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Characterization of dominant cataract mutations in mice: penetrance, fertility and homozygous viability of mutations recovered after 250 mg/kg ethylnitrosourea paternal treatment

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SUMMARY

Seventeen dominant cataract mutations of the mouse recovered in ethylnitrosourea mutagenesis experiments have been genetically characterized as to penetrance, fertility, and homozygous viability. Nine mutations were shown to be fully penetrant with no fertility effects, four mutations were classified as having reduced penetrance with no fertility effects, one mutation had reduced penetrance and reduced fertility, two mutations were shown to have a reduced frequency of mutant offspring due to penetrance and viability effects, and one mutation most likely has a reduced viability of carrier individuals. Of the eleven mutations for which definitive homozygous viability data were obtained, ten were shown to be homozygous viable and only one was shown to be homozygous lethal. In similar experiments in which dominant cataract, dominant skeletal or dominant visible mutations were recovered after radiation treatment, comparable frequencies of mutations with reduced penetrance were observed but there was a strikingly higher frequency of homozygous lethal mutations. These observations support the hypothesis of a qualitative difference in the mutations recovered after ethylnitrosourea as compared to radiation treatment. Finally, it is argued that a systematic comparison of the induced mutation rates to dominant and recessive alleles with subsequent genetic characterization of the recovered mutations provides a critical set of data necessary for an improvement in the indirect and direct procedures of genetic risk estimation.

1. INTRODUCTION

Due to the relatively simple and fast examination procedures using live animals, the search for dominant mutations causing cataracts in mice has been shown to be practical (Kratochvilova & Ehling, 1979). Such experiments have the advantages that dominant mutations affecting one phenotype are systematically studied, and presumed mutations are genetically confirmed. To date, results have been reported for dominant cataract mutations recovered after radiation or ethylnitrosourea (ENU) parental male treatment (Kratochvilova & Ehling, 1979; Kratochvilova, 1981; Ehling *et al.* 1982; Favor, 1983).

The subsequent characterization of recovered dominant cataract mutations will provide information for a better understanding of the genetic system controlling lens formation and maintenance of lens clarity. Of particular interest is the determination of the number of mutable loci screened in the dominant cataract mutation test, the classes of inherited human disease to which the recovered dominant cataract mutations would be homologous, and the fate and effects of such mutations should they arise in a human population. The present results are an attempt to determine the transmissibility of dominant cataract mutations recovered after parental ENU treatment (Favor, 1983) as well as an estimate of the penetrance, fertility, and homozygous viability of the mutant alleles.

2. MATERIALS AND METHODS

(i) *First outcross generation*

Presumed mutant individuals were recovered in a combined specific locus and dominant cataract mutation experiment (Favor, 1983). The recovered presumed mutations were subjected to an outcross to strain 101/El and either confirmed as a dominant mutation or classified as not due to a dominant mutation. For confirmed mutations, the original outcross was maintained to determine the frequency of transmission of the mutant phenotype when outcrossed to strain 101/El. The frequency of mutant individuals observed was compared to that expected. The observed frequency of offspring exhibiting the mutant phenotype relative to that expected was calculated and will be referred to as the penetrance of the mutation for the particular cross.

Mean litter size of the first three litters produced for each confirmed mutation was calculated. To determine if fertility of the mutation carriers was affected, the mean litter size of each mutation carrier was compared to the mean litter size of the same sex presumed mutations subsequently classified as not due to a dominant mutation. Such a statistical comparison is therefore controlled for time, space, and genotype of animals.

(ii) *Subsequent outcrosses*

Male and female heterozygous mutant mice recovered in outcrosses were further outcrossed to either strain 101/El mice or $(101/El \times C3H/El)F_1$ hybrid mice. As in the first outcross generation, the frequency of transmission of the mutation was determined, compared to the Mendelian expectation, and penetrance calculated as defined. For those mutations which were shown to have an effect on litter size in the original outcross generation, a one-tailed *a priori* comparison of the mean litter size of the first three litters of heterozygous carrier individuals was made to the mean litter size of those mutations shown not to have effects on litter size in the original outcross generation, controlling for sex of heterozygous carrier and genotype of outcross partner.

(iii) *Homozygous viability*

Intercrosses of heterozygotes were established and mean litter size of the first three litters determined. A one-way analysis of variance was carried out to determine if litter size differences were present. A Student–Newman–Keuls *a posteriori*

comparison of the mean litter size of the mutation intercrosses was performed to determine from which mutations the significant differences arose.

Mutant offspring, resulting from an intercross, were either outcrossed or crossed *inter se* to determine if they were homozygous for the mutant allele. When both mutant and normal phenotypes were observed in the resulting offspring, the possible homozygous individuals were concluded to be heterozygotes and the cross was immediately terminated. When only mutant phenotypes were observed in

Table 1. *Experimental protocol for the genetic determination of homozygotes for a dominant trait using an outcross or intercross mating procedure*

Cross type	Possible mating types†	Probability of wild-type offspring (p)	Probability of observing all mutant offspring in n offspring (P)	Minimal n for $P < 0{\cdot}05$
Outcross	$H_0 = */+ \times +/+$	1/2	$(1-p)^n$	5
	$H_1 = */* \times +/+$	0	1	—
Intercross	$H_0 = */+ \times */+$	1/4	$(1-p)^n$	11
	$H_1 = */* \times */+$ or $*\|* \times */*$	0	1	—

† +/+, homozygous wild-type; */+, heterozygote; */*, homozygous mutant.

resultant offspring, enough offspring were classified so that the probability of such an observation occurring from heterozygous carriers would be less than 0·05, before classifying the carrier individual in an outcross as homozygote or at least one individual in an *inter se* cross as a homozygote (Table 1). Crosses, in which homozygous carriers were identified, were maintained and additional offspring were examined. Therefore, the actual probability of erroneously classifying a cross as involving a homozygous individual is less than the stated 0·05.

(iv) *General procedures*

Animal husbandry and biomicroscopic examination procedures were as previously described (Kratochvilova & Ehling, 1979; Kratochvilova, 1981; Ehling *et al.* 1982; Favor, 1983). Two mutations (ENU-143, -189) confirmed by Favor (1983) have been maintained subsequent to the confirmation outcross solely by intercrosses. Therefore, data are reported here only for the seventeen mutations with complete data for genetic characterization.

Statisitical comparison of the observed ratio of mutant and wildtype offspring to that expected for a particular mating type were by the Chi-square test with Yates' correction. All litter size means comparisons were by t-test. Although litter size data are not normally distributed, the t-test comparison was considered robust, so that errors in statistical conclusion should not occur. Handling and calculation of original outcross productivity data for all tested presumed mutations were carried out by use of computer programs available from BMDP statistical software version 6·2 (Dixon *et al.* 1981).

3. RESULTS

(*l*) *First outcross generation*

Table 2 gives the transmission and fertility data of the originally recovered mutations, when outcrossed to strain 101/El, as well as the litter size of those variants subsequently classified as not due to a dominant mutation. Both the confirmed mutations and the control populations have been categorized as to sex of carrier.

Eight mutations (ENU-68, -228, -262, -378, -411, -415, -449, -464) have reduced penetrance, ranging from 0·18 to 0·76, of which all but two (ENU-378, -449) are statistically different from the 1:1 Mendelian expectation for a dominant trait of mutant to wild-type offspring resulting in the outcross of a heterozygote. The

Table 2. *Penetrance and fertility effects of dominant cataract mutations recovered in mice after paternal ENU treatment.*

(Penetrance and fertility were calculated for the cross: Original Mutant × Strain 101/El)

Mutant	Offspring mutant:wild type	Penetrance	Mean litter size ($\bar{x}$, S.D., n)	Relative fertility
+/+* (♂)	—	—	6·86 ± 2·29 (470)	1·00
+/+* (♀)	—	—	7·61 ± 2·53 (371)	1·00
	Mutants with penetrance less than 0·80			
ENU- 68 (♂)	9:64†	0·25	6·66 ± 0·57 (3)	0·97
-228 (♂)	3:31†	0·18	6·66 ± 2·51 (3)	0·97
-262 (♀)	24:48†	0·66	9·00 ± 1·00 (3)	1·18
-378 (♂)	6:16	0·54	5·33 ± 0·57 (3)	0·78
-411 (♀)	20:75†	0·42	9·66 ± 3·05 (3)	1·27
-415 (♀)	14:30†	0·64	4·66 ± 0·57‡ (3)	0·61
-449 (♀)	11:18	0·76	6·00 ± 2·00 (3)	0·79
-464 (♀)	6:18†	0·50	3·33 ± 2·08‡ (3)	0·44
	Mutants with full penetrance			
ENU- 65 (♀)	12:11	1·04	7·66 ± 3·05 (3)	1·01
-288 (♂)	18:16	1·05	7·66 ± 2·51 (3)	1·12
-326 (♀)	45:29	1·22	9·00 ± 2·00 (3)	1·18
-369 (♀)	28:13†	1·37	5·66 ± 3·21 (3)	0·74
-410 (♀)	20:21	1·07	4·33 ± 1·52‡ (3)	0·57
-413 (♂)	13:15	0·93	6·66 ± 2·08 (3)	0·97
-418 (♀)	15:17§	0·93	7·33 ± 1·15 (3)	0·96
-424 (♂)	27:21	1·12	5·00 ± 2·64 (3)	0·73
-436 (♀)	12:16	0·86	5·33 ± 1·53 (3)	0·70

* Simultaneous control data taken as productivity data of presumed mutants recovered in the same ENU experiment subjected to a confirmation cross to strain 101/El mice and subsequently shown not to be a dominant mutation.

† Significantly different from the 1:1 Mendelian ratio of mutant and wild-type offspring resulting from the outcross of a heterozygote to homozygote wild-type for a dominantly expressed trait (chi-square with Yates' correction > 3·841).

‡ Mean litter size significantly different from the corresponding control litter size (*t* test).

§ In two subsequent litters in the confirmation cross 0 mutations were classified in 14 offspring. In view of the close approximation of the classification of the offspring in the initial litters to the Mendelian expectation and that the expressivity of this mutant is variable, misclassification of offspring may have occurred in the subsequent two litters. Results from these last two litters have not been included.

remaining nine mutations (ENU-65, -288, -326, -369, -410, -413, -418, -424, -436) have a calculated penetrance value approximating 1·0, ranging from 0·86 to 1·37, of which ENU-369 shows a significantly greater frequency of mutant phenotypes than the Mendelian expectation. Mutant ENU-418 has varying expressivity ranging from an anterior polar opacity to a total lens opacity. Carriers expressing a phenotype other than that described for the original mutation may have been misclassified in two litters compromising a total of fourteen offspring. When these two litters are ignored, ENU-418 is classified as having penetrance approximating 1·0. When compared to the appropriate control, two mutations (ENU-415, -464) with reduced penetrance and one mutation (ENU-410) with penetrance approximating 1·0 have significantly reduced litter size. The mean litter size relative to the corresponding control for mutants ENU-415, -464 and -410 was 0·61, 0·44 and 0·57 respectively. The remaining mutations have relative mean litter sizes ranging from 0·70 to 1·27.

(ii) *Subsequent outcrosses*

Table 3 contains penetrance and fertility data of further outcrosses of male and female mutant carriers to strain 101/El mice as well as male mutant carriers outcrossed to (101/El × C3H/El) F_1 hybrid mice. The first group of eight mutations corresponds to those determined in the original outcross to have reduced penetrance. This reduced penetrance was confirmed for all mutants when subsequent male carriers were outcrossed to strain 101/El. Mutants ENU-228, -262 and -464 were shown to have a significantly reduced number of mutant carriers when either male or female carriers were outcrossed to strain 101/El as well as when male carriers were outcrossed to (101/El × C3H/El)F_1 hybrids. For mutants ENU-68, -411, and -415, a significantly reduced number of mutant carriers were demonstrated in all instances where enough data were available to allow a statistically meaningful comparison. Mutation ENU-68 was lost and data are not available for outcrosses to (101/El × C3H/El)F_1 hybrids. Only two litters each were observed for the outcross of female carriers of mutants ENU-411 and -415 to strain 101/El. Two mutations, ENU-378 and 449, have variable results depending on the genotype of outcross partner. ENU-378 showed a reduced number of mutant offspring when male carriers were outcrossed to strain 101/El, but a good approximation of the expected Mendelian ratio was observed when either female carriers were outcrossed to strain 101/El or male carriers were outcrossed to (101/El × C3H/El)F_1 hybrids. The number of mutant offspring observed was reduced when mutant ENU-449 male or female carriers were outcrossed to strain 101/El, but full penetrance was observed when male carriers were outcrossed to (101/El × C3H/El)F_1 hybrids.

The nine mutations listed in the second group in Table 3 correspond to those mutations classified in Table 2 as having a penetrance value approximating 1·0. All outcrosses of male and female carriers to strain 101/El subsequent to the outcross of the original mutation were also shown to have penetrance values approximating 1·0 and, with the exception of mutant ENU-424, all outcrosses of male mutant carriers to (101/El × C3H/El)F_1 hybrids were also shown to have penetrance values approximating 1·0.

Table 3. *Penetrance and fertility effects of dominant cataract mutations in mice in subsequent outcrosses to strain 101/El or (101/El × C3H/El)F_1 hybrids*

	♂ */+ × 101/El			♀ */+ × 101/El			♂ */+ × (101/El × C3H/El)F_1		
Mutant	Offspring mutant:wild-type	Penetrance	Litter size $\bar{x}$, S.D., n	Offspring mutant:wild-type	Penetrance	Litter size $\bar{x}$, S.D., n	Offspring mutant:wild-type	Penetrance	Litter size $\bar{x}$, S.D., n
Mutations having reduced penetrance in original outcross									
ENU- 68	1:59*	0·03	8·57 ± 1·71 (7)	10·66*	0·26	5·50 ± 2·92 (8)	—	—	—
-228	19:57*	0·50	5·41 ± 1·62 (12)	9:22*	0·58	6·00 ± 1·41 (4)	14:53*	0·42	8·37 ± 3·58 (8)
-262	2:41*	0·09	5·28 ± 3·35 (7)	7:97*	0·13	8·00 ± 1·58 (5)	0:14*	0·00	7·00 ± 2·82 (2)
-378	21:46*	0·62	5·33 ± 1·96 (6)	20:16	1·11	6·00 ± 2·28 (6)	40:27	1·19	7·00 ± 3·61 (3)
-411	37:96*	0·56	5·16 ± 2·19 (19)	9:9	1·00	9·00 ± 1·41 (2)	23:86*	0·42	7·64 ± 3·20 (11)
-415	8:28*	0·44	5·66 ± 1·52 (3)	3:8	0·54	5·50 ± 0·70 (2)	56:102*	0·71	8·00 ± 2·52 (13)
-449	23:41*	0·72	5·11 ± 2·84 (9)	5:19*	0·42	6·33 ± 1·52 (3)	86:80	1·04	9·07 ± 2·39 (13)
-464	0:35*	0·00	2·83 ± 2·13 (6)†	1:22*	0·09	3·66 ± 1·15 (3)†	1:48*	0·04	4·50 ± 2·58 (6)†
Mutations having full penetrance in original outcross									
ENU- 65	43:59	0·84	6·71 ± 1·97 (14)	86:80	1·03	6·53 ± 2·77 (19)	82:84	0·99	6·81 ± 2·66 (16)
-288	48:41	1·07	5·91 ± 1·68 (12)	52:38	1·15	7·75 ± 2·60 (8)	47:50	0·97	8·60 ± 3·21 (5)
326	26:22	1·08	3·60 ± 1·83 (10)	31:45	0·82	7·50 ± 3·11 (4)	27:22	1·10	6·00 ± 2·16 (4)
-369	19:26	0·84	5·28 ± 1·70 (7)	37:31	1·08	5·87 ± 2·23 (8)	80:82	0·99	8·72 ± 2·33 (11)
-410	29:39	0·85	5·77 ± 2·90 (9)	5:7	0·83	6·00 ± 1·41 (2)	54:71	0·86	6·60 ± 2·59 (10)
-413	22:14	1·22	4·50 ± 2·00 (8)	51:69	0·85	8·50 ± 2·39 (8)	47:43	1·04	7·62 ± 2·56 (8)
418	27:31	0·93	4·66 ± 2·50 (6)	16:19	0·91	6·50 ± 3·78 (4)	65:57	1·06	8·00 ± 1·87 (9)
-424	41:56	0·85	4·57 ± 2·82 (7)	16:24	0·80	6·25 ± 1·25 (4)	25:53*	0·64	9·25 ± 3·59 (4)
-436	25:32	0·88	5·70 ± 2·40 (10)	42:50	0·91	5·28 ± 2·43 (7)	26:36	0·84	10·33 ± 1·15 (3)

* Significantly different from 1:1 mutant to wild-type Mendelian ratio expectation of the outcross of a heterozygote for a dominantly expressed trait to a homozygote wild-type (chi-square with Yates' correction > 3·841).

† Mean litter size significantly different from the mean litter size of those mutations shown not to have litter size effects in the original outcross generation (t test).

Of the three mutations shown to have a reduced litter size in the outcross to strain 101/El of the original mutation, only mutant ENU-464 showed litter size effects in subsequent outcross generations. This reduction in litter size was observed for both male ($t = 2{\cdot}69$, D.F. = 138) and female ($t = 2{\cdot}03$, D.F. = 91) mutant ENU-464 carriers outcrossed to strain 101/El as well as male mutant carriers outcrossed to (101/El × C3H/El)F_1 hybrids ($t = 3{\cdot}14$, D.F. = 99).

(iii) *Homozygous viability*

Table 4 lists for each mutation the number of possible homozygotes tested, the minimal number of homozygotes recovered, and the mean litter size of an intercross of heterozygotes. For two reasons, caution should be exercised with the

Table 4. *The minimal number of homozygotes recovered from the possible homozygotes tested for the various dominant cataract mutations in mice*

(Included is the mean litter size from the cross heterozygote × heterozygote.)

Mutant	Number of individuals tested for homozygosity	Minimal† number of homozygotes recovered	Litter size in cross (*/+ × */+) $\bar{x}$, S.D., n
	Mutations having reduced penetrance in original outcross		
ENU- 68	2	0	4·25 ± 1·50 (4)
-228	—	—	3·40 ± 1·14 (5)
-262	2	0	3·20 ± 1·64 (5)
-378	4‡	2	7·00 ± 1·00 (3)
-411	8	0	2·66 ± 2·42 (6)
-415	2	0	2·00 ± 1·31 (8)
-449	13	3	4·33 ± 2·25 (6)
-464	—	—	—
	Mutations having full penetrance in original outcross		
ENU- 65	15	0	4·81 ± 2·13 (11)
-288	17	4	6·83 ± 3·31 (6)
-326	16‡	9	7·33 ± 2·08 (3)
-369	10	2	9·66 ± 0·57 (3)
-410	9	2	7·00 ± 1·67 (6)
-413	4‡	3	8·50 ± 3·50 (6)
-418	6	1	9·67 ± 1·53 (3)
-424	6	1	3·43 ± 1·40 (7)
-436	8	2	6·17 ± 2·86 (6)

† In the cross of two possible homozygotes, when positive, it is concluded that at least one individual is homozygous.

‡ Homozygous phenotype can be recognized.

upper group of mutations shown to have reduced penetrance in Tables 2 and 3. First, since the mutations are not fully penetrant not as many carrier individuals are available for crosses. Older mice, often previously used in outcrosses, were used for intercrosses. The litter size data are, therefore, not comparable. Second, since the mutations are not fully penetrant, the expectations listed in Table 1 of observing wild-type offspring do not apply. The calculations in Table 1 assume full penetrance. One would expect, in crosses involving a homozygote for a mutation

with reduced penetrance, to observe wild-type offspring. Although one could make similar calculations taking penetrance into account, experiments to identify homozygotes for mutations with penetrance effects by the reduced number of wild-type offspring are impractical.

Homozygotes have been recovered for two mutations originally classified as having reduced penetrance (ENU-378, -449). For ENU-378, homozygous mutants are phenotypically recognizable and the genetic tests for homozygosity represent *inter se* crosses of individuals exhibiting the homozygous phenotype. The resulting offspring would be homozygous, thus avoiding penetrance effects of heterozygous individuals. Mutant ENU-449 was shown to have full penetrance when outcrossed to $(101/El \times C3H/El)F_1$ hybrids. Interestingly, the intercross mice from which homozygous mutants were recovered were derived from outcrosses to $(101/El \times C3H/El)F_1$ hybrids.

Of the nine mutations with penetrance approximating 1·0, homozygotes have been recovered from eight. It is therefore concluded that homozygotes for these mutations are viable and fertile. From one of the nine mutations with penetrance approximating 1·0 (ENU-65), homozygotes have not been recovered. Since fifteen possible homozygotes were tested, one should expect one-third, i.e. five homozygotes, to have been recovered among the mutant offspring tested.

One-way analysis of variance indicated significant variability of mean litter size for intercrosses of heterozygotes among the mutants classified as having penetrance approximating 1·0 ($F = 2{\cdot}94$, D.F. $= 8, 42$). *A posteriori* means comparisons indicate that the variability exists among the mutants with extremely small litter sizes (ENU-65, -424) and those with extremely large litter sizes (ENU-369, -413, -418). Of the mutations with extremely small litter sizes, ENU-65 was shown not to have produced homozygotes and it is therefore concluded to be homozygous lethal. ENU-424 also was shown to have a reduced litter size although homozygotes were recovered. This may represent reduced fitness of homozygous and heterozygous offspring, the magnitude such that litter size effects are only evident by *inter se* crosses where 0·75 of the offspring would be expected to be either homozygous or heterozygous for the mutant allele.

4. DISCUSSION

(i) *Penetrance and viability estimates of mutations*

The data clearly show for one group of nine mutations (ENU-65, -288, -326, -369, -410, -413, -418, -424, -436) that neither penetrance nor viability is affected. Since the distortion in Mendelian segregation observed in the outcross of the originally recovered mutant ENU-369 was not observed in subsequent outcrosses, these results were concluded to be spurious. Similarly, the reduced litter size observed in the original outcross of ENU-410 was concluded to be due to chance. The single subsequent outcross (ENU-424 males outcrossed to $(101/El \times C3H/El)F_1$ hybrids) in which a distortion of the expected Mendelian ratio was observed among the twenty-seven subsequent outcross comparisons made for a mutation originally classified as fully penetrant, may also be a chance observation. Alternatively, this may be an instance in which the penetrance effects of a mutation are dependent on

the genetic background. Mutants ENU-378 and ENU-449 also show a distortion in the observed Mendelian ratio dependent on the genetic background to which they were outcrossed. Originally classified as having reduced penetrance when outcrossed to strain 101/El, this classification was upheld for mutant ENU-449 when either males or females were outcrossed to strain 101/El but not when males were outcrossed to (101/El × C3H/El)F_1 hybrids. For mutant ENU-378, subsequent outcrosses of males to strain 101/El showed less mutant offspring than expected, similar to that seen in the outcross of the original male mutant to strain 101/El. However, neither outcrosses of females to strain 101/El nor outcrosses of males to (101/El × C3H/El)F_1 hybrids showed a reduction in the expected number of mutant offspring observed.

The remaining six mutations (ENU-68, -228, -262, -411, -415, -464) classified as having reduced penetrance in the outcross of the original recovered mutation to strain 101/El, consistently showed a reduction in penetrance in subsequent outcrosses of either males or females to strain 101/El or males to (101/El × C3H/El)F_1 hybrids, when sufficient data were available for a meaningful statistical comparison. The reduced litter size for ENU-464 was observed in the outcross of the original mutant as well as all subsequent outcrosses, whereas for ENU-415 the litter-size reduction was only observed in the outcross of the original mutation and, therefore, concluded to be spurious. The data are reassuringly consistent for fifteen of the seventeen mutations characterized: nine mutations have full penetrance with no fertility effects as heterozygotes (ENU-65, -288, -326, -369, -410, -413, -418, 424, -436), five mutations have reduced penetrance with no fertility effects (ENU-68, -228, -262, -411, -415) and one mutation has reduced penetrance and reduced fertility (ENU-464).

The reduced penetrance and viability of mutant ENU-464 has prevented the recovery of a large enough number of mutant carriers for a satisfactory characterization of this mutation. However, it should be emphasized that this mutation causes a severe phenotype, total corneal opacity with iris anomaly (Favor, 1983). The expressivity of this mutation has been constant in subsequent outcrosses, and although the frequency of offspring exhibiting the mutant phenotype is low, it should not be confused with the phenotypic variants, neither severe nor unique, shown not to be due to dominant mutations (Favor, 1983).

That the distortion in the expected Mendelian ratio has been interpreted and calculated as a penetrance effect is, admittedly, simplistic. Observed distortions may arise due to a true penetrance effect in which all individuals with the mutant genotype do not express the mutant phenotype. Alternatively, the distortion in the observed frequency of mutant individuals may be due to a reduction in the viability of mutant individuals (referred to as s) or to meiotic drive. Table 5 indicates the expected relative frequencies of mutant and wild-type phenotype offspring resulting from the outcross of a heterozygote, assuming a dominant allele with either penetrance (P) or viability (s) effects. For those mutations which show a distortion in the Mendelian segregation ratio with normal relative fertility, the penetrance value should be assumed correct. However, to disprove the alternative hypothesis of a meiotic drive effect, extensive breeding experiments of classified wild-type offspring are required. For those mutations with a distortion in the

Mendelian ratio and a reduced relative fertility, a comparison of the calculated penetrance value (P) with that which one would observe if the segregation distortion were due to a viability effect of the mutant offspring (P^*), may differentiate between the alternative hypotheses. The calculations are given as follows:

P = Observed Mutant Offspring/Expected Mutant Offspring

P = Observed Mutant Offspring/[0·5 × (Total Offspring)]

$P^* = (1/2)(1-s)/(1/2)[1{\cdot}0-1/2(s)]$

where s is the average reduction in viability of mutant individuals.

Table 5. *Expected frequencies of offspring classified as mutant and wild type resulting from the outcross of a heterozygote to homozygote wild-type for a dominant trait assuming reduced penetrance* (P) *or reduced viability* (s).

Genotype	Penetrance (P)	Viability (s)
*/+	1/2 (P)	1/2 (1-s)
+/+	1/2+1/2 (1-P)	1/2
Total	1·0	1·0–1/2 (s)

Since $(1/2)(s)$ is the relative reduction in litter size of the mutant as compared to the corresponding control, s can be estimated from the fertility data. The penetrance value which would be calculated (P^*) should the distortion in the Mendelian ratio be due to a viability effect rather than a penetrance effect can therefore be made. These calculations for the mutants classified as having reduced penetrance are given in Table 6. Of the mutants classified as having reduced penetrance, four (ENU-68, -228, -262, -411) have a relative fertility approximating 1·0 and the calculation has not been made. For two mutants (ENU-378, -415) the expected penetrance values calculated assuming a viability reduction are at variance with the observed penetrance values calculated. The observed distortion in the Mendelian ratio is, therefore, not due exclusively to a reduced viability of the mutant individuals. Both penetrance and viability effects of the mutant may be present. For ENU-449 the expected penetrance value and the observed penetrance value calculated are in good agreement. Although statistically one cannot prove a hypothesis, these results are not inconsistent with the hypothesis that the distortion in the observed Mendelian ratio is due to a reduced viability of the mutant carriers. Also consistent with this hypothesis is the observation that, when mutant ENU-449 is outcrossed to $(101/El \times C3H/El)F_1$ hybrids, the fertility of the crosses is higher relative to similar outcrosses of the other recovered mutations and a distortion in the Mendelian ratio is not observed. The reduction in fertility observed for ENU-464 is too large to indicate only a reduced viability of mutant offspring, but rather a reduction in the number of both mutant and wild-type offspring which survive to weaning. As in the case of mutations ENU-378 and ENU-415, ENU-464 may have both penetrance and viability effects resulting in a reduced frequency of mutant offspring.

(ii) *Comparison to previously recovered dominant mutations*

Previous results have been reported for dominant mutations recovered in mice after parental X- or gamma-radiation treatment in which breeding tests of

transmissibility were subsequently performed. Of ten dominant cataract mutations recovered after gamma-irradiation treatment, Kratochvilova (1981) has shown four mutants to have 100 per cent penetrance with no viability effects, three mutants to have reduced penetrance with no viability effects, and three mutants to have reduced viability. Three mutations were shown to be homozygous viable and fertile. No homozygotes were found for the remaining mutations, but from the data it cannot yet be concluded that the mutations were homozygous lethal.

Table 6. *Expected calculation of penetrance should the distortion in Mendelian ratio result from reduced viability of mutants*

Mutant	Penetrance (P)	Relative fertility $1-(1/2)(s)$	$(1-s)/[1-(1/2)(s)]$ P^*
ENU- 68	0·25	0·97	—
-228	0·18	0·97	—
-262	0·66	1·18	—
-378	0·54	0·78	0·71
-411	0·42	1·27	—
-415	0·64	0·61	0·36
-449	0·76	0·79	0·73
-464	0·50	0·44	—

Of an additional three dominant cataract mutations recovered by Kratochvilova, one mutant was shown to have full penetrance and no viability effects and two mutants were characterized as having reduced penetrance with normal viability (Ehling *et al.* 1982).

Selby & Selby (1978) have shown that of thirty-one dominant mutations affecting the skeleton recovered after gamma-irradiation, nine had reduced penetrance and twenty-two had normal penetrance. Further, Selby (1982) has indicated seven of eight dominant skeletal mutations tested were homozygous lethal.

Of the dominant visible mutations recovered after X-irradiation treatment at Harwell which were subsequently classified, two were homozygous viable and four homozygous lethal (Lyon, Phillips & Fisher, 1979). Batchelor, Phillips & Searle (1966) reported six dominant visible mutations recovered after gamma-irradiation of which three were homozygous lethal. The remaining mutations failed to produce homozygotes but were not conclusively shown to be homozygous lethal. Together, the results indicate that of the dominant mutations recovered after parental radiation treatment approximately one-third to one-half have normal penetrance, one-third have reduced penetrance, and the remainder have reduced viability and, therefore, cannot be classified for penetrance effects. Of those mutations tested for homozygous viability, there are approximately equal numbers of mutants which are homozygous viable and lethal. However, there may be differences in the frequency of homozygous lethal mutations depending upon the genetic test system employed to recover dominant mutations and/or the experimental protocol used to characterize the recovered mutations. In the dominant cataract test possibly as high as two-thirds of the mutations tested were homozygous lethal, most of the dominant skeletal mutations tested were homozygous lethal, while more than half of the dominant visible mutations tested were homozygous lethal.

By comparison, of the dominant cataract mutations recovered after parental ENU treatment one-half to two-thirds have normal penetrance and one-half to one-third have reduced penetrance (depending upon how the variable mutants are classified). This frequency is only slightly different from the frequency of fully penetrant dominant mutations recovered after radiation treatment. Many more mutations in both treatment groups would have to be characterized before any meaningful comparison could be made. More striking is the low frequency of homozygous lethal mutations recovered after ENU treatment. Only one mutation was homozygous lethal of eleven mutations tested, in contrast to the radiation results in which more than one-third of the mutations tested, regardless of the mutation test by which they were recovered, were homozygous lethal. These results support the suggestion from specific locus experiments that the mutations recovered after ENU treatment may be mainly intragenic changes, whereas radiation-induced mutations may represent intergenic changes (Ehling *et al.* 1982).

(iii) *Use of dominant cataract mutations in genetic risk estimations*

Two methods have been employed to estimate the genetic risk to a human population associated with an increased mutation rate due to radiation or mutagen exposure. The direct approach (Ehling, 1976; Ehling & Neuhäuser, 1979; Selby & Selby, 1977; UNSCEAR, 1982) uses experimental mutation rate data to dominant alleles systematically determined for a genetic system controlling phenotype at predetermined tissues or organs. The mutation rate data are corrected by the reciprocal of the percentage of mutable loci controlling dominant deleterious effects which the systematically screened genetic system represents in order to express the mutation rate for all dominant deleterious alleles. The mutation rate data are further corrected for physical and biological factors, such as dose rate, dose fractionation, and germ-cell stage sensitivity differences. In the absence of the corresponding experimental data, the assumption is made that the mutation rate to dominant alleles is similarly dependent upon such factors as the mutation rate to recessive specific loci alleles from which the factors were calculated (Ehling, 1983).

The indirect approach of genetic risk estimation (Lüning & Searle, 1971; Childs, 1981; UNSCEAR, 1982) uses the experimentally determined exposure dose which results in an observed treatment group mutation rate to recessive specific loci alleles double the spontaneous mutation rate. The doubling dose determined for the specific loci is assumed to be representative for all different classes of mutations of genetically determined diseases in man.

The relative frequency of observed genetically affected individuals for a class of genetic disease due to newly occurring spontaneous mutations under mutation-selection equilibrium must be determined or assumed. The expected increase in affected individuals due to increased mutation rate may be calculated (Childs, 1981; UNSCEAR, 1982) given the relative increase in the mutation rate and the selection coefficient of mutant genotypes for a particular class of genetic disease.

One assumption is common to both genetic risk estimation procedures – that results from specific locus experiments (factors affecting the mutation rate or doubling dose) are representative for all classes of mutations. The dominant

cataract mutation test has been designed to screen simultaneously for recessive specific locus and dominant cataract mutations. Thus, in experiments which measure induced mutation rates to dominant alleles, direct comparisons between these two classes of mutation are made and have shown for radiation treatment that the factors of germ cell stage and dose fractionation similarly affect both classes of mutation (Ehling *et al.* 1982). Future dominant cataract mutation experiments will continue this comparison to include other factors shown in specific locus experiments to affect the yield of induced mutations.

5. CONCLUSIONS

The subsequent genetic characterization of presumed dominant cataract mutations has many advantages. First, results represent confirmed dominant mutations. Second, penetrance and fertility effects of the recovered mutations are obtained. Such information is important for a more accurate estimation of the genetic risk of an increased mutation rate.

For the direct estimation procedure, knowledge of the penetrance effects of recovered mutations could be used to indicate the number of dominant mutations induced but not observed due to reduced penetrance. One would expect the true number of mutations with reduced penetrance which were induced to be at least as great as the reciprocal of the average penetrance times the number of mutations with reduced penetrance actually observed. In the present example, the 17 mutants characterized consist of one group of nine with normal penetrance in the original outcross generation, one mutation with normal penetrance and reduced viability of mutant carriers, and one group of seven mutations with a distortion in the Mendelian ratio. Assuming this distortion to be due to a penetrance effect, the average penetrance value of the seven mutations is 0·48. Multiplying the number of mutations with reduced penetrance (seven) times the reciprocal of their average penetrance value (2·10) indicates that at least 15 mutations with reduced penetrance were induced. Thus, the experimental protocol may underestimate by a factor of at least one-third the true mutation rate. This correction procedure relies upon an accurate estimation of the penetrance effects of recovered mutations and a differentiation between an observed distortion in the Mendelian ratio being due to a true penetrance effect as opposed to a viability effect. Mutations with a reduced viability of mutant offspring will show a distortion in the Mendelian ratio but would not be missed in a screening procedure. By including such mutations in the group of mutations with penetrance effects, the correction would be overestimated.

In calculating the increased incidence of human hereditary disorders by the indirect estimation procedure, fertility effects of the occurring mutations have either been assumed (UNSCEAR, 1982) or calculated from epidemiological data (Childs, 1981). Although selective factors in humans against deleterious mutations are probably both biological and social, such data in animal models may be of interest. The average fertility of the 17 characterized dominant cataract mutations is 0·88, a value which agrees with that used for autosomal dominant deleterious mutations (Childs, 1981; UNSCEAR, 1982).

Third, information about penetrance effects may be of interest as animal models of the classes of human genetic diseases as outlined in UNSCEAR (1982). Mutants with severely reduced penetrance may correspond to that class of human dominant hereditary disorders with complex aetiology while mutants with normal penetrance would correspond to simple dominant disorders.

Fourth, these mutations represent a collection of systematically recovered dominant mutations which may be of general usefulness as dominant markers for mouse genetics or particular usefulness as a tool in analysing the developmental sequences and metabolic system of the lens. Finally, through characterization of further recovered dominant cataract mutations, a large enough group of fully penetrant and fully fertile mutations which are homozygous viable and fertile will be obtained with which to genetically identify the mutable loci screened by the dominant cataract test.

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