Effects of H-2 and Vitamin A on Micrognathia in Congenic Mice (43451)

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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 eighteenth day of gestation, and the fetuses were sexed and examined for defects in mandibular development.**

On average, micrognathia occurred five times more frequently in female (1.5%) than male (0.3%) fetuses. The addition of vitamin A to the diet affected only females, reducing the frequency of this defect to that observed in males from dams fed the control diet. Micrognathia was strongly associated with micro- or anophthalmia, but not with defects of the palate. C57BL/10 fetuses had the highest frequency of micrognathia (3.2%) and B10.D2 and B10.A(5R) fetuses had the lowest (0.1%). The results suggest that a locus distal to C4 and perhaps proximal to Qa-1 may exert a moderate influence on mandibular development and a second locus proximal to E_β may have a weak effect.

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A severely underdeveloped mandible (micrognathia) is a relatively uncommon birth defect that may occur as an isolated finding, as a component of several rare syndromes, or as a result of teratogenic agents (1–3). In reviewing studies on cleft palate in congenic strains of mice done in this laboratory since 1976 (4), it appeared that the frequency of micrognathia varied among strains which should differ only in the region of the major histocompatibility complex (H-2) on Chromosome 17 (5), between the sexes, and with the amount of vitamin A in the diet.

Presented below are data gleaned from these studies which suggest that there is an H-2-associated locus distal to C4 that determines to some degree the frequency of micrognathia. This defect was found to be approximately five times more frequent in females, and the addition of vitamin A to the diet reduced the frequency in females to that observed among males. Micrognathia and microphthalmia, but not cleft palate, were strongly associated traits.

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Materials and Methods

In previously reported studies on cleft palate (reviewed in [4]), mice were sacrificed on the eighteenth day of pregnancy, and the number of living fetuses and resorptions was recorded. Living fetuses were weighed and examined for gross external abnormalities including micrognathia and micro- 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 micrognathia; since that time, the mandible has been considered underdeveloped when it is less than 70% the size found in littermates.

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 (MHC) and vitamin A on the frequency of eye abnormalities and extending or confirming older observations. The May 1989 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 I (see also Ref. 5). In the experiments, one male and two virgin 10–12-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 eighteenth 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 (5001) 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 Fisher exact test and by log-linear analyses (6) using the NCSS statistical program (NCSS, Kaysville, Utah). Parenthetically, the injection of dexamethasone on the twelfth day of gestation to induce cleft palate in the progeny did not appear to influence the frequency of micrognathia 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.

Results

On average, micrognathia was observed five times more often in females than males when the mice were fed the regular diet (1.5% vs 0.3%, $P = 0.0000$, Table I). The addition of vitamin A to the diet affected only females, reducing the frequency to that observed in males from dams fed the control diet (1.5% vs 0.2%, $P = 0.0009$).

The frequency of micrognathia varied moderately among the strains given the regular diet. C57BL/10 had the highest frequency (3.2%, male and female) and B10.A(5R) and B10.OL had the lowest (0.0%). There were relatively few fetuses observed among B10.OL, B10.A(15R), and B10.A(1R) strains on the regular diet and B10.A(5R), B10.OL, B10.A(1R), and B10.A(2R) strains given added vitamin A; these data were not used for comparisons among strains or diets, but where noted they were combined with those from genetically similar strains or identical diets for comparisons among groups.

The frequency of micrognathia in C57BL/10 mice was significantly higher than in B10.A(5R) ($P = 0.025$, differs from C57BL/10 between E_B and Qa-1) and B10.A(18R) mice ($P = 0.004$, differs from C57BL/10 from a point between C4 and H-2D and Qa-1). The incidence of this defect in C57BL/10 was not significantly different from that noted in B10.BR, B10.A, and B10.A(2R), as individual strains or as a group.

The incidence of micrognathia was similar in B10.BR (2.3%), B10.A (2.2%), and B10.A(2R) (2.2%) mice, and these frequencies did not differ significantly from those of the other strains when tested individually as male and female combined or as females only. However, when the two strains that have H-2^k alleles centromeric to E_B (B10.BR and B10.A) are combined and compared with those strains that do not and have *k* or *d* alleles but not *b* alleles between E_B and Qa-1 (B10.A(5R), B10.OL, and B10.D2), the frequency of the defect was significantly lower in the latter group (22/1984 vs 1/779, respectively; $P = 0.004$).

The frequency of micrognathia in the progeny of reciprocal crosses selected for studies on microphthalmia (7) tended to be intermediate between those noted in the parental strains (Table II), which suggests that the trait is recessive or codominant. A maternal effect was not noted (data not shown). Approximately 50% of the fetuses with micrognathia also had microphthalmia or anophthalmia; there was no association with cleft palate in groups given dexamethasone on Day 12 of gestation (Table III).

Discussion

Malformations such as isolated cleft palate (4), microphthalmia (7), otocephaly, micrognathia, ventricular septal defects (8), lateral cleft lip, open eye lids, and atrial septal defects (9, 10) normally are noted infrequently in inbred strains of mice. Most likely, these defects are the result of the interaction early in the midgestational period of genetic and environmental factors.

As was noted previously with isolated cleft palate (4) and microphthalmia (7), the results reported here suggest that both H-2-associated genes and dietary vitamin A influence to some degree the development of the mouse mandible. The frequency of the malformation varied significantly between the strains, which differ only in the region of the MHC: The frequency of the defect was five times higher in females fed a regular diet than in males and was strongly associated with microphthalmia but not isolated cleft palate (11). The addition of vitamin A to the diet affected only female fetuses, decreasing the frequency of the defect to that observed in males from dams fed the regular diet. In contrast to studies on dexamethasone-induced cleft palate in which added vitamin A increased the frequency of the defect only in strains that bore *b* alleles between C4 and H-2D (4), the frequency of micrognathia was

Table I. Frequency of Micrognathia in Male and Female 18-Day-Old Congenic Mouse Fetuses from Mothers Fed Laboratory Chow with and without Added vitamin A (200 IU/day)

Strain[a]	H-2 haplotype				Number/total (percentage)			
					Male[b]		Female	
	K	Eβ	S	D	Regular diet[c]	Added vitamin A	Regular diet	Added vitamin A
B10.A(5R)	*b*	*b/k*	*d*	*d*	0/144 (0.0)	1/45 (2.2)	0/168 (0.0)	0/57 (0.0)
B10.OL	*d*	*d*	*k*	*k*	0/49 (0.0)	0/50 (0.0)	0/54 (0.0)	0/50 (0.0)
B10.D2	*d*	*d*	*d*	*d*	0/209 (0.0)		1/155 (0.6)	
B10.A(18R)	*b*	*b*	*b*	*d*	0/682 (0.0)	0/159 (0.0)	7/672 (1.0)	0/164 (0.0)
B10.A(15R)	*k*	*k*	*d*	*b*	1/73 (1.3)	0/66 (0.0)	0/98 (0.0)	0/81 (0.0)
B10.A(1R)	*k*	*k*	*d*	*b*	0/49 (0.0)	1/65 (1.5)	1/60 (1.7)	0/46 (0.0)
B10.A(2R)	*k*	*k*	*d*	*b*	1/274 (0.4)	0/27 (0.0)	5/284 (1.8)	0/33 (0.0)
B10.BR	*k*	*k*	*k*	*k*	1/360 (0.3)	0/127 (0.0)	7/355 (2.0)	1/154 (0.6)
B10.A	*k*	*k*	*d*	*d*	2/652 (0.3)	0/141 (0.0)	12/617 (1.9)	0/145 (0.0)
C57BL/10	*b*	*b*	*b*	*b*	3/397 (0.7)	0/93 (0.0)	10/393 (2.5)	1/111 (0.9)
Totals					8/2889 (0.3)	2/773 (0.3)	43/2856 (1.5)	2/841 (0.2)

[a] B10.A(5R) versus B10.A(18R), $P = 0.36$; B10.A(18R) versus C57BL/10, $P = 0.0039$ (two-tailed Fisher exact test).
[b] Male versus female: all strains, regular diet, $P = 0.0000$; added vitamin A, $P = 0.625$.
[c] Regular diet versus added vitamin A: all strains, male and female, $P = 0.0077$; females only, $P = 0.0009$; males only, $P = 0.698$.

Table II. Incidence of Micrognathia among the Progeny of Crosses Between Congenic Strains of Mice with High and Low Frequency[a]

F_1 Hybrid	Number/total (percentage)	
	Male	Female
B10.A(1R) × B10.A(2R)	0/153 (0.0)	1/167 (0.6)
B10.A(1R) × B10.A(18R)	0/59 (0.0)	1/87 (1.1)
B10.A(15R) × B10.A(18R)	0/117 (0.0)	0/111 (0.0)
B10.BR × B10.D2	0/94 (0.0)	0/95 (0.0)
B10.A × B10.BR	0/87 (0.0)	3/105 (2.9)
B10.A × B10.D2	0/69 (0.0)	0/70 (0.0)
C57BL/10 × B10.A	1/68 (1.5)	1/67 (1.5)

[a] Mothers were fed laboratory chow.

Table III. Association of Micrognathia with Microphthalmia but not with Cleft Palate[a]

Micrognathia	Microphthalmia[b]		Cleft palate[c]	
	Yes	No	Yes	No
Yes	23	28	8	62
No	281	5413	938	5855

[a] Mothers were fed laboratory chow.
[b] $P = 0.0000$, Fisher exact test.
[c] $P = 0.569$. Dams given 80 mg/kg of dexamethasone on Day 12 (4).

decreased in virtually all strains, which suggests that in this malformation, the locus responsible for the effect is not associated with the MHC. The same vitamin A-supplemented diet increased the frequency of microphthalmia in male and female mice of all strains reported here (7).

The highest incidence of micrognathia was found in C57BL/10 mice and the frequencies were significantly lower in the two strains that share a large number of MHC-associated *b* alleles with the host, i.e., B10.A(5R) and B10.A(18R). These results strongly suggest that a locus distal to the proximal crossover point in B10.A(18R) mice, i.e., between C4 and tumor necrosis factor, and Qa-1 exerts a moderate effect on the development of the mouse mandible. The other strains that share H-2^b alleles with C57BL/10 at some point distal to C4 (B10.A(1R), B10.A(2R), and B10.A(15R)) do not differ significantly from the host strain and, therefore, are of no help in mapping.

No significant differences in the frequency of micrognathia are noted when strains bearing *k* and/or *d* alleles in the MHC are compared, i.e., B10.BR, B10.OL, B10.D2, and B10.A. However, when those strains that have *k* alleles proximal to E_B (B10.BR and B10.A) are combined and compared with those that have *d* alleles in the same region (B10.D2 and B10.OL), the results ($P = 0.023$) suggest the possibility of a locus proximal to E_B that may have a weak effect on mandibular development. This conclusion is viewed with some caution, because the comparison was selective and the difference was marginally significant.

This caveat not withstanding, the results reported here are consistent with a model of complementation between H-2-associated genes similar to that noted in the cases of isolated cleft palate (4), microphthalmia (7), and immune response products (12, 13); that is, one locus proximal to E_B and the second distal to C4 act together to influence mandibular development. From this model, high frequencies of micrognathia would be found in strains with *b-b*, *k-k*, and *k-d* alleles, low frequencies with *d-d*, *d-k*, and *b-d* alleles, and intermediate (to high) frequencies with *k-b* alleles.

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