fr} (Muggleton-Harris & Higbee, 1986), but whether this is a true reflexion of differences between them or is due to differences in genetic background cannot be determined at this stage. As the *Cat*^{Fr} gene is carried on a unique inbred genetic background, in accordance with the rules of nomenclature of inbred strains of mice (Lyon, 1979) it is proposed that the strain should be designated CAT.

A brief description is:

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CAT: inbred F?+26. Albino: A, B, c, D. Origin: homozygous for the *Cat*^{Fr} mutation which arose in strain A mice before 1966. Outcrossed to an unknown laboratory stock, then inbred by A. L. Muggleton-Harris. All mice develop bilateral cataract prenatally.

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