Effects of H-2 and Vitamin A on Eye Defects in Congenic Mice (43339)

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Abstract. **Pregnant mice congenic with C57BL/10 (B10.A, B10.BR, B10.D2, B10.A(2R), B10.A(5R), B10.A(15R), B10.A(1R), B10.A(18R), and B10.OL) were fed Purina Mouse Chow or the same diet plus 200 IU of vitamin A daily. The pregnant dams were sacrificed on the 18th day of gestation and the fetuses were sexed and examined for defects in eye development. It was found that the frequency of microphthalmia and anophthalmia in the female progeny of mice fed Mouse Chow was 7.4–9.2% in B10.A and B10.BR, 4.0–5.5% in B10.A(18R), B10, B10.A(5R), B10.A(1R), B10.A(15R), and B10.A(2R), and 0.8% and 1.4% in B10.D2 and B10.OL mice, respectively. On average, the frequency of these defects in the female progeny was 6.2 times greater than that in males ($P < 0.001$). The right eye was 5.8 times more often affected than the left ($P < 0.001$). The addition of vitamin A to the diet increased the frequency of these eye abnormalities in all strains, suggesting that this effect is not mediated by loci associated with H-2, as is the case with vitamin A-enhanced cleft palate. The addition of vitamin A to the diet did not affect the ratios of affected males to females, affected right to left eye, or microphthalmia to anophthalmia. The results suggest that there are two loci on chromosome 17, one centromeric to E_β and one telemeric to C4, that interact to determine to some degree the frequency of microphthalmia and anophthalmia.** [P.S.E.B.M. 1992, Vol 199]

In the mouse, microphthalmia and anophthalmia have been associated with mutations on chromosome 1 (1) in the region of the λ-crystallin cluster (lens obsolescence), chromosome 2 (2) (small eye and alleles Dickie small eye and Harwell small eye), and chromosome 6 (3) (osteopetrosis, mi), and with the ZRDCT/Ch strain (4). In reviewing the studies on cleft palate in congenic strains of mice done in this laboratory since 1976 (5), it was evident that the frequency of these eye defects varied considerably among strains that should have differed only in the region of the major histocompatibility complex (H-2) on chromosome 17 (6).

Presented below are the data gleaned from these studies which suggest that there are two loci on chromosome 17, one proximal to E_β and the other distal to C4, that interact to determine the frequency of microphthalmia and anophthalmia. Females were affected 6.2 times more frequently than were males, the right eye was affected 5.8 times more often than the left, and the addition of 200 IU of vitamin A per day to the mothers' diet increased the frequency of these defects in all strains without affecting the ratios of male to female, right eye to left eye, or microphthalmia to anophthalmia.

Materials and Methods

In previously reported studies on cleft palate (reviewed in Ref. 5), mice were sacrificed on the 18th day of pregnancy, and the number of living fetuses and resorptions was recorded. Living fetuses were weighed and examined for gross external abnormalities, including coloboma and microphthalmia and anophthalmia. The oral cavity was inspected for the presence of cleft palate, and the internal organs were examined for defects and to determine the sex. Between 1976 and 1984, five individuals performed fetal examinations. Since 1985, I have conducted all studies. Prior to 1985, there were no specific criteria for microphthalmia and anophthalmia; since that time, an eye is called microphthalmic when it is less than 70% the size found in littermates, and anophthalmia is indicated when there is no external evidence of eye formation.

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The data reported here have been gathered from noninjected or sham-injected groups that were controls for studies on glucocorticosteroid-induced cleft palate conducted between 1976 and 1988. Studies performed since 1988 have been for the sole purpose of investigating the roles of the major histocompatibility complex and vitamin A on the frequency of eye abnormalities and extending and/or confirming the older observations. The recent fire at the Jackson Laboratory (Bar Harbor, ME) has prevented confirmation of earlier work in B10.D2 and B10.A mice.

The congenic strains B10.D2, B10.OL, C57BL/10, B10.A(18R), B10.A(5R), B10.A(1R), B10.A(15R), B10.A(2R). B10.BR, and B10.A were maintained in this laboratory by brother × sister matings. The differences in H-2 haplotypes are shown in Table II (see also Ref. 6). In the experiments, one male and two virgin 10- to 12-week-old females were placed in each cage. The day a vaginal plug was detected was considered to be Day 0 of pregnancy. On the 18th day of gestation, the pregnant mice were sacrificed and the fetuses were examined as noted above.

The average pregnant female mouse consumes approximately 5 g of food per day (i.e., the equivalent of one average Purina Mouse Laboratory Chow biscuit, which contains 12 IU/g of vitamin A). In indicated experiments, 200 IU of vitamin A were added to each biscuit by soaking the biscuit in 0.2 ml of vitamin A palmitate (Sigma Chemical Co., St. Louis, MO) in vegetable oil (1,000 IU/ml). The biscuits fed to the control groups in the studies on the effects of vitamin A were soaked in 0.2 ml of vegetable oil only. The mice in the control groups consumed approximately 60 IU of vitamin A daily (2,400 IU/kg) and the experimental group consumed approximately 260 IU (10,400 IU/kg).

The sampling unit in these studies is the individual fetus. Frequencies were compared by means of the χ^2, Yates correction χ^2 tests, and log linear analyses (7) using the NCSS statistical program (NCSS, Kaysville, UT). Parenthetically, the injection of dexamethasone on the 12th day of gestation to induce cleft palate in the progeny did not appear to influence the frequency of eye defects until the dose reached very high levels (>280 mg/kg in most strains; usual dose, 80 mg/kg). These data have not been included in order to eliminate a potentially confounding factor.

Table I. Association of Microphthalmia with Anophthalmia but not with Coloboma

Microphthalmia	Anophthalmia[a]		Coloboma[b]	
	Yes	No	Yes	No
Yes	17	141	1	157
No	57	5692	20	5729

[a] χ^2, 118.6, $P < 0.0001$.
[b] χ^2, 0.35.

Results

The frequencies of microphthalmia, anophthalmia, and coloboma and their concurrence among 5907 fetuses of all strains are shown in Table I. The results indicate that coloboma and the other two eye defects are not associated traits; however, there appears to be a strong association between microphthalmia and anophthalmia. Similar results were obtained when the data from the individual strains were analyzed. On this basis, microphthalmia and anophthalmia will be considered to be manifestations of perturbations along a common final pathway.

The frequency of microphthalmia and anophthalmia varied considerably among the strains studied (Table II). B10.D2 and B10.OL mice had low frequencies, B10.A and B10.BR mice had high frequencies, and the other strains were intermediate. As will be discussed

Table II. Frequency and Side (Right or Left) of Microphthalmia and Anophthalmia in Male and Female 18-Day-Old Congenic Mouse Fetuses from Mothers Fed Purina Laboratory Chow[a]

Strain	H-2 haplotype				Male					Female				
	K	Eβ	S	D	Number/total	(%)	R	R&L	L	Number/total	(%)	R	R&L	L
B10.D2	d	d	d	d	0/170	(0.0)	0	0	0	1/122	(0.8)	1	0	0
B10.0L	d	d	k	k	0/59	(0.0)	0	0	0	1/70	(1.4)	1	0	0
C57BL/10	b	b	b	b	0/232	(0.0)	0	0	0	11/263	(4.2)	4	5	2
B10.A(18R)	b	b	b	d	5/539	(0.9)	1	1	3	22/547	(4.0)	10	10	2
B10.A(5R)	b	b/k	d	d	2/218	(0.9)	2	0	0	11/225	(4.9)	7	3	1
B10.A(1R)	k	k	d	b	1/130	(0.8)	1	0	0	7/133	(5.3)	5	2	0
B10.A(15R)	k	k	d	b	2/140	(1.4)	1	1	0	9/171	(5.3)	6	3	0
B10.A(2R)	k	k	d	b	3/272	(1.1)	2	0	1	16/290	(5.5)	8	6	2
B10.BR	k	k	k	k	3/311	(0.9)	3	0	0	31/335	(9.2)	14	13	4
B10.A	k	k	d	d	5/375	(1.3)	3	1	1	27/364	(7.4)	20	5	2

[a] B10.D2 vs B10.A: χ^2, 10.7; $P < 0.01$; B10.D2 vs C57BL/10: χ^2, 4.32; $P < 0.05$; B10.A(18R) vs B10.A: χ^2, 10.4; $P < 0.01$; and B10.A(2R) vs B10.BR: χ^2, 2.54.

below, females had higher frequencies of eye defects than males, and the right eye was affected more often than the left.

The progeny of reciprocal crosses between strains with low and high frequencies of eye defects had intermediate values (Table III). A maternal effect was not noted.

The progeny of pregnant mice of all strains given added vitamin A in the diet had higher frequencies of microphthalmia and anophthalmia (Tables IV and V). The incidence of coloboma was not affected by vitamin A (data not shown). In general, the lower the frequency of eye defects on the control diet, the greater the increase in incidence with added vitamin A. Exception to this was noted in the B10.A(15R) and B10.A(2R) strains.

Data shown in Table VI indicates that the added vitamin A did not affect the ratio of affected females to males. Similarly, vitamin A did not appear to change the ratios of right to left eye defects (Table VII) or of anophthalmia to microphthalmia (Table VIII). These findings were true for all strains (data not shown).

Log linear analyses of multiway tables confirmed the results of the χ^2 tests reported in the tables: Regular diet versus added vitamin A, $P = 0.0001$; male versus female, $P = 0.0001$; right eye versus left eye, $P = 0.0014$; B10.D2 and B10.OL versus C57BL/10, B10.A(18R), and B10.A(5R), $P = 0.016$; C57BL/10, B10.A(18R), and B10.A(5R) versus B10.A(1R), B10.A(2R), and B10.A(15R), $P = 0.21$; B10.A(1R), B10.A(2R), and B10.A(15R) versus B10.BR and B10.A, $P = 0.15$; B10.BR and B10.A versus C57BL/10, B10.A(18R), and B10.A(5R), $P = 0.0054$.

Table III. Incidence of Microphthalmia and Anophthalmia among the Progeny of Crosses between Congenic Strains of Mice with High and Low Frequencies[a]

Strain (F_1)	Male	Female	χ^{2b}
	Number/ total (%)	Number/ total (%)	
B10.BR × B10.D2	0/53 (0.0)	2/55 (3.6)	
B10.D2 × B10.BR	0/41 (0.0)	0/40 (0.0)	1.05
B10.D2 × B10.A	0/24 (0.0)	1/31 (3.2)	
B10.A × B10.D2	0/45 (0.0)	1/38 (2.6)	0.18
C57BL/10 × B10.A	2/29 (6.9)	1/36 (2.7)	
B10.A × C57BL/10	0/40 (0.0)	2/31 (6.4)	0.13
B10/A × B10.BR	0/45 (0.0)	1/46 (2.1)	
B10.BR × B10.A	0/48 (0.0)	3/52 (5.7)	0.17
B10.A(18R) × B10.A	0/38 (0.0)	1/32 (3.2)	

[a] Mothers were fed Laboratory Chow.
[b] Yates correction.

Discussion

Clearly, the frequency of microphthalmia and anophthalmia varied considerably among these strains of mice, which are assumed to differ only in the region of H-2 (6). Strains that have H-2^d alleles proximal to E_β were found to have low frequencies of eye defects (B10.D2 and B10.OL), and strains that have H-2^b alleles in the same region (C57BL/10, B10.A(5R), and B10.A(18R)) had intermediate frequencies. The B10.BR strain (H-2^k) had the highest incidence. B10.A mice that have H-2^k alleles proximal to E_β, but H-2^d alleles distal, had a high frequency of eye defects, but B10.A(1R), B10.A(2R), and B10.A(15R) mice, which are identical to B10.A mice proximal to S but have H-2^b alleles distal to an undetermined point between C4 and tumor necrosis factor (5) had intermediate values, suggesting that a locus in this region has some effect on the incidence of these eye defects. A candidate for the locus proximal to E_β is the αA crystallin gene (*Acry*-1), which maps between glyoxylase (*Glo*-1) and H-2K (8). Restriction fragment length polymorphism analysis of inbred strains places H-2^d and H-2^q strains in *Acry*-1^a and H-2^a, H-2^k, and H-2^b strains in *Acry*-1^b. Exceptions to this are the strains 129/J (H-2^b) and MA/My (H-2^K), which have *Acry*-1^a alleles. Data are not available on the Acry allotypes of the congenic strains used in these studies.

Table IV. Frequency of Microphthalmia and Anophthalmia in Male and Female 18-Day-Old Congenic Fetuses from Mothers Fed Laboratory Chow plus 200 IU Vitamin A Daily

Strain	Male					Female				
	Number/total	(%)	R	R&L	L	Number/total	(%)	R	R&L	L
B10.0L	0/61	(0.0)	0	0	0	7/60	(11.7)	3	2	2
C57BL/10	1/45	(2.2)	1	0	0	5/41	(12.2)	4	0	1
B10.A(18R)	1/30	(3.3)	1	0	0	6/33	(18.2)	6	0	0
B10.A(5R)	1/66	(1.5)	1	0	0	7/74	(9.4)	4	2	1
B10.A(1R)	2/73	(2.7)	1	1	0	5/52	(9.6)	4	1	0
B10.A(15R)	2/101	(1.9)	2	0	0	26/128	(20.3)	16	9	1
B10.A(2R)	0/17	(0.0)	0	0	0	7/18	(38.8)	4	3	0
B10.BR	4/139	(2.9)	4	0	0	21/149	(14.1)	15	4	2
B10.A	0/55	(0.0)	0	0	0	7/44	(15.9)	2	4	1

Table V. Effect of Added Dietary Vitamin A (200 IU/day) on the Frequency of Microphthalmia and Anophthalmia in 18-Day-Old Congenic Mouse Fetuses

Strain	Frequency (%)[a]					
	Male			Female		
	Added vitamin A	Regular diet	Increase (X)	Added vitamin A	Regular diet	Increase (X)
B10.0L	0.0	0.0		11.7	1.4	8.3
C57BL/10	2.2	0.0		12.2	4.2	2.9
B10.A(18R)	3.3	0.9	3.7	18.2	4.0	4.5
B10.A(5R)	1.5	0.9	1.7	9.4	4.9	1.9
B10.A(1R)	2.7	0.8	3.4	9.6	5.3	1.8
B10.A(15R)	1.9	1.4	1.3	20.3	5.3	3.8
B10.A(2R)	0.0	1.1		38.8	5.5	7.0
B10.BR	2.9	0.9	3.2	14.1	9.2	1.5
B10.A	0.0	1.3		15.9	7.4	2.1

[a] Mean: 16.68 ± 8.56%; B10.A(5R) vs B10.A(15R): χ^2, 4.04, $P < 0.05$; B10.A(1R) vs B10.A(2R): χ^2, 7.57, $P < 0.01$.

Table VI. Effect of Added Dietary Vitamin A (200 IU/day) on the Male to Female Incidence of Microphthalmia and Anophthalmia

Added vitamin A?	Frequency of eye defects	
	Males[a] (Number/total)	Females[a] (Number/total)
No	21/2446	136/2520
Yes	12/587	91/599

[a] χ^2, 0.16.

Table VII. Effect of Added Dietary Vitamin A (200 IU/day) on the Incidence of Right, Left, and Bilateral Eye Defects

Added vitamin A?	Males			Females		
	R	R&L	L	R	R&L	L
No[a]	13	3	5	76	47	13
Yes[a]	11	1	0	58	25	8

[a] R vs L, male and female: χ^2, 2.76.

The addition of vitamin A to the diet increased the frequency of microphthalmia and anophthalmia in all strains studied, suggesting that this effect is mediated by a locus not associated with H-2, as was the case with cleft palate, where only strains that had H-2^b alleles between C4 and tumor necrosis factor had increased frequencies when the mothers were fed added vitamin A (5). Although, the added vitamin A increased the frequency of these eye defects, it did not have any significant effect on the ratio of microphthalmia to anophthalmia, which was somewhat surprising if one considers the latter to be merely a more severe form of the former. One possible explanation for this may be that the vitamin A affects a developmental process early in the pathway, but not at the very beginning, and that anophthalmia may represent atrophy independent of disrupted development. That is, excess vitamin A may initiate prematurely, delay, or interupt an early process in the development of the eye, and atrophy may occur in a proportion of the affected eye through a process not dependent upon vitamin A. Alternatively, the vitamin A may affect a step in development which, if totally interrupted, leads to failure of external eye formation or, if delayed, produces a smaller eye.

The studies reported here confirm the findings that females are affected more often than males (6 times more frequently), and that the right eye is affected more often than the left (5.8 times more often) (9); at the dose used, vitamin A did not materially alter these ratios.

Table VIII. Effect of Added Dietary Vitamin A (200 IU/day) on the Ratio of Microphthalmia to Anophthalmia[a]

	Laboratory Chow				Laboratory Chow plus vitamin A			
	Male		Female		Male		Female	
	M	A	M	A	M	A	M	A
Total	13/2446	8/2446	99/2520	37/2520	8/587	3/587	62/599	29/599
Percentage	0.53	0.33	3.92	1.46	1.36	0.50	10.3	4.84
M:A	1.60		2.68		2.72		2.13	

[a] Males, χ^2, 0.37; Females, χ^2, 3.82, $0.1 > P > 0.05$; Male and female, χ^2, 2.29. M, microphthalmia; A, anophthalmia.

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