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Genetic localization and phenotypic expression of X-linked cataract (*Xcat*) in *Mus musculus*

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Summary

Linkage data relative to the markers tabby and glucose-6-phosphate dehydrogenase are presented to locate X-linked cataract (*Xcat*) in the distal portion of the mouse X-chromosome between jimpy and hypophosphatemia. The human X-linked cataract-dental syndrome, Nance–Horan Syndrome, also maps closely to human hypophosphatemia and would suggest homology between mouse *Xcat* and human Nance–Horan Syndrome genes. In hemizygous males and homozygous females penetrance is complete with only slight variation in the degree of expression. Phenotypic expression in *Xcat* heterozygous females ranges from totally clear to totally opaque lenses. The phenotypic expression between the two lenses of a heterozygous individual could also vary between totally clear and totally opaque lenses. However, a correlation in the degree of expression between the eyes of an individual was observed. A variegated pattern of lens opacity was evident in female heterozygotes. Based on these observations, the site of gene action for the *Xcat* locus is suggested to be endogenous to the lens cells and the precursor cell population of the lens is concluded to be small. The identification of an X-linked cataract locus is an important contribution to the estimate of the number of mutable loci resulting in cataract, an estimate required so that dominant cataract mutagenesis results may be expressed on a per locus basis. The *Xcat* mutation may be a useful marker for a distal region of the mouse X-chromosome which is relatively sparsely marked and the X-linked cataract mutation may be employed in gene expression and lens development studies.

1. Introduction

The dominant cataract mutation test has been developed as a relatively efficient method to systematically screen for dominant mutations affecting a specific phenotype in the mouse (Kratochvilova, 1981; Kratochvilova & Ehling, 1979). The lens was chosen as the indicator phenotype since live animals could be screened for phenotypic variants indicative of presumed cataract mutations and the recovered phenotypic variants would be available for breeding test following examination. The method has proven to be useful as evidenced by the germ cell mutagenicity results which have been generated recently for radiation and ethylnitrosourea (Ehling *et al.* 1982; Favor, 1983, 1986; Favor *et al.* 1987, 1988; Graw *et al.* 1986; Kratochvilova, 1981; Kratochvilova & Ehling, 1979). It is of utmost importance that the number of loci screened for dominant cataract mutations be identified so that the observed mutation rates may be expressed per locus. Further, the identification of dominant cataract loci may provide additional genetic markers in the mouse and such genetic variants may be of use in future mammalian genetic studies.

It is fortuitous that the lens was chosen as the indicator phenotype with which to screen for dominant mutations. The mutagenicity experiments carried out have resulted in a collection of mutations affecting lens and eye development. The development of the eye has been one of the organs of choice in classical organogenesis studies. In contrast to the manipulative procedures possible for chick and frog developmental studies, the study of development in placental mammals has relied by necessity on genetic variants affecting normal differentiation. Thus, our collection of dominant cataract mutations may prove useful for studies of eye development in the mouse.

Here we present the genetic linkage results to identify and localize X-linked cataract, *Xcat*, in the distal portion of the X-chromosome. Phenotypic expression studies have been carried out and are useful to determine the site of gene action as well as to characterize the development of the mammalian lens.

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2. Methods

Original crosses and radiation exposure resulting in the induction of the original cataract mutation have been previously described (Favor *et al.* 1987). Routine ophthalmological examinations of mice were at 48x magnification with slit lamp illumination employing a Zeiss 30 SL/M microscope. Pupils were dilated with 1 % atropine solution. For the gene expression studies, the degree of lens opacity was estimated subjectively by visual examination.

Linkage studies were carried out with the standard tabby allele obtained from Harwell and maintained at Neuherberg, which was kindly provided by Professor J. H. Schröder. The glucose-6-phosphate dehydrogenase enzyme activity mutation was recovered in an ethylnitrosourea mutagenesis experiment carried out at Neuherberg (Charles & Pretsch, 1987). Animals were classified for glucose-6-phosphate dehydrogenase enzyme activity of erythrocytes following previously described procedures (Charles & Pretsch, 1987; Pretsch *et al.* 1988).

3. Results

(i) *Identification of an X-linked cataract mutation*

The original mutation was recovered in an F_1 female descendent from the cross T-stock female × DBA/2 male in which the DBA/2 male parent was irradiated with 3+3 Gy and offspring were systematically screened for newly induced dominant cataract as well as forward and reverse specific locus mutations. The original mutation carrier expressed a unilateral posterior cortical opacity and was designated D-454 (Favor *et al.* 1987). Table 1 presents the transmission results of the genetic confirmation cross for the original presumed mutation. It was noted that the resulting male carriers expressed a bilateral total opacity whereas the phenotypic expression in those females classified as carriers varied from total lens opacity to cortical opacity. Further, some females were classified as wild type although they expressed minor lens opacities. It should be noted from the transmission results of the original mutant female crossed to a wild-type male that in male offspring a 1:1 ratio of mutant to wild-type was observed, while in female offspring fewer mutant carriers were classified than the expected 50%. Recovered mutations are routinely maintained in our laboratory by male to male transmission. Results of the succeeding generation in which a male mutation carrier was mated to a wild-type female are given in Table 2. All male offspring were wild-type and all female offspring expressed a lens opacity ranging from a total lens opacity as seen in male carriers to a minor lens opacity. These results indicate X-chromosome linkage for the induced cataract mutation and explain the discrepancies in phenotypic expression between hemizygous males and heterozygous female carriers as well as the variability of phenotypic expression among heterozygous female carriers.

Table 1. *Classification of offspring resulting from the cross of the presumed cataract mutation female with a wild-type male*

	Phenotype	
Offspring	Cataractous	Wild type
Male	15	16
Female	9	16

Table 2. *Classification of offspring resulting from the cross of a male cataract mutation carrier with a wild-type female*

	Phenotype	
Offspring	Cataractous	Wild type
Male	0	34
Female	27	0

(ii) *Localization of the cataract mutation on the X-chromosome*

Two X-linked genetic variants, tabby (*Ta*) and glucose-6-phosphate dehydrogenase (*G6pd*), were utilized to locate the X-linked cataract gene. It was first determined that cataract carriers expressed normal *G6pd* enzyme activity and *G6pd* deficiency mutation carriers did not result in cataractous lenses (data to be published elsewhere). Tables 3 and 4 give the results of the three point cross. Discrepancies in the alternative haplotypes for the various crossover or non-crossover classes were observed. In females this is likely due to misclassification, while in males it is most likely due to differential viability of the mutation carriers. The loci for X-linked cataract and *G6pd* are shown to flank the tabby locus. Since *G6pd* was previously shown to be proximal to the tabby locus (Peters *et al.* 1988), this indicates the cataract locus to be distal to tabby. The estimated genetic distance between tabby and the cataract locus was $21{\cdot}0 \pm 1{\cdot}6$ for the cross in which the *G6pd* variant was in repulsion to the cataract and tabby mutations, and $21{\cdot}4 \pm 2{\cdot}8$ for the cross in which mutants for all three loci were in coupling. Together with previously obtained data for the two-point cross with tabby and the cataract mutation (Favor & Pretsch, 1987), the genetic distance was estimated to be $21{\cdot}8 \pm 1{\cdot}1$. This locates the cataract locus to be between jimpy and hypophosphatemia in the distal portion of the X-chromosome based on the most recent mouse genetic map (Davisson & Roderick, 1989).

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FIGURE 1. Photomicrograph of an eye from an *Xcat* hemizygote male (48 x magnification, slit lamp illumination). The two vertical red-brown bands are iris tissue. The lens is totally opaque. The spherical structures within the lens are lenticular vacuoles associated with the opacity.

Table 3. *Linkage analysis of the X-linked cataract mutation in the cross:*

$$\frac{Xcat\text{–}Ta\text{–}+}{+\text{–}+\text{–}G6pd} \times \frac{+\text{–}+\text{–}+}{\longleftarrow}$$

Class	Maternal meiotic product	Offspring Male	Offspring Female	Total (%)
Non-CO	*Xcat–Ta–+*	58	118	
	+–+–G6pd	129	145	450
Single CO-I	*Xcat–Ta–G6pd*	9	17	
	+–+–+	17	30	73 (11·0 ± 1·2)
Single CO-II	*+–Ta–+*	20	48	
	Xcat–+–G6pd	38	27	133 (20·1 ± 1·6)
Double CO	*Xcat–+–+*	1	2	
	+–Ta–G6pd	1	2	6 (0·9 ± 0·4)

Table 4. *Linkage analysis of the X-linked cataract mutation in the cross:*

$$\frac{Xcat\text{–}Ta\text{–}G6pd}{+\text{–}+\text{–}+} \times \frac{+\text{–}+\text{–}+}{\longleftarrow}$$

Class	Maternal meiotic product	Offspring Male	Offspring Female	Total (%)
Non-CO	*Xcat–Ta–G6pd*	7	36	
	+–+–+	47	47	137
Single CO-I	*Xcat–Ta–+*	7	17	
	+–+–G6pd	2	2	28 (13·3 ± 2·3)
Single CO-II	*+–Ta–G6pd*	6	9	
	Xcat–+–+	12	12	39 (18·6 ± 2·7)
Double CO	*Xcat–+–G6pd*	0	0	
	+–Ta–+	1	5	6 (2·9 ± 1·1)

Two previously described X-linked mutations have been shown to affect the eye; eye-ear reduction, *Ie*, and bare patches, *Bpa*. The presently described cataract mutation has a phenotype distinct from either *Ie* or *Bpa*. Further, both *Ie* and *Bpa* are located proximally to tabby. Thus, it may be concluded that the presently described dominant cataract mutation is a newly described locus and has been designated *Xcat*, X-linked cataract.

(iii) *Phenotypic expression of the* Xcat *mutation*

Hemizygous males as well as homozygous and heterozygous female *Xcat* carriers were classified as to the degree of lens opacity in the right and left eye. Tables 5, 6 and 7 indicate the number of animals observed to express the indicated degree of lens opacity in the right and left eye. The phenotypes for hemizygous males and homozygous females were similar, expressed as a total lens opacity in both eyes with only minor deviation in the degree of opacity (Tables 5 and 6). The phenotype is depicted in Fig. 1.

Table 5. *Phenotypic expression of* Xcat *as measured by the degree of lens opacity in hemizygous male mice. Results indicate the number of animals expressing the indicated degree of lens opacity in the right and left eye*

		Right eye 0%	25%	50%	75%	100%
Left eye	0%	—	—	—	—	—
	25%	—	—	—	—	—
	50%	—	—	1	—	—
	75%	—	—	1	41	4
	100%	—	—	—	3	231

Table 6. *Phenotypic expression of* Xcat *as measured by the degree of lens opacity in homozygous female mice. Results indicate the number of animals expressing the indicated degree of lens opacity in the right and left eye*

		Right eye 0%	25%	50%	75%	100%
Left eye	0%	—	—	—	—	—
	25%	—	—	—	—	—
	50%	—	—	—	—	—
	75%	—	—	—	4	1
	100%	—	—	—	3	115

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Table 7. *Phenotypic expression of* Xcat *as measured by the degree of lens opacity in heterozygous female mice. Results indicate the number of animals expressing the indicated degree of lens opacity in the right and left eye*

		Right eye				
		0%	25%	50%	75%	100%
Left eye	0%	25	7	2	2	3
	25%	6	22	5	5	5
	50%	2	7	33	10	2
	75%	1	3	7	17	5
	100%	1	2	4	3	17

All individuals expressed an altered phenotype indicating complete penetrance.

The variability of expression in heterozygous female carriers is indicated in Table 7. Female carriers were observed with phenotypes ranging from no noticeable opacities to totally opaque lenses as expressed in hemizygous males and homozygous females. Further, the variability of phenotypic expression between the two eyes of an individual heterozygous female was observed to be as extreme, although there is a clear tendency for a correlated expression of phenotype in the two eyes of an individual. For heterozygous females in which an intermediate degree of lens opacity expression was observed, the opacity was manifested variably. Lenses were observed in which the opacity was confined to particular regions such as the cortex, nucleus or patches throughout the lens. Further, some lenses were observed in which there was an intermediate degree of homogeneous lens opacity.

4. Discussion

The *Xcat* mutation was recovered in an irradiation mutagenesis experiment. The fact that the parental male population was irradiated and the original mutation occurred in an F_1 female is consistent with the assumption that the *Xcat* mutation was induced by radiation exposure.

We have employed the dominant cataract mutation test procedures as a method to systematically screen for dominant mutations in mice (Kratochvilova & Ehling, 1979; Ehling *et al.* 1982; Favor, 1983; Kratochvilova, 1981). At present the number of mutable loci resulting in dominant cataract is not known and has been estimated to be 30 based upon the number of phenotypically distinct mutations known in man (Ehling, 1985). This is a useful first estimate. However, dominant cataract mutations of similar phenotype were shown to be non-allelic as well as the converse, dominant cataract mutations with different phenotypes were shown to be allelic (J. Kratochvilova, personal communication; Kratochvilova & Favor, 1988). This knowledge underlines the importance that the estimate of the number of mutable cataract loci screened in the dominant cataract mutation test should be based upon the identification of genes through linkage studies. In the most recent list of known mutations in the mouse (Green, 1989) a total of 62 mutations have been identified which affect the eye. Of these 62, 26 mutations have been mapped and 5 of the mapped mutations are dominant mutations which result in cataract or abnormal lens development. Recently Kratochvilova & Favor (1988) have reported initial results of allelism test of dominant cataract mutations recovered in radiation mutagenesis experiments. To date 15 mutations have been analysed and 7 different loci identified (J. Kratochvilova, personal communication). The recovered mutations will be valuable genetic variants with which to identify dominant cataract loci in the mouse. More than 85 independent mutations have been recovered in a series of mutagenesis experiments and have been maintained in our laboratory (Kratochvilova & Ehling 1979; Kratochvilova, 1981; Ehling *et al.* 1982; Favor, 1983, 1986; Graw *et al.* 1986; Favor *et al.* 1987, 1988; Kratochvilova *et al.* 1988).

In humans three X-linked genetic disorders associated with cataract have been listed although it is not certain if they represent three distinct loci (McKusick, 1989). Linkage results have located the human X-linked cataract-dental syndrome (Nance–Horan Syndrome) close to the human X-linked hypophosphatemia locus (D. Stambolian, personal communication). Given the evolutionary conservation of X-linkage in mammals, there may be homology between the mouse *Xcat* gene shown to map close to the mouse X-linked hypophosphatemia locus and the human locus for Nance–Horan Syndrome. Thus, the *Xcat* mutation may be a mouse model for this human genetic defect.

The use of X-linked genetic variants to study gene regulation as well as the process of tissue and organ differentiation in mammals is most useful. Although the analysis of gene expression for the *Xcat* mutation as presented here is based on only a crude estimation of the degree of lens opacity, the results are of interest. The variable expression in heterozygous females, especially the patchy distribution of opacity throughout the lens suggests variegation. This observation would imply the site of *Xcat* gene action to be endogenous to the lens cells. The variability in the level of *Xcat* expression in the lenses of heterozygous females depends upon the sampling of a number of lens precursor cells from a mosaic cell population in which either the X-chromosome bearing the mutation or the wild-type allele is active. Deviation from the expected 50% cells in which the mutant bearing X-chromosome is active would be due to sampling variance. The fact that the phenotype in the lenses of heterozygotes could vary to such extremes would

suggest high sampling variance due to a rather small population of precursor cells which eventually differentiate into the lens. In contrast, for a tissue in which a very large precursor cell population exists, sampling variance would be low and an intermediate phenotype with little variation would be expected. This is what is observed for *G6pd* activity in erythrocytes (Pretsch *et al.* 1988). We have independently concluded a small lens precursor cell population based on the observation of an all or none lens cataract phenotype in mutation mosaics (Favor *et al.* 1990). The strong correlation of lens cataract phenotype between the eyes of a heterozygote individual is surprising. The lens placode develops from epithelial cells following induction by the optical vesicle on day 10 of gestation. Since the development of the two lens placodes is an independent process on contralateral sides, the observation of a strong correlation of phenotype in the two lenses suggests a common cell pool for the epithelium in the anterior region of the embryo or a non-random probability of X-chromosome inactivation in the cell pools of the two lenses. Finally, although the induction of the lens placode involves a considerable number of cells, the small number of lens precursor cells as deduced from the phenotypic expression studies may indicate the population of epithelial cells induced to develop into the lens placode to be descendent from a small precursor cell pool. This is likely due to the local clonal expansion of epithelial cells prior to induction of the lens placode cell population. The interaction between local clonal growth and cell intermingling and the resulting local mosaicism have been previously discussed (West, 1978).

In conclusion, the recovery of an X-linked cataract mutation has been useful in studying X-linked gene expression as well as the developmental biology of the mammalian lens. The identification of an additional locus resulting in dominant cataract is an important step in determining the number of loci screened in the dominant cataract mutation test. That the *Xcat* locus maps to a region of the mouse X-chromosome with relatively few mapped loci is useful in genetically marking this region. Finally, since the *Xcat* locus is a distant distal genetic marker from the X-chromosome inactivation center, *Xce*, with a relatively simple phenotype, it may be a useful locus with which to study X-chromosome inactivation.

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