

Allelism tests of 15 dominant cataract mutations in mice

J. KRATOCHVILOVA* AND J. FAVOR

GSF-Institut für Säugetiergenetik, D-8042 Neuherberg, Federal Republic of Germany

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Summary

Fifteen autosomal dominant mutations that cause cataract of lenses in mice were tested for allelism. The outcrosses of double mutants revealed three allelism groups, consisting of 5, 4 and 2 mutations as well as 4 mutations which segregated independently. The results indicated 7 different cataract loci in the sample of 15 mutations. The biomicroscopic examination of the eyes showed that phenotypically similar as well as very distinct cataract mutations can be alleles of the same gene. Conversely, phenotypically similar mutations were shown to be non-allelic.

1. Introduction

Numerous mouse mutants with cataract of the lens have been recovered in Neuherberg by the systematic examination of F₁ offspring after parental mutagenic treatment (Kratochvilova, 1981; Ehling *et al.* 1982; Graw *et al.* 1986; Favor, 1983, 1984, 1986; Favor *et al.* 1987, 1989). In breeding tests it was demonstrated that the mutant phenotypes were caused by single dominant genes. Results from these experiments have been employed for the direct estimation of human genetic risk in the first generation after mutagenic treatment (Ehling, 1982, 1983, 1988). The cataract mutations were analysed further for penetrance, fertility and viability. Previous publications reported data obtained from samples of 17 and 20 dominant cataract mutations induced by ethylnitrosourea or radiation (Favor, 1984; Kratochvilova & Favor, 1988).

It is of particular interest to identify the loci at which the cataract mutations occurred. Confronted with such a large sample of recovered mutations a first step would be the testing for allelism. At present, tests with 15 dominant autosomal cataract mutations have been completed. The analyses included 9 mutations from the sample of 20 radiation-induced mutations previously studied (Kratochvilova & Favor, 1988), 1 additional mutation found after spermatogonial exposure to 2 + 2 Gy, 1 mutation found in a procarbazine experiment (Kratochvilova *et al.* 1988) and 4 mutations that arose spontaneously in breeding stocks or

control groups. All selected mutations showed full penetrance and no effects on viability and fertility of heterozygotes. Therefore, these mutations were readily suitable for conducting allelism tests.

2. Materials and methods

The mutations tested were designated provisionally with gene symbols based on their phenotypic characteristics. The origin and the phenotype of the dominant cataract mutants are summarized in Table 1. Congenic mutant lines were established by at least ten backcross generations to strain 102/El. Presumed double heterozygotes were obtained by crosses of homozygotes from two different mutant lines. When homozygotes were lethal, heterozygotes were used. The presumed double heterozygotes (A +/+B) were outcrossed to normal mice (+ +/+ +). A ratio of 3:1 for cataractous (A +/+ +, +B/+ +, A +/+B) and normal (+ +/+ +) or a ratio of 1:1:1:1 if the four phenotypes can be distinguished would indicate non-allelism and non-linked genes. To test the data for the independent assortment of non-alleles the χ^2 test was used. The outcrosses of presumed double heterozygotes which produced only cataractous offspring indicated mutations at the same locus or at two closely linked loci. The minimal linkage distance that could be excluded between two different loci was calculated from n equal to the number of offspring classified and the binomial probability x equal to zero at 0.05 significance level (Green, 1981).

The phenotype of the offspring was classified when they were 3 wk old. The eyes were examined with the

* Corresponding author.

Table 1. Origin and phenotype of the cataract mutants

Provisional gene symbol		Treatment of the male parent	Phenotype	
Now	Former		Heterozygotes	Homozygotes
<i>Cat-2^{nz}</i>	<i>Nzc</i> (6)*	Gamma-rays 4.55 + 4.55 Gy	Nuclear and zonular opacity	Nuclear opacity
<i>Cat-2^t</i>	<i>R-324</i> (2)	X-rays 5.10 + 5.10 Gy	Total opacity, dysplasia of lens and iris, microphthalmia	Same as heterozygote
<i>Cat-2^{no}</i>	<i>Nop</i> (1)	None	Nuclear opacity	Same as heterozygote
<i>Cat-2^{ns}</i>	<i>Scat</i> (3)	None	Nuclear and anterior suture opacity	Total opacity microphthalmia
<i>Cat-2^{ro}</i>	<i>Rop</i> (7)	Procarbazine 5 × 200 mg/kg	Radial opacity	Homogenous opacity
<i>Cat-3^{vl}</i>	<i>Vlm</i> (6)	Gamma-rays 4.55 + 4.55 Gy	Vacuoles, microphthalmia	Same as heterozygote
<i>Cat-3^{va}</i>	<i>R-341</i> (2)	X-rays 5.10 + 5.10 Gy	Vacuoles and axial opacity	Same as heterozygote
<i>Cat-4^a</i>	<i>Apyc</i> (6)	Gamma-rays 4.55 + 4.55 Gy	Anterior polar cataract	Closed eyes, microphthalmia
<i>Cat-4^b</i>	<i>Anc</i> (4)	Gamma-rays 5.34 Gy	Anterior polar cataract	(Lethal)
<i>Cat-4^c</i>	<i>R-7, Pcs-2</i> (2)	X-rays 3.00 + 3.00 Gy	Anterior polar cataract, microphthalmia	(Lethal)
<i>Cat-4^d</i>	— (5)	None	Anterior polar cataract, microphthalmia	(Lethal)
<i>Coc</i>	<i>R-322</i> (2)	X-rays 5.10 + 5.10 Gy	Coraliform flecks	Same as heterozygote
<i>Tcm</i>	<i>X-11</i> (2)	X-rays 3.00 + 3.00 Gy	Total cataract, dysplasia of lens and iris, microphthalmia	Same as heterozygote
<i>Hfi</i>	538 (5)	X-rays 2.00 + 2.00 Gy	Hydropic fibres	Total opacity
<i>Cle</i>	<i>H-9</i> (5)	None	Closed eyes, dysplasia of lens and iris, microphthalmia	Same as heterozygote

* Figures in parentheses represent references of original article reporting mutation as well as former designations:

- (1) Graw, J. *et al.* (1984). *Experimental Eye Research* **39**, 37–45.
- (2) Graw, J. *et al.* (1986). *Mutation Research* **159**, 47–54.
- (3) Graw, J. *et al.* (1989). *Experimental Eye Research* **49**, 469–477.
- (4) Kratochvilova, J. (1981). *Journal of Heredity* **72**, 302–307.
- (5) Kratochvilova, J. (Unpublished.)
- (6) Kratochvilova, J. & Ehling, U. H. (1979). *Mutation Research* **63**, 221–223.
- (7) Kratochvilova, J. *et al.* (1988). *Mutation Research* **198**, 295–301.

aid of a slit lamp commonly used in human ophthalmology. Mydriasis was achieved with a drop of a 1% solution of atropin applied to the eyes at least 10 min before the examination.

3. Results

The results of the allelism tests are summarized in Table 2. Many mutant combinations were not tested, once a series of allelic or closely linked mutations was established. The *Cat-2* series consists of 5 mutations (*Cat-2^{nz}*, *Cat-2^t*, *Cat-2^{no}*, *Cat-2^{ro}*, *Cat-2^{ns}*), the *Cat-3* series of 2 mutations (*Cat-3^{vl}*, *Cat-3^{va}*) and the *Cat-4* series of four mutations (*Cat-4^a*, *Cat-4^b*, *Cat-4^c*, *Cat-4^d*). Any combination of the remaining four mutations (*Coc*, *Tcm*, *Hfi*, *Cle*) assorted at random. Tables 3 and

4 show numbers and phenotypes of outcross offspring. The results that were in accordance with the one-locus hypothesis are given in Table 3. The outcrossed mice produced only cataractous offspring. In cases where the types of cataracts were phenotypically distinguishable from each other a satisfactory agreement with the 1:1 expected ratio was found ($\chi^2 < 3.84$). The minimal excluded linkage distance ranged between 0.5 and 2.2 cM. Table 4 shows results that indicate double heterozygotes at two unlinked loci. There was no instance in which results indicated double heterozygotes of linked loci.

Table 2. Results of allelism tests

Mutant alleles	Cat-2					Cat-3		Cat-4				Coc	Tcm	Hfi	Cle	
	nz	t	no	ns	ro	vl	vao	a	b	c	d					
<i>Cat-2^{nz}</i>	+															
<i>Cat-2^t</i>	+	+														
<i>Cat-2^{no}</i>	+	+	+													
<i>Cat-2^{ns}</i>	+	+	+	+												
<i>Cat-2^{ro}</i>	+	+	+	+	+											
<i>Cat-3^{vl}</i>	-	nt	nt	-	-	+										
<i>Cat-3^{vao}</i>	-	-	nt	nt	-	+	+									
<i>Cat-4^a</i>	-	nt	-	nt	-	-	-	+								
<i>Cat-4^b</i>	nt	-	-	nt	nt	-	nt	+	l							
<i>Cat-4^c</i>	nt	nt	nt	nt	nt	nt	nt	+	l	l						
<i>Cat-4^d</i>	nt	nt	nt	nt	nt	nt	nt	+	l	l	l					
<i>Coc</i>	nt	nt	-	nt	-	-	-	-	nt	nt	nt	+				
<i>Tcm</i>	-	-	-	nt	nt	-	-	-	nt	nt	nt	-	+			
<i>Hfi</i>	-	nt	nt	-	nt	-	nt	-	nt	nt	nt	-	-	+		
<i>Cle</i>	nt	nt	nt	nt	-	nt	-	-	nt	nt	nt	-	-	-	-	+

+, in outcrosses only cataractous offspring that indicate allelism.
 -, in outcrosses cataractous and normal offspring that indicate independent mutations.
 nt, not tested.
 l, lethal.

Table 3. Outcrosses of presumed double heterozygotes that indicate allelism

Mutant alleles		Number of offspring in phenotypic groups				Excluded linkage distance > cM
A	B	A+	B+	AB	++	
<i>Cat-2^{nz}</i>	<i>Cat-2^t</i>	323	310*	—	0	0.94
<i>Cat-2^{nz}</i>	<i>Cat-2^{no}</i>	205	227	0	0	0.69
<i>Cat-2^{nz}</i>	<i>Cat-2^{ro}</i>	143	135	0	0	1.07
<i>Cat-2^{nz}</i>	<i>Cat-2^{so}</i>	262	301	0	0	0.53
<i>Cat-3^{vl}</i>	<i>Cat-3^{vao}</i>	318	303	0	0	0.48
<i>Cat-4^a</i>	<i>Cat-4^b</i>	227†	—	0	0	1.08
<i>Cat-4^a</i>	<i>Cat-4^c</i>	134†	—	0	0	2.21
<i>Cat-4^a</i>	<i>Cat-4^d</i>	148†	—	0	0	2.00

* B+ and AB phenotypically not distinguishable.
 † A+ and B+ phenotypically not distinguishable, but AB distinguishable from both.

4. Discussion

The results from the sample of 15 mutations indicate seven definite loci on autosomes for dominant cataracts in mice. The number of cataract loci is crucial for the calculation of mutation rates per locus, which is necessary for a comparison of the dominant cataract and recessive specific locus mutation rates (Ehling & Favor, 1984).

In the literature, from the 62 dominant genes that affect lens transparency or lens differentiation there are only few that could be mapped. The following loci are given in the catalogue of mutant genes of the mouse (Green, 1989): *Bld* on chromosome 15, *Bpa* on

X chromosome, *Cat* on chromosome 10, *Elo* on chromosome 1, *Ie* on X chromosome, *Lop-4* on chromosome 4, *Sey* on chromosome 2, and *Xcat* on X chromosome. Thus, three cataract genes on the X chromosome and five on autosomes are already known. In order to map the cataract mutations detected in our laboratory or to reveal possible allelism with the already localized genes, linkage tests with standard stocks carrying morphological markers (Phillips & Cattanaach, 1975) have been started.

The presumptive allelic mutations at *Cat-2*, *Cat-3* and *Cat-4* loci originated from treated as well as untreated animals. Several alleles have been already reported for the loci *Bld* (Watson, 1962), *Cat* (Verrusio

Table 4. Outcrosses of presumed double heterozygotes that indicate independent assortment of non-alleles

Mutant alleles		Number of offspring in phenotypic groups				χ^2 test§
A	B	A +	B +	AB	+ +	
<i>Cat-2^{nz}</i>	<i>Cat-3^{vt}</i>	43	70†	—	39	0.04
<i>Cat-2^{nz}</i>	<i>Hfi</i>	26	32	30	34	0.53
<i>Cat-2ⁱ</i>	<i>Tcm</i>	—	—	135‡	41	0.27
<i>Cat-2^{ro}</i>	<i>Cat-4^a</i>	56	41	47	54	0.55
<i>Cat-2^{ro}</i>	<i>Coc</i>	38	34	14	36	1.32
<i>Cat-2^{ro}</i>	<i>Cle</i>	16	30†	—	16	0.02
<i>Cat-3^{vt}</i>	<i>Cat-4^a</i>	41	45	39	45	0.20
<i>Cat-3^{vt}</i>	<i>Hfi</i>	33*	26	—	23	0.41
<i>Cat-3^{va0}</i>	<i>Coc</i>	75*	34	—	30	0.87
<i>Cat-3^{va0}</i>	<i>Tcm</i>	25	41†	—	20	0.18
<i>Cat-3^{va0}</i>	<i>Cle</i>	16	27†	—	20	1.53
<i>Cat-4^a</i>	<i>Coc</i>	9	16	10	12	0.01
<i>Cat-4^a</i>	<i>Tcm</i>	10	16†	—	11	0.44
<i>Cat-4^a</i>	<i>Hfi</i>	15	13	11	19	1.86
<i>Cat-4^a</i>	<i>Cle</i>	11	37†	—	15	0.05
<i>Coc</i>	<i>Tcm</i>	21	39†	—	26	1.26
<i>Coc</i>	<i>Hfi</i>	10	29†	—	8	1.60
<i>Coc</i>	<i>Cle</i>	11	27†	—	18	1.42
<i>Tcm</i>	<i>Hfi</i>	14*	7	—	10	0.87
<i>Tcm</i>	<i>Cle</i>	—	—	69‡	32	2.41
<i>Hfi</i>	<i>Cle</i>	22	55†	—	32	1.10

* A + and AB phenotypically not distinguishable.
 † AB and B + phenotypically not distinguishable.
 ‡ A +, AB and B + phenotypically not distinguishable.
 § Calculated for 3:1 ratio of cataractous and normal mice.

& Fraser, 1966; Muggleton-Harris *et al.* 1987) and *Sey* (Hogan *et al.* 1986, 1987). One of the three *Sey* alleles (*Sey^H*) was radiation induced (Hogan *et al.* 1986). There was no difference between the severity of induced and spontaneous mutations. Although results are limited, it appears that anterior polar cataracts were most frequent in radiation experiments (Kratochvilova & Favor, 1988), whereas nuclear cataracts occurred predominantly in ethylnitrosourea experiments (Favor, 1983, 1986). The spectrum of cataract mutations may be dependent on treatment as is known for the specific locus mutations (Ehling & Favor, 1984).

The manifestation of lens opacities was almost uniform and characteristic for each mutant line. In the *Cat-4* series, all mutations showed anterior polar cataracts. Only the extent of the opacities varied between individuals as well as between the eyes of one individual. Two mutations caused microphthalmia. In contrast to the *Cat-4* mutations, the mutations in the *Cat-2* series differed in form, structure and topography of the opacities. Similarly, the alleles reported at the *Cat* locus, *Cat* (Paget, 1953), *Cat^{Fr}* (Zwaan and Williams, 1968, 1969) and *Lop* (Lyon *et al.* 1981) and at the *Sey* locus, *Sey* (Roberts, 1967), *Sey^H* (Hogan *et al.* 1986) and *Sey^{dey}* were distinct from each other in some morphologic features. The variability may be

partly due to the different genetic backgrounds of the animals and also to the varying criteria used by the investigators. In our experiments, all mutations were transferred to a homogenous genetic background. Therefore, it can be concluded that independent cataract mutations that are alleles at a locus can lead to very distinct lens opacities. The finding of the several unique opacities in the *Cat-2* series suggests that the *Cat-2* mutations affect various functional regions of a complex or affect gene expression in a temporally specific way. Alternatively, phenotypically similar mutations have been shown to be non-allelic. These findings indicate that our estimate of the number of cataract loci, which is based on phenotypically distinct mutations in man, is only an initial estimate. There is as yet no way of knowing if this is an over- or underestimate. This may only be determined by further genetic analyses. With more than 85 independent dominant cataract mutations, which we have recovered in mutagenicity experiments (Favor *et al.* 1989), we may be close to obtaining mutations at all possible loci. Combined with independent mutations already mapped, these genetic resources may be useful in identifying loci responsible for cataract. Not only do these mutations provide a means of identifying the loci responsible for cataract development, such studies will likely result in mutations valuable for the

study of mammalian eye development with possible homologies to the human situation.

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