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Phenotypic characterization and genetic analysis of twenty dominant cataract mutations detected in offspring of irradiated male mice

JANA KRATOCHVILOVA AND JACK FAVOR

Institut für Säugetiergenetik, Gesellschaft für Strahlen- und Umweltforschung, D-8042 Neuherberg, Federal Republic of Germany

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Summary

Twenty autosomal dominant cataract mutations were detected among the offspring of male mice irradiated with γ- or X-rays. The single or fractionated doses ranged from 5.34 to 10.2 Gy. The phenotypic manifestation and penetrance of the mutations as well as fertility and viability of the mutants were studied by extensive breeding. Manifestation of 4 mutations was limited to the lens. Sixteen mutations were characterized by multiple ocular anomalies, of which 4 mutations also affected other organs of the body. Seventy per cent of the mutations caused severe opacity of the lens or lens and cornea. Homologous hereditary diseases in man would be juvenile cataracts with serious impairment of vision or blindness. Expressivity of the lens opacities was almost constant whereas the accompanied defects varied with respect to their severity in different individuals as well as in eyes of an individual. Phenotypic differences dependent on the genetic background could not be observed. Fourteen mutations were classified as mutations with complete penetrance without any effect on viability and fertility of heterozygotes. Four other mutations were shown to be fully penetrant but the viability of the heterozygotes was impaired. Two mutations had incomplete penetrance with no viability or fertility effects. Of the 14 mutations with complete penetrance and normal viability and fertility 6 were shown to be homozygous viable, 1 semi-lethal and 7 lethal.

1. Introduction

In order to detect dominant mutations that cause cataract of the lens a method was developed (Kratochvilova & Ehling, 1979) which makes possible the examination of large populations of mice. On the basis of the frequencies of dominant cataract mutations induced by mutagenic agents in mice, Ehling (1983, 1987) estimated human genetic risk in the first generation after a mutagenic exposure.

In the course of several experiments in our laboratory 20 autosomal dominant cataract mutations have been recovered after irradiation of male mice with different high doses at high-dose rate. The main scope of the previous studies (Kratochvilova, 1981; Ehling *et al.* 1982; Graw *et al.* 1986) was directed to the mutation frequencies. Therefore only preliminary results of genetical tests were given. In the present paper, re-examination of the mutations has been made on the basis of extensive breeding. Congenic lines have been established. Thus phenotype, penetrance, fertility and viability could be studied on a homogeneous genetic background.

2. Materials and methods

(i) *Mutations*

Table 1 shows the origin of the cataract mutants including radiation doses, treated germ cell stages, numbers of offspring examined, numbers of mutants and their symbols. For the 7 mutations recovered by Graw *et al.* (1986) provisional gene symbols are assigned here and correspond to the reference numbers of the originally tested variants as follows: X-11 = *Tcm*; R-7 = *Pcs-2*; R-59 = *Asc-1*; R-60 = *Asc-2*; R-322 = *Coc*; R-324 = *Cat-2*^{t}; R-341 = *Cat-3*^{vao}. The previously described mutations *Nzc* and *Vlm* (Kratochvilova, 1981; Peters, 1987) will be shown here to be allelic to *Cat-2*^{t} and *Cat-3*^{vao}, respectively. We therefore propose the allele symbol *Cat-2*^{nz} for the *Nzc* mutation and *Cat-3*^{vl} for the *Vlm* mutation.

(ii) *Ophthalmological examination*

The slit lamp commonly used in human ophthalmology was employed for examination of the mouse eyes. Pupils were dilated with a drop of 1% atropin

Table 1. *Dominant cataract mutations recovered in mice after paternal irradiation*

Dose (Gy)	Germ cell stage[a]	Number of offspring	Number of mutations	Provisional gene symbols	References
4·55 + 4·55[b]	pg	272	1	*Vlm* to *Cat-3*^{vl}	Kratochvilova (1981); Peters (1987)
4·55 + 4·55[b]	g	5231	6	*Nzc* to *Cat-2*^{nz}, *Iac*, *Nuc*, *Apyc*, *Apoc*, *Acc*	Kratochvilova (1981; Peters (1987)
5·34[b]	pg	1721	1	*Alm*	Kratochvilova (1981); Peters (1987)
5·34[b]	g	10212	2	*Anc*, *Idc*	Kratochvilova (1981); Peters (1987)
6·0[b]	g	11095	3	*Enc*, *Apo*, *Pcs*-1	Ehling *et al.* (1982); Peters (1987)
3·0 + 3·0[c]	pg	1120	1	*x-11* to *Tcm*	Graw *et al.* (1986)
3·0 + 3·0[c]	g	15551	3	*Pcs-2*, *Asc-1*, *Asc-2*	Graw *et al.* (1986)
5·1 + 5·1[c]	g	11205	3	*Coc*, *R-324* to *Cat-2*^{t}, *Vao* to *Cat-3*^{vao}	Graw *et al.* (1986)

[a] pg, postspermatognial cells; g, spermatogonia.
[b] Gamma-rays.
[c] X-rays.

solution applied at least 10 min before examination. An optical section of the eye was achieved by use of a narrow beam of light at a 20–40° angle from the direction of observation. Abnormalities involving the lens, iris and cornea were sufficiently distinct at ×48 magnification.

(iii) *Inheritance tests*

Generally, recommendations for inheritance tests given by Phillips & Cattanach (1975) were used. Congenic lines were established by backcrossing the heterozygous mutants to non-cataractous mice of the inbred strain 102/El, recently examined genetically by West *et al.* (1985). The segregation and fertility data were computed from the 11th and later backcross generations. In addition, heterozygous mutants were repeatedly crossed with (102/El × C3H/El)F_1 hybrids which were usually better breeders. All animals were 10–12 weeks old at the time matings were initiated. Only the first 1–3 litters from a mating were taken for the evaluation of segregation ratio and litter size. The ophthalmologic examination and ensuing categorization to mutant and normal animals were performed at weaning age. The obtained segregation ratio was compared with the expected Mendelian ratio by χ^2 test with Yates' correction.

The mean litter size in mutant lines was compared to the litter size in controls. As the control value for the backcrosses to 102/El, the litter size in the 102/El inbred strain breeding stock was employed ($\bar{x} = 5.7$, S.D. = 2·5, $n = 1052$). The control data for the crosses to (102/El × C3H/El)F_1 mice were obtained in crosses of the hybrids with presumed mutants being subsequently classified to be non-heritable variants (females $\bar{x} = 6{\cdot}8$, S.D. = 2·3, $n = 62$; males $\bar{x} = 7{\cdot}4$, S.D. = 2·5, $n = 49$). Penetrance for the cross of mutant heterozygote to homozygous wild type and relative litter size were determined for the mutant lines that showed distortion of the Mendelian ratio. Penetrance is defined and calculated as the percent carriers observed relative to the Mendelian expectation.

In order to obtain homozygotes, intercrosses of heterozygotes were performed. The number of fertile matings, segregation ratio, litter size and any possible phenotypic differences between the heterozygous and homozygous mutants were recorded. At least 12 presumed homozygotes resulting from the intercrosses were mated to wildtype mice. Animals producing only mutant offspring were confirmed to be homozygous for a fully penetrant gene. Each presumed homozygote was allowed to produce at least 11 offspring, such that the probability of erroneous classification was less than 0·05 (Favor, 1984). Homozygous mutant lines were established by *inter se* matings of the confirmed homozygotes.

The available homozygous individuals were further employed for allelism tests. Two different homozygotes were mated together and their offspring outcrossed to normal mice. If the genes were allelic or closely linked only mutants would be obtained. If they were not allelic and unlinked 25% normal offspring would be expected. Should the genes be linked, the expected frequency of normal offspring would be a function of the genetic distance between the two genes. When homozygotes were not available, heterozygotes were used to obtain double heterozygotes. The double heterozygotes were either phenotypically identifiable or were identified by the segregation of both mutant phenotypes in an outcross.

3. Results

(i) *Description of the phenotypes*

The phenotypic characteristics of the mutants by macroscopic and slit lamp observation are given in Table 2. All phenotypic features were already developed at weaning age and were stationary. The lens opacities as well as the associated anomalies were typical for every mutant line. The phenotypes observed

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Table 2. *Phenotypic characteristics of heterozygous mutants*

Mutant						
Provisional name	Gene symbol	Lens	Cornea	Iris	Other ocular defects	Other system defects
Total cataracts						
Vacuolated lens with microphthalmia	*Cat-3^{vt}*	Vacuolization Dysplasia	—	Small pupils Dysplasia	Microphthalmia	—
Vacuolated lens with axial opacity	*Cat-3^{vao}*	Vacuolization and axial opacity	—	Small pupils Dysplasia	Microphthalmia	—
Total cataract with microphthalmia	*Tcm*	Total opacity Dysplasia	—	Small pupils Dysplasia	Microphthalmia	—
Total cataract with microphthalmia	*Cat-2^{t}*	Total opacity Dysplasia	—	Small pupils Dysplasia	Microphthalmia	—
Iris anomaly with cataract	*Iac*	Cortical or total opacity	—	Corectopia Coloboma	Microphthalmia	Long teeth
Iris dysplasia with cataract	*Idc*	Total opacity Lenticonus Dysplasia	Opacity	Dysplasia	Microphthalmia	Belly spot Reduced body size
Anterior lenticonus with microphthalmia	*Alm*	Total opacity Lenticonus Dysplasia	Opacity	Dysplasia	Microphthalmia	Belly spot Reduced body size
Anterior polar cataracts						
Anterior pyramidal cataract	*Apyc*	Anterior polar opacity	Opacity	Pupillary membrane	—	—
Anterior polar cataract	*Apoc*	Anterior polar opacity	Opacity	Pupillary membrane Synechiae	—	—
Anterior capsular cataract	*Acc*	Anterior polar opacity of the capsule and of the embryonic suture	Opacity	Pupillary membrane	—	—
Anisocoria with polar cataract	*Anc*	Anterior polar opacity	Opacity	Anisocoria Pupillary membrane	—	—
Anterior polar opacity	*Apo*	Anterior polar opacity	Opacity	Pupillary membrane	—	Circling behaviour
Polar cataract and small eyes	*Pcs-1*	Anterior polar opacity	Opacity	Dysplasia	Microphthalmia	—
Polar cataract and small eyes	*Pcs-2*	Anterior polar opacity	Opacity	Dysplasia	Microphthalmia	—
Anterior suture cataract	*Asc-1*	Anterior polar opacity with a suture anomally	—	Pupillary membrane	—	—
Anterior suture cataract	*Asc-2*	Anterior polar opacity with a suture anomaly	—	Pupillary membrane	—	—
Central cataracts						
Nuclear and zonular cataract	*Cat-2^{nz}*	Opacity of the nucleus and perinuclear zone	—	—	—	—
Nuclear cataract	*Nuc*	Opacity of the foetal nucleus and the posterior embryonic suture	—	—	—	—
Embryonic nucleus cataract	*Enc*	Opacity of the embryonic nucleus and the posterior suture	—	—	—	—
Coralliform cataract	*Coc*	Small roundish opacities in the central part	—	—	—	—

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in the later outcross generations were identical with those of the original mutants and no differences were found on the 102/El or (102/El × C3H/El)F_1 genetic backgrounds. With respect to the localization of the lens opacities 7 total cataracts (*Cat-3^{vl}*, *Cat-3^{vao}*, *Tcm*, *Cat-2^{t}*, *Iac*, *Idc*, *Alm*), 4 central cataracts (*Cat-2^{nz}*, *Nuc*, *Enc*, *Coc*) and 9 anterior polar cataracts (*Apyc*, *Apoc*, *Acc*, *Anc*, *Apo*, *Pcs-1*, Pcs-2, *Asc-1*, *Asc-2*) were observed.

(*a*) *Total cataracts*. The mutants with total cataracts showed opacity of whole lenses and macroscopically obvious microphthalmia. The reduction in eye size was documented by weighing the eyeballs of heterozygous mutants and normal siblings at 3 weeks of age. In Table 3, results of 6 mutant lines with total cataracts and 1 microphthalmic line with polar cataracts are given. Unfortunately, 2 of the microphthalmic lines (*Alm*, *Pcs-1*) could not be included in the table, because they have been already lost at the time of examination. The weight of the eyeballs of the mutants was 35–70% compared to the non-mutant siblings. The most pronounced microphthalmia was found in *Cat-2^{t}* line. Different forms of opacities could be distinguished in the different mutant lines. Thus, roundish vacuoles of various size (*Cat-3^{vl}*), small vacuoles with axial opacity (*Cat-3^{vao}*), cloudy opacity (*Tcm*, *Cat-2^{t}*, *Iac*) and protruding opacity adhering to opaque cornea (*Idc*, *Alm*) were observed. Iris malformations were always present. In 2 mutant lines (*Idc*, *Alm*) the ocular defects were associated with a white belly spot and reduced body size. The weight of those mutants at weaning age was 60–70% of the weight of the normal siblings. In 1 mutant line (*Iac*) about half of the mutants had long teeth leading to starvation if not corrected. There was variation in severity of the iris defects and corneal opacity observed in mutants of a mutant line as well as in eyes of one individual.

(*b*) *Central cataracts*. The cataract was the exclusive anomaly of the mutants in this group. No other ocular or other system defects were observed. Three mutants showed diffuse or powdery opacities in the foetal or embryonic nucleus being combined with opacities of the posterior embryonic suture (*Nuc*, *Enc*) or an opacity of the perinuclear zone (*Cat-2^{nz}*). One mutant (*Coc*) had small roundish irregularly shaped opacities resembling corals which were located in the foetal nucleus. There was no variation in the form or intensity of the opacities among mutants in 3 mutant lines (*Cat-2^{nz}*, *Nuc*, *Enc*). Only in 1 mutant line (*Coc*) could variation in the number and size of the spots be observed.

(*c*) *Anterior polar cataracts*. Seven cataract mutants (*Apyc*, *Apoc*, *Acc*, *Anc*, *Apo*, *Pcs-1*, *Pcs-2*) showed an opacity at the anterior pole of the lens with some associated opacities located axially. The opaque area showed a more or less prominent pyramidal form. The lens opacities were frequently associated with corneal opacities. The corneal opacities ranged from polar opacity to complete opacity with vascularization.

In general, associated ocular defects varied in the degree of severity much more than the polar lens opacities. Occasionally, there was a persistent connection between the anterior surface of the lens and the central cornea. Two mutants (*Asc-1*, *Asc-2*) were characterized by a subcapsular disk opacity and vacuoles along the anterior suture. The remnants of the pupillary membrane on the lens capsule were often concomitant with the polar cataracts. Synechiae of the iris with the lens or the cornea were frequently present in 2 mutants (*Apoc*, *Anc*). In another 2 mutants (*Pcs-1*, *Pcs-2*) the polar cataracts were associated with microphthalmia and dysplasia of the iris. In 1 mutant line (*Apo*) about 30% of mutants showed circling behaviour. There was some variation in the form and extent of the polar opacities between the eyes of one individual or of different individuals. In 3 mutant lines (*Apyc*, *Apoc*, *Acc*) 5–50% of mutants showed cataract only unilaterally. Mutants in 1 of the mutant lines

Table 3. *Eye weights of 3-week-old siblings in microphthalmic lines*

	Eye weights (mg)				
	Normal		Mutants		
Mutant line	$\bar{x}\pm$S.D.	*n*	$\bar{x}\pm$S.D.	*n*	% Normal
Cat-3^{vl}	15·23 ± 0·52	10	7·64 ± 0·53*	8	50
Cat-3^{vao}	14·28 ± 0·48	4	9·30 ± 0·97*	14	65
Tcm	15·51 ± 0·30	6	8·87 ± 1·72*	8	57
Cat-2^{t}	14·76 ± 0·56	6	5·16 ± 0·37*	10	35
Iac	14·16 ± 0·47	6	8·47 ± 1·37*	8	60
Idc	15·35 ± 1·20	12	7·50 ± 1·91*	6	49
Pcs-2	14·09 ± 0·91	12	9·87 ± 0·95*	12	70

* Significantly different from the weight of the normal siblings (*t*-test, $P < 0·01$).

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Table 4. *Segregation in outcrosses of double mutants*

Mutant alleles		Numbers of offspring in phenotypic groups			
A	B	A+	AB	B+	++
Cat-3^{vl}	*Cat-3^{vao}*	318	0	303	0
Cat-3^{vl}	*Tcm*	11	—	17[b]	13
Cat-3^{vl}	*Iac*	6	—	10[b]	6
Cat-3^{vl}	*Cat-2^{nz}*	70[a]	—	43	39
Cat-3^{vl}	*Coc*	32[a]	—	15	17
Cat-3^{vl}	*Apyc*	41	39	45	45
Cat-3^{vl}	*Asc-2*	23[a]	—	8	10
Cat-3^{vao}	*Tcm*	25	—	41[b]	20
Cat-3^{vao}	*Cat-2^{t}*	8	—	19[b]	10
Cat-3^{vao}	*Cat-2^{nz}*	18[a]	—	10	12
Cat-3^{vao}	*Asc-2*	19[a]	—	9	14
Tcm	*Cat-2^{t}*	—	69[c]	—	22
Tcm	*Cat-2^{nz}*	16[a]	—	10	11
Tcm	*Coc*	8[a]	—	3	5
Cat-2^{t}	*Cat-2^{nz}*	145	0	162	0
Cat-2^{t}	*Coc*	14[a]	—	9	8
Cat-2^{t}	*Anc*	35[a]	—	20	16
Iac	*Cat-2^{nz}*	30[a]	—	12	18
Idc	*Cat-2^{nz}*	12[a]	—	7	8
Cat-2^{nz}	*Coc*	22	17	20	17
Cat-2^{nz}	*Enc*	20[a]	—	13	11

[a] A+ and AB phenotypically not distinguishable.
[b] AB and B+ phenotypically not distinguishable.
[c] A+, AB and B+ phenotypically not distinguishable.

(*Apyc*) could be occasionally recognized only by the remnants of the pupillary membrane.

The mutants in the lines *Idc* and *Alm* showed very similar phenotypic features. Also the pairs of mutant lines *Tcm*, *Cat-2^{t}*, *Pcs-1*, *Pcs-2* and *Asc-1*, *Asc-2* were phenotypically indistinguishable from each other suggesting that they might be allelic. Therefore, allelism and linkage studies have been started. The results of the already completed allelism tests are given in Table 4. Outcrossing the double mutants *Cat-3^{vl}/Cat-3^{vao}* and *Cat-2^{t}/Cat-2^{nz}* produced only cataractous offspring of parental phenotypes in a 1:1 ratio. Due to the large numbers of offspring examined in the allelism tests it was concluded that the pairs of the genes *Cat-3^{vl}–Cat-3^{vao}* and *Cat-2^{t}–Cat-2^{nz}* were allelic. In the outcrosses of the other double mutants a segregation ratio of 3:1 of cataractous to normal animals was found which was expected for non-allelic genes.

(ii) *Segregation*

In Table 5 results of the crosses of heterozygous mutants to homozygous wild-type mice are summarized. In 14 mutant lines the yields of mutant and normal offspring corresponded to the 1:1 expected Mendelian ratio. In the other 6 mutant lines (*Idc*, *Alm*, *Nuc*, *Apoc*, *Acc*, *Apo*) a significant reduction in the number of mutants was found. There were no differences in segregation between males and females nor for crosses with 102/El or (102/El × C3H/El)F_1. Our previous data (Kratochvilova, 1981) that indicated sex differences in 4 mutant lines (*Alm*, *Nuc*, *Acc*, *Anc*) could not be confirmed in this study. Also distorted segregation found formerly in a mutant line (*Apyc*) has not been observed in the present results due to improved recognition of the mutants. The reduced numbers of mutants found in the confirmation crosses for three mutations (*Cat-2^{t}*, *Coc*, *Asc-1*) (Graw *et al.* 1986) could not be observed in the established mutant lines.

(iii) *Litter size*

The mean litter size for crosses of the mutants is also given in Table 5. A significant reduction was found for 2 mutants (*Nuc*, *Apo*) in all crosses with the exception of heterozygous (*Apo*) males crossed to (102/El × C3H/El)F_1. In another 2 mutant lines (*Alm*, *Idc*) only females produced smaller litters. In the most affected mutant line (*Nuc*) litter size ranged between 42–63% of control (Table 6).

(iv) *Penetrance*

Penetrance was calculated as the percentage of observed mutants relative to that expected (total number of the offspring in the backcross times 0·5). This calculation assumes that the viability of the mutant and normal offspring is the same. Impaired

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Table 5. *Segregation and litter size in crosses of heterozygous mutants to 102/El and (102/El × C3H/El)F_1 mice*

Mutant		Crosses with 102/El					Crosses with (102/El × C3H/El)F_1				
		Offspring normal mutant			Litter size		Offspring normal mutant			Litter size	
Provisional gene symbol	Sex	*n*	*n*	%	$\bar{x} \pm$ S.D.	*n*	*n*	*n*	%	$\bar{x} \pm$ S.D.	*n*
Cat-3^{vl}	♀	65	55	45·8	6·0 ± 2·1	20	62	56	47·5	5·9 ± 2·6	20
	♂	50	52	51·0	5·1 ± 2·0	20	81	80	49·7	8·1 ± 2·8	20
Cat-3^{vao}	♀	60	58	49·2	5·9 ± 3·6	20	24	23	48·9	7·8 ± 2·7	6
	♂	15	23	60·5	6·3 ± 1·0	6	18	14	43·8	8·0 ± 2·6	4
Tcm	♀	42	37	46·8	5·3 ± 2·1	15	25	30	54·5	5·0 ± 1·9	11
	♂	58	52	47·3	6·9 ± 1·8	16	36	33	47·8	8·6 ± 1·9	8
Cat-2^{t}	♀	34	32	48·5	6·6 ± 2·0	10	19	23	54·8	6·0 ± 2·7	7
	♂	14	15	51·7	4·1 ± 0·9	7	37	27	42·2	8·0 ± 2·5	8
Iac	♀	42	47	52·8	4·5 ± 2·0	20	55	51	48·1	5·3 ± 2·0	20
	♂	55	45	45·0	5·0 ± 1·8	20	83	61	42·4	7·2 ± 2·2	20
Idc	♀	36	19	34·5[a]	2·8 ± 1·8[b]	20	42	20	32·3[a]	3·1 ± 1·8[b]	20
	♂	70	18	20·5[a]	4·4 ± 1·4	20	81	39	32·5[a]	6·0 ± 1·9	20
Alm	♀	24	14	36·8[a]	2·5 ± 2·0[b]	15	32	10	23·8[a]	2·5 ± 1·8[b]	17
	♂	70	29	29·3[a]	5·0 ± 2·1	20	87	21	19·4[a]	5·4 ± 2·2	20
Cat-2^{nz}	♀	57	47	45·2	5·2 ± 2·0	20	79	78	49·7	7·9 ± 2·5	20
	♂	59	51	46·4	5·5 ± 2·0	20	81	89	52·4	8·5 ± 1·8	20
Nuc	♀	59	12	16·9[a]	3·6 ± 1·5[b]	20	52	15	22·4[a]	3·4 ± 1·4[b]	20
	♂	23	9	28·1[a]	3·2 ± 1·2[b]	10	41	21	33·9[a]	3·1 ± 2·0[b]	20
Enc	♀	67	54	44·6	6·1 ± 1·8	20	49	42	46·2	5·7 ± 1·7	16
	♂	68	52	43·3	6·0 ± 1·6	20	44	38	46·3	7·5 ± 1·5	11
Coc	♀	35	49	58·3	6·5 ± 2·9	13	35	26	42·6	6·1 ± 1·7	10
	♂	25	28	52·8	5·3 ± 2·2	10	42	32	43·2	7·4 ± 1·8	10
Apyc	♀	56	58	50·9	5·7 ± 2·3	20	83	58	41·1	7·1 ± 1·8	20
	♂	49	54	52·4	5·2 ± 2·2	20	75	81	51·9	7·8 ± 2·4	20
Apoc	♀	84	18	17·7[a]	5·1 ± 1·3	20	96	21	17·9[a]	5·9 ± 2·6	20
	♂	94	17	15·3[a]	5·6 ± 2·0	20	112	29	20·6[a]	7·1 ± 3·0	20
Acc	♀	94	23	19·7[a]	5·9 ± 2·3	20	98	16	14·0[a]	5·7 ± 1·7	20
	♂	74	29	28·2[a]	5·2 ± 1·9	20	105	27	20·5[a]	6·6 ± 2·3	20
Anc	♀	47	50	51·5	4·9 ± 2·1	20	69	59	46·1	6·4 ± 2·0	20
	♂	56	40	41·7	4·8 ± 1·7	20	99	71	41·8	8·5 ± 2·2	20
Apo	♀	44	21	32·3[a]	3·3 ± 1·5[b]	20	65	26	28·6[a]	4·6 ± 1·5[b]	20
	♂	59	14	19·2[a]	3·7 ± 1·6[b]	20	93	30	24·4[a]	6·2 ± 2·4	20
Pcs-1	♀	56	61	52·1	5·9 ± 2·0	20	40	31	43·7	5·9 ± 1·8	12
	♂	55	53	49·1	5·4 ± 2·1	20	77	66	46·2	7·2 ± 2·5	20
Pcs-2	♀	60	62	50·8	6·1 ± 1·9	20	31	26	45·6	5·7 ± 1·8	10
	♂	67	52	43·7	6·0 ± 2·0	20	42	40	48·8	7·5 ± 2·7	11
Asc-1	♀	38	31	44·9	5·3 ± 1·8	13	22	25	53·2	5·2 ± 2·2	9
	♂	30	34	53·1	4·9 ± 2·1	13	29	25	46·3	6·8 ± 2·0	8
Asc-2	♀	22	21	48·8	7·2 ± 1·2	6	32	27	45·8	7·4 ± 1·3	8
	♂	61	51	45·5	5·6 ± 2·7	20	84	67	44·4	7·9 ± 1·7	19

[a] Significantly different from the 1:1 expected ratio (χ^2 test with Yates' correction).
[b] Significantly different from the litter size in controls (*t* test).

viability in one or both Mendelian classes will also distort the segregation ratios and would be recognized as a reduction in the litter size. Therefore, Table 6 gives the penetrance values as well as the litter size as a percent control for those mutations in which an abnormal segregation ratio or litter size was observed. In the 2 mutant lines (*Apoc*, *Acc*) the penetrance ranged between 31–56%. In these cases the values are reliable measures of penetrance because there was no significant effect on fertility. Further, these mutants often expressed unilateral cataracts. In the other 4 mutant lines (*Idc*, *Alm*, *Nuc*, *Apo*) the litter size in certain backcrosses was about half of the control litter size. The high deficit in the number of mutants could be caused by incomplete penetrance or impaired viability of the mutants or both phenomena simultaneously. The bilateral manifestation of the cataracts and constant expressivity of all phenotypic features in the four mutant lines suggested that the mutations have full penetrance. The reduced transmission of mutants, the breeding data and the difficulties in perpetuating the mutant lines are likely a result of reduced viability and fertility of the carriers of the 4 mutations.

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Table 6. *Dominant cataract mutations with abnormal segregation or fertility effects*

Mutant		Backcrosses to 102/El		Backcrosses to (102/El × C3H/El)F_1	
Provisional gene symbol	Sex	Penetrance[a] %	Litter size[b] %	Penetrance[a] %	Litter size[b] %
Apoc	♀	35·3	89·5	35·9	86·8
	♂	30·6	98·3	41·1	94·6
Acc	♀	39·5	103·5	28·1	86·4
	♂	56·3	91·2	40·9	89·2
Idc	♀	69·1	49·1	64·5	45·6
	♂	40·9	77·2	65·0	81·1
Alm	♀	73·7	43·9	47·6	36·8
	♂	58·6	87·7	38·9	73·0
Nuc	♀	33·8	63·2	44·8	50·0
	♂	56·3	56·1	67·7	41·9
Apo	♀	64·6	57·9	57·1	67·7
	♂	38·4	64·9	48·8	83·8

[a] [(mutant offspring)/(total offspring × 0·5)] × 100.
[b] [(mean litter size of heterozygotes)/(mean litter size in controls)] × 100.

(v) *Homozygous viability*

Table 7 contains the results of the intercrosses of heterozygotes and the numbers of homozygotes which were confirmed by outcrossing presumed homozygotes for the 14 mutations with complete penetrance. Six mutations (*Cat-3^{vao}*, *Tcm*, *Cat-2^{t}*, *Cat-2^{nz}*, *Coc*, *Apyc*) exhibited the expected 1:3 segregation ratio and homozygotes were confirmed by outcrossing presumed homozygotes.

In 1 mutant line (*Cat-3^{vl}*) there was a significant reduction in the 1:3 expected ratio. However, a few viable and fertile homozygotes were obtained. The homozygotes in this line were considered to be semi-lethal. A distortion in the segregation was also observed in 4 other mutant lines (*Anc*, *Enc*, *Asc-1*, *Asc-2*). The negative results in the outcrosses of presumed homozygotes suggested that the homozygotes were lethal.

In 3 mutant lines (*Iac*, *Pcs-1*, *Pcs-2*) no significant difference from the 1:3 ratio could be demonstrated. However, no homozygotes could be confirmed in outcrosses of a significant number of presumed homozygotes and it is concluded that the mutations are homozygous lethal. This apparent discrepancy is surely a statistical artifact since the segregation ratio was also in accordance with the 1:2 ratio which would be expected when the class of homozygotes was missing.

Table 7. *Segregation in inter-crosses and number of confirmed homozygotes in outcrosses*

Mutant provisional gene symbol	Fertile matings (%)	Offspring			Litter size		Presumed homozygotes	
		Normal (*n*)	Mutant (*n*)	Mutant (%)	$\bar{x} \pm$ S.D.	(*n*)	Tested (*n*)	Confirmed (*n*)
Cat-3^{vl}	81·8	20	22	52·4[a]	4·2 ± 2·0	10	22	1
Cat-2^{nz}	100	13	46	78·0	5·9 ± 1·5	10	12[b]	12
Apyc	100	11	36	76·5	4·7 ± 1·5	10	12[b]	12
Iac	35·7	16	25	61·0	4·1 ± 1·6	10	25	0
Anc	83·3	13	14	51·9[a]	2·7 ± 1·5[c]	10	14	0
Enc	100	18	23	56·1[a]	4·1 ± 1·3	10	15	0
Pcs-1	85·7	16	32	66·7	4·8 ± 1·4	10	30	0
Pcs-2	55·6	19	29	60·4	4·8 ± 2·0	10	17	0
Asc-1	71·4	26	26	50·0[a]	5·8 ± 3·3	10	16	0
Asc-2	50·0	20	20	50·0[a]	4·0 ± 1·5	10	12	0
Coc	100	21	45	68·2	6·6 ± 2·8	10	20	8
Cat-2^{t}	100	15	45	75·0	6·0 ± 3·6	10	15	6
Cat-3^{vao}	100	19	62	76·5	8·1 ± 2·4	10	12	4
Tcm	100	20	44	68·8	6·4 ± 1·7	10	10	4

[a] Significantly different from 1:3 expected ratio (χ^2 with Yates' correction).
[b] Phenotypically different from heterozygotes.
[c] Significantly different from the mean litter size in the 102/El strain $\bar{x} = 5·7$, S.D. = 2·5, $n = 1052$.

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4. Discussion

(i) *Phenotypic comparisons*

The dominant cataract mutations which were recovered in the F_1 generation after irradiation of male mice caused lens opacities with constant expressivity. The phenotypes were characteristic for every mutant line. Approximately 45% of the opacities found in the radiation experiments were polar cataracts, 35% were total cataracts and 20% central cataracts. A similar distribution in the phenotypic groups was found by Favor *et al.* (1987) for mutations recovered in the progeny of irradiated BALB/c and DBA/2 male mice. After treatment of $(102/El \times C3H/El)F_1$ males with ethylnitrosourea, however, mutations with central cataracts (40%) were most frequent (Favor, 1983, 1984, 1986). At least 2 possibilities may explain these observed differences. It is known from specific locus experiments that for a particular locus the sensitivity to mutation induction may vary for radiation or ENU treatment (Ehling *et al.* 1982). Thus, the difference in the relative frequency of recovered dominant cataract mutations of a particular phenotypic class may result from a difference in the sensitivity to mutation induction following radiation or ENU treatment. Alternatively, this difference may be due to the type of DNA lesion induced by radiation or ENU. The homozygous viability of recovered specific locus mutations suggests that radiation induces mainly small deletions whereas ENU treatment induces mostly point mutations (Ehling & Favor, 1984). Possibly intragenic lesions result in a variant phenotype confined to a single trait, while larger intergenic deletions affect additional traits.

There are 23 recessive and 15 dominant alleles associated with cataract that have been discovered by other investigators and are listed in *Mouse News Letters* (Peters, 1987). About half of the genes have been localized to chromosomes and have been described in detail. All these alleles have phenotypic affects detectable macroscopically and characterized as a total opacity usually combined with microphthalmia (Green, 1981). They are similar to the total cataracts recovered in the present study. The polar cataracts are comparable to the mutation dysgenetic lens (*dyl*) described by Sanyal & Hawkins (1979). The mouse polar cataracts seem to be analogous to the anterior chamber cleavage syndrome in man (Waring *et al.* 1975). Also central cataracts are known from slit lamp diagnosis in human ophthalmology (Waardenburg *et al.* 1961) and the Nakano cataract (*nct*) of the mouse (Fukui *et al.* 1980).

Recently, West & Fisher reported 2 spontaneous (1985) and 6 ethylnitrosourea-induced (1986) cataract mutations detected in a small-scale experiment. In contrast to our reported mutations, the mutations recovered by West and Fisher resulted in lens opacities which were relatively small in extent and varied in their form among individuals. This may be a consequence of the genetic confirmation procedures employed by West and Fisher, whereby a presumed mutation expressing a particular lens opacity was concluded to be a confirmed mutation if among its offspring individuals were observed with a lens opacity of any kind.

From the 20 radiation-induced mutations, 16 showed gene dosage effects observable as intensive ocular defects or lethality in homozygotes. The semidominant effect has been also reported for all dominant cataract mutations cataloged by Green (1981).

The allelism test revealed that phenotypically similar as well as phenotypically different mutations can be alleles of the same gene. From the finding of allelism of the phenotypically different alleles *Cat-2*^{t} and *Cat-2*^{nz}, one could conclude that the primary lesion may be the same and the remainder of the eye might become abnormal secondarily. Such gene action was already proposed by Zwaan (1980) for the cataract genes *ak*, *dyl* and *Sey*^{Dey} [formerly designated *Dey* but recently shown to be allelic to *Sey* (Hogan, Hetherington & Lyon, 1987)].

(ii) *Penetrance*

The yield of the mutations with complete penetrance and normal litter size was 70%. A similar percentage was obtained in the radiation experiments with other strains of mice (Favor *et al.* 1987), as well as for ethylnitrosourea induced dominant mutations (Favor, 1984; 1986).

For cataract mutations recovered by other investigators, complete penetrance was reported for eye lens obsolescence (*Elo*) (Oda *et al.* 1980), cataract and small eye (*Cts*) (Kobayashi, 1980), small eyes (*Sey*) (Roberts, 1967) and sightless (*Sig*) (Searle, 1965). The cataract genes blind (*Bld*) and lens opacity (*Lop*) had complete penetrance on certain genetic background while in some crosses to other strains abnormal segregation was found (Teicher & Caspari, 1978; Lyon *et al.* 1981). A reduced number of mutants was also reported for the mutations *Sey*^{Dey} and *Sey*^{H} (Hogan *et al.* 1986; Hogan, Hetherington & Lyon, 1987; Theiler *et al.* 1978). The authors stated that both, reduced viability or penetrance may be involved in the effect.

(iii) *Homozygous viability*

The radiation-induced mutations showed a considerably lower proportion (6 from 14) of viable homozygotes than the ethylnitrosourea-induced mutations (10 from 11) recovered by Favor (1984). This observation is in accord with results from specific locus studies which indicate a qualitative difference between radiation- and procarbazine- or ethylnitrosourea-induced mutations (Ehling & Neuhäuser, 1979; Ehling *et al.* 1982; Russell, 1983). Homozygous

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lethality was considered to be an indicator for deletion rather than point mutation. Also 3 cataloged cataract mutations (*Cm*, *Sey*^{H}, *Sig*), which were discovered in offspring of irradiated mice, are homozygous lethal. From the cataract mutations of spontaneous origin, 4 (*Cts*, *Elo*, *Lop*, *Lop*^{Cat}) were homozygous viable, and 3 (*Bld*, *Sey*, *Sey*^{Dey}) were homozygous lethal (Green, 1981; Hogan *et al.* 1987; Lyon *et al.* 1981; Muggleton-Harris, Festing & Hall, 1987). No correlation between the severity of the phenotype and homozygous lethality could be observed among the cataract mutations in this study as well as among the cataract mutations recovered by other investigators.

5. Conclusions

The dominant cataract mutations have well-defined phenotypes which are relatively easily recognizable. Eighty per cent of the mutations had pleiotropic effects. The expression of the lens opacities is constant in comparison to the associated defects. The allelism tests which are still in progress have revealed so far that repeat mutations occurred at 2 loci.

From the high yield of cataract mutations in mice that cause total opacity of the lens or almost total opacity of the cornea it could be concluded that the majority of the radiation-induced cataract mutations in man would result in serious impairment of vision.

In the sample of 20 mutations, only 2 showed incomplete penetrance. Therefore, the mutation rates obtained in the radiation experiments may not be essentially underestimated by failure to observe mutations with low penetrance.

The heterozygous mutants in 4 mutant lines had impaired viability and fertility leading to difficulties in perpetuating them. If one presumes that the present sample of cataract mutations is representative for all radiation induced dominant mutations, then 20% of the mutations would have selective disadvantage and will be eliminated from the population.

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