

The murine cataractogenic mutation, Cat Fraser, segregates independently of the gamma crystallin genes

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Summary

The murine mutation, Cat Fraser (*Cat^{Fr}*), causes dominantly inherited ocular cataracts. Lenses of adult mice bearing this mutation contain reduced amounts of all seven γ -crystallin proteins and their corresponding transcripts. Levels of other lens proteins and transcripts appear normal and no extra-ocular effects of the mutation have been observed. The selective effect of this mutation on the γ -crystallins is consistent with the possibility that the site at which it occurs is involved in the coordinated regulation of the family of genes which encodes them. We have shown that several restriction fragment length polymorphisms in the γ -crystallin genes segregate independently of the *Cat^{Fr}* mutation. Therefore, despite its selective effect on the expression of the γ -crystallin genes, the mutation is not linked to them. This observation rules out the possibility that the mutation is in a *cis*-acting regulatory site.

1. Introduction

The crystallins are the major structural proteins of the vertebrate lens and are confined to that organ. Their presence in the lens is thought to be necessary for its transparency (Delaye & Tardieu, 1983). In mammals, they constitute 90% of total soluble lens protein and comprise three antigenically distinct classes: α , β and γ (Harding & Dilley, 1976).

In the mouse, there are seven different γ -crystallins (Garber & Gold, 1982) encoded by at least six distinct genes. Four of these genes have been characterized (Shinohara *et al.* 1982; Lok *et al.* 1984), and are clustered on chromosome 1 (Quinlan, 1987). Two other γ -crystallin genes have recently been identified (Quinlan, personal communication). Those γ -crystallin genes which have been sequenced exhibit 80–90% base homology between them (Shinohara *et al.* 1982; Breitman *et al.* 1984). In addition to nucleotide sequence homology, the genes also show structural similarities (Lok *et al.* 1984; Breitman *et al.* 1984). All that have been characterized have 3 exons, a small 9 basepair (bp) exon followed by two larger exons of about 250 bp each. The exons are separated by two introns, the first comprising 80–100 bp, the second, 800 to several thousand bp. There is little

homology between the intron sequences or the 3' flanking regions. In the 5' flanking region, however, there are highly conserved sequences upstream of the TATA box (Lok *et al.* 1984). In addition to the intraspecific homologies noted above, the γ -crystallin genes exhibit a high degree of interspecific homology (Schoenmakers *et al.* 1984; Meakin *et al.* 1985).

There is an interesting relationship between the structure of the γ -crystallin genes and of the proteins they encode (Blundell *et al.* 1981; Breitman *et al.* 1984). The tertiary structure of the γ -crystallins comprises four domains, each of which is in the shape of a Greek key. Two of these domains are encoded by the second exon and two by the third exon. The first (5') half of the third exon, which encodes the third domain, is the region of the gene which exhibits the least intergenic homology.

During development, the different classes of crystallins are to be found at a very characteristic and reproducible sequence of sites. The most obvious example of this is the fact that the crystallins as a whole are, with rare exceptions, confined to the lens. Lens tissue specific promoter activity has been shown for the α - and γ -crystallins in mice (Piatigorsky *et al.* 1984; Lok *et al.* 1985) and for the δ -crystallins in chickens (Piatigorsky *et al.* 1984). Moreover, different families of crystallins are differently distributed within

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the lens during development (Piatigorsky, 1981; Zigman, 1985). In the murine lens, the α -crystallins are detectable on the 12th day of development and are found thereafter in both the lens epithelium and the presumptive fibre cells. The β - and γ -crystallins appear half a day later and are both confined to presumptive fibre cells. This localization is maintained throughout pre- and post-natal lens development. It has also been demonstrated, both *in vitro* and *in vivo*, that the initiation of γ -crystallin synthesis coincides with fibre cell differentiation (McAvoy, 1978, 1980a). Recent evidence indicates that, for a given γ -crystallin gene, the amount of its transcript present in the lens rises and falls during ontogeny in a characteristic and reproducible manner and that this ontogenetic pattern of variation in amount of transcript is different for different genes (Murar-Orlando *et al.* 1987). This latter observation suggests that, in addition to the putative mechanisms which regulate this family as a whole, there are also specific mechanisms which regulate transcription of individual members, or subsets, of the γ -crystallin gene family. In summary, there is strong evidence that the family of genes which encodes the crystallins is under the control of complex regulatory mechanisms.

These mechanisms are not yet understood but whatever they are, there is a possibility that the murine mutation *Cat^{Fr}*, which causes dominantly inherited cataracts, interacts with them. This possibility is suggested by the selective effect of this mutation on γ -crystallin gene products. Lenses of adult mice homozygous for the mutation contain reduced amounts of all of the γ -crystallins and of their corresponding transcripts. The mutation does not affect the other crystallins nor does it appear to have any extra-ocular effects. Restriction analysis has not revealed any changes in the structure or methylation of the γ -crystallin genes in mice bearing the mutation (Garber *et al.* 1985). Although these observations are consistent with the hypothesis that the *Cat^{Fr}* mutation interferes with the regulation of the γ -crystallin gene family, they can be explained by other conceivable modes of action which are noted in the discussion.

As part of our attempt to determine whether or not this mutation impairs the regulation of the γ -crystallin genes, we wanted to find out if it is linked to them. Tight linkage of the *Cat^{Fr}* mutation to the γ -crystallin gene cluster would suggest that it is in a *cis*-acting regulatory site. Absence of linkage would lead to the conclusion either that the mutation is at a transacting regulatory site or that it is not at a regulatory site at all. Since DNA polymorphisms occur in the γ -crystallin genes (Lok *et al.* 1984) it is possible to observe the segregation of this gene cluster with respect to the *Cat^{Fr}* mutation by means of informative crosses. Here we reported the results of a linkage study which makes use of this technique.

2. Methods

Detection of RFLP markers

Genomic DNA was prepared from Swiss Webster (SW) mice (the inbred strain in which the *Cat^{Fr}* mutation is maintained) and from mice belonging to other inbred strains. The DNA was, in each case, isolated from liver and kidney tissue by phenol extraction (Maniatis *et al.* 1982). Samples of the resulting DNA were then digested with various restriction endonucleases, run on 1% agarose gels and blotted onto ICN Biotrans nylon membrane. Southern blotting (Southern, 1975) and hybridizations were performed as described in the protocols provided with the membranes. Hybridizations were performed in 50% formamide at 42° and blots were washed to a final stringency of $0.1 \times \text{SSC}$, 0.1% SDS at 50°. The blots were probed with nick translated γ 2-crystallin cDNA originally prepared from the plasmid pM γ 2Cr1 (Shinohara *et al.* 1982). Under the hybridization conditions used in these experiments, this probe will cross-hybridize to all other members of the γ -crystallin family. This allows the detection, with a single probe, of polymorphisms in any of the γ -crystallin genes. The polymorphisms detected with this probe were assigned to specific γ -crystallin genes by comparing them to those detected with probes specific for single members of the γ -crystallin family. The preparation of these probes from the less homologous 5' end of the second exon has previously been described (Murar-Orlando *et al.* 1987).

Pedigree analysis

Mice from strains exhibiting γ -crystallin polymorphisms were crossed with SW mice carrying the *Cat^{Fr}* mutation. The F₁ offspring of these crosses, heterozygous for both the *Cat^{Fr}* mutation and the RFLP phenotype were backcrossed to the wild-type parent. The resulting backcross progeny (F₂'s) were then screened visually for the cataractic phenotype and, by Southern blotting, for their RFLP phenotype. F₂ animals without cataracts, carrying the SW DNA polymorphism and F₂ animals with cataracts not carrying the SW DNA polymorphism were scored as recombinants. All other F₂ animals were scored as non-recombinants.

3. Results

Of the strains of mice tested for polymorphisms in the γ -crystallin genes, polymorphisms (different from those present in Swiss Webster) were detected in two, namely CBA/J and C57BL. Polymorphisms were detected in Taq I and Bam HI digests of DNA prepared from C57BL mice and in Bgl II digests of DNA prepared from CBA/J mice. These polymorphisms are shown and described in Fig. 1. The polymorphisms detected in C57BL DNA were subse-

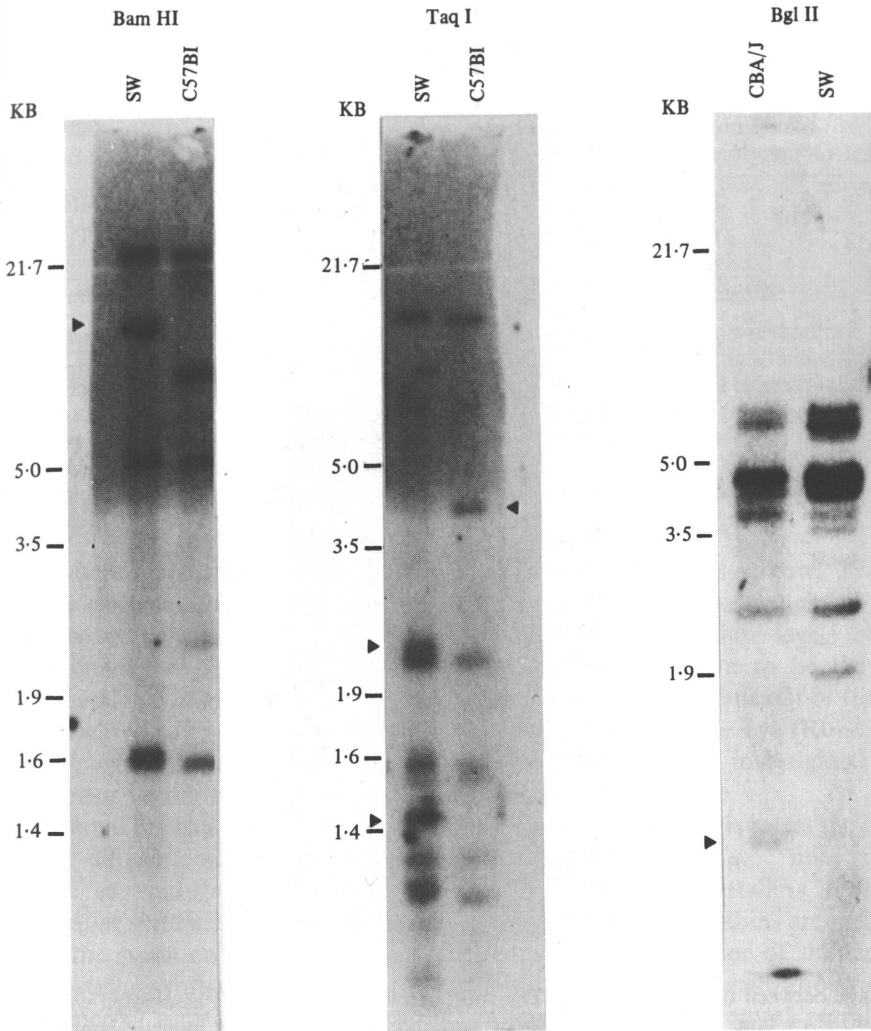


Fig. 1. DNA polymorphic markers used in linkage analysis of SW \times C57BL pedigrees. SW is Swiss Webster, the mouse strain the *Cat^{Fr}* mutation is maintained in. For all blots, 8 micrograms of liver DNA were digested with excess enzyme and, after electrophoresis, blotted onto Biotrans (ICN) nylon membrane. Nick translated

γ 2-crystallin cDNA probes were hybridized in 50% formamide at 42 °C. The conditions of final washing were 0.1 \times SSC, 0.1% SDS at 50° for 30 minutes. Arrows indicate the polymorphic bands used as markers in the backcrosses.

quently identified as being in two different γ -crystallin genes, γ 2 (detected with Bam HI) and γ 4 (detected with Taq I) (Paul Quinlan and L.-C. Tsui, personal communication). Both of these genes have been mapped to the γ -crystallin cluster on chromosome 1 (Quinlan, unpublished data). The CBA/J polymorphism is more complex than those detected in C57BL and may be the result of changes in more than one γ -crystallin gene. The band at approximately 2.0 kb (see Fig. 1) may correspond to a polymorphism previously detected in the γ 1 gene (Paul Quinlan & L.-C. Tsui, personal communication).

Both C57BL and CBA/J males were crossed with female Swiss Webster mice carrying the *Cat^{Fr}* mutation. Offspring of this cross (F_1) were backcrossed with the normal male parent and the progeny (F_2) scored both for cataracts and for the presence or absence of the polymorphic markers (Table 1). Of a total of 56 F_2 mice screened, 31 showed a recombinant phenotype and 25 a parental phenotype (Table 2).

This result is not significantly different (at 95%) from the 1:1 ratio which would result from independent segregation of unlinked markers. The *Cat^{Fr}* mutation is therefore not linked to the γ -crystallin genes.

4. Discussion

The results indicate that the *Cat^{Fr}* mutation is not linked to the γ -crystallin gene cluster. This follows from the fact that the polymorphisms which were shown to segregate independently of it in this study can be confidently assigned to members of this tightly linked cluster on chromosome 1. In any case, it is now known that there are no murine γ -crystallin genes that are unlinked to this cluster. It can therefore be said that the *Cat^{Fr}* mutation is not linked to any member of the family of genes whose products are affected by the mutation. Hence, the mutation is either non-regulatory or it is at a site involved in the synthesis of some trans-acting regulatory factor.

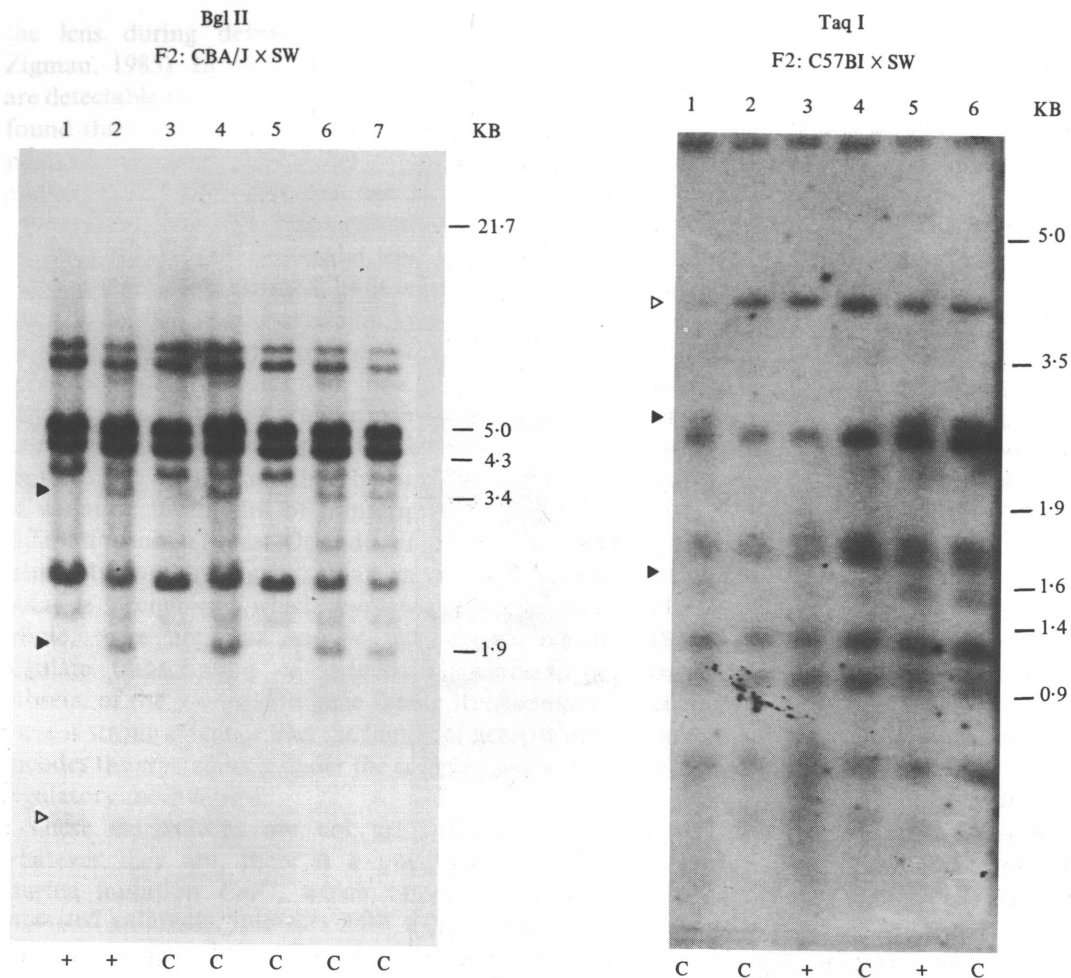


Fig. 2(a) Southern blot analysis of linkage between the γ -crystallin genes and the Cat^{Fr} mutation. Each lane represents DNA prepared from the backcross progeny (F_2) of CBA/J x SW. The phenotype of the F_2 , with respect to the Cat^{Fr} mutation, is given at the bottom of each lane: C is cataractic, + is wild type. The DNA was digested with BglII, blotted onto ICN Biotrans nylon membrane and probed with nick translated γ -crystallin

cDNA. The solid arrows indicate bands associated with the cataractic parental, the open arrows indicate bands associated with the wild-type parental. Lanes 2, 3 and 5 therefore demonstrate recombination between the two markers. (b) As above except the progeny are from a C57BL x SW cross and the DNA was digested with TaqI. Lanes 2, 4 and 5 demonstrate recombination.

Table 1. Results of crosses

Parental genotypes Swiss Webster x C57BL c/c 1/1 +/+ 2/2 F ₁ genotype: c/+ 1/2 Backcross to C57BL			Parental genotypes Swiss Webster x CBA/J c/c 1/1 +/+ 3/3 F ₁ genotype: c/+ 1/3 Backcross to CBA/J		
Genotype	Type of segregant	No. of animals	Genotype	Type of segregant	No. of animals
+/+ 2/2	Non-recomb.	2	+/+ 3/3	Non-recomb.	8
c/+ 1/2	Non-recomb.	4	c/+ 1/3	Non-recomb.	11
+ , + 1/2	Recomb.	4	+ /+ 1/3	Recomb.	12
c/+ 2/2	Recomb.	3	c/+ 3/3	Recomb.	12

+ : Wild-type allele at Cat^{Fr} locus. c: Mutant allele at Cat^{Fr} locus. +/+ : Wild-type homozygote without cataract. +/c: Heterozygote with cataract. c/c: Homozygote with cataract. 1: Band pattern of DNA from SW mouse (bearing Cat^{Fr} mutation) when restricted with BamHI, TaqI or Bgl II. 2: Band pattern of DNA from C57BL mouse when restricted with TaqI or BamHI. 3: Band pattern of DNA from CBA/J mouse when restricted with Bgl II.

Table 2. Pooled results of crosses

F ² with Parental cross	Polymorphism	F ₂ No. tested	F ₂ No. recombinant	Recombinant phenotype (%)
C57BL × SW	Taq I, BamHI	13	7	53.8
CBA/J × SW	Bgl II	43	24	55.8
	Total	56	31	55.3

Results of analysis of linkage between the *Cat^{Fr}* mutation and the γ -crystallin genes. SW is the mouse strain in which the *Cat^{Fr}* mutation is maintained. Restriction enzymes listed under polymorphism heading are those used to generate polymorphisms used as markers in Southern blots. In the C57BL × SW pedigree the polymorphisms detected with both enzymes were tested independently and gave identical results. A 55.3% frequency of recombinant phenotypes is not significantly different from the 50% recombination frequency expected if the markers were not linked.

It is probable that such trans-acting factors exist. Their existence is suggested by reports of lens specific promoters associated with crystallin gene expression (Piatigorsky *et al.* 1984; Lok *et al.* 1985). Crystallin synthesis in cultured chicken lens cells has been shown to be activated by lentropin, a hormone-like substance which can be extracted from the vitreous humour posterior to the lens (Beebe *et al.* 1980). Studies on explanted rat lens epithelium have demonstrated that some diffusible substance, released by neural retina tissue can induce epithelial cell differentiation and crystallin synthesis *in vitro* (McAvoy, 1980b).

If the mutation is non-regulatory, the reduction of γ -crystallin synthesis may be a secondary result of the presence of a cataract. In this respect, an important question is whether, in the course of development, the cataract appears before or after the initiation of γ -crystallin synthesis. Several cytological studies of *Cat^{Fr}* mice report that morphological abnormalities in their lens cells are detectable at approximately the same time as the γ -crystallins appear (about 12 days into development) (Zwann & Williams, 1968, 1969; Konyukhov & Kolesova, 1975). These observations are neutral with respect to the question of whether the cataract causes the reduction in γ -crystallins or whether the converse is true. In one cytologic study, however, the mutation was reported to be causing definite aberrations in the cells forming the presumptive lens vesicle at only 9 days into development, well before the γ -crystallins appear (Hamai & Kuwabara, 1975). This observation, if correct, would strongly support the hypothesis that the effects of the mutation on γ -crystallin synthesis are secondary results of an earlier developmental abnormality.

This possibility is also supported by the fact that there are other cataractogenic mutations in mice which selectively affect the γ -crystallin proteins. The mutation, *Elo* (eye lens obsolescence) (Oda *et al.* 1980a) appears to affect γ -crystallin synthesis in mice (Oda *et al.* 1980b; Watanabe *et al.* 1980). However, the mechanism by which it does so is unknown and the mutation appears to be linked to the γ -crystallin

genes (Skow, 1982; Quinlan, 1987). The Nakano mouse, which has a recessively inherited cataract, also has low levels of the γ -crystallins. This has been shown to be the result of selective leakage of the proteins out of the lens and into the aqueous humour of the eye (Russell *et al.* 1982). This mechanism has been investigated and ruled out for *Cat^{Fr}* (Garber, 1984).

This raises the question of how the cataract present in *Cat^{Fr}* mice causes the selective reduction in γ -crystallins, if this is indeed the case. Because γ -crystallins are present in the fibre cells which are in the interior of the lens (McAvoy, 1978; Layden, 1985), the localization of cell degradation by the cataract to the central region of the lens (Zwann & Williams, 1968) could result in the selective effects on the γ -crystallins. It is also possible that there are subsets of fibre cells responsible for producing γ -crystallins. A selective effect of the mutation on such cells could result in the observed phenotype. These possibilities are currently being investigated by *in situ* hybridization.

Note added in proof: The observations reported in this paper are consistent with the conclusion of Muggleton-Harris *et al.* (*Genetical Research* (1987), 49:235–238), which is that the *Cat^{Fr}* mutation is closely linked to, or allelic to, the *Lop* locus on chromosome 10.

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