Effects of H-2 and Dietary Vitamin A on the Frequency of Dorsoventral Vaginal Septum

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Abstract. **The frequency of dorsoventral vaginal septum (DVS) in mice is determined in part by genes associated with the major histocompatibility complex H-2. Data presented here confirm that one locus (*DVS-1*) maps centromeric to Eα and that the second (*DVS-2*), previously shown to be associated with the S-to-D region, maps to the C4:B144 interval, most likely between *Dcp-2* which contributes to glucocorticoid-induced cleft palate susceptibility and *Acp* which enhances cleft palate susceptibility through the action of vitamin A. Comparisons of data obtained in this laboratory in the periods 1981–1983 and 1985–1990 and observations from the production colony from which many of the strains were purchased revealed minor variations in frequencies of DVS within strains which may be due to differences in ascertainment and/or to environmental factors. The addition of vitamin A to the diet of pregnant mice at a dose that increases the frequency of cleft palate in susceptible strains had no effect on the incidence of DVS.** [P.S.E.B.M. 1993, Vol 203]

Dorsoventral vaginal septum (DVS) is a spontaneously occurring birth defect that results in a decrease in fertility but has no effect on fecundity (1). The septa are longitudinal bands of tissue of varied thickness that bisect the vaginal canal and most likely are the result of failure of programmed cell death in the fused müllerian ducts which form the upper two thirds of the murine vagina.

Studies with H-2 congenic strains of mice have indicated that (i) the mode of inheritance is dominant for high frequency, (ii) there is no maternal effect, and (iii) two H-2-associated genes, one centromeric to Eα (*DVS-1*) and the other in the S-to-D subregion (*DVS-2*), contribute significantly to the occurrence of this anomaly (2, 3).

In the experiments presented in this report, the original observations are expanded by measuring DVS frequency in H-2 congenic strains with haplotypes that are recombinants of H-2^a and H-2^b where the recombinant event has occurred in the C4:B144 interval, thus allowing for more accurate mapping of *DVS-2*. In addition, the frequencies of DVS obtained in the original studies (1981–1983) are compared with the results obtained over a subsequent 5-year period. The effects of dietary vitamin A on the incidence of DVS were studied because a vitamin-A-responsive gene (*Acp*) which affects the incidence of cleft palate maps to the C4:B144 region, as does *DVS-2* (4). Finally, the incidence of DVS was examined in the progeny of reciprocal crosses between strains with low and intermediate or high frequencies.

Materials and Methods

The breeding stock of the H-2 congenic strains C57BL/10 (B10), B10.A, B10.BR, B10.D2, B10.A(5R), and B10.A(2R) were obtained from Jackson Laboratory, Bar Harbor, ME; strains B10.A(1R), B10.A(15R), and B10.A(18R) were from Jack Stimpfling, Columbus Hospital, Great Falls, MT; and B10.OL was from D. C. Shreffler, Washington University School of Medicine, St. Louis, MO. The H-2 haplotypes of these strains are shown in Table I (see also Refs. 4–7). The mice were maintained in this laboratory by brother × sister matings. The virgin female mice were examined with a blunt probe for the presence of DVS at 6 weeks of age.

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Table I. MHC Alleles of Congenic Strains Used in These Studies and Relative Frequency of DVS[a]

Strain	Centromeric to H-2K	K	A	Eβ	Eα	C4	Presumed			B144	TNFα	D	*Qa-1*	Telomeric to *Qa-1*	DVS[b]
							Acp	*DVS-2*	*Dcp-2*						
B10.A	*k* or *b*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*a*	*b*	L
B10.A(15R)	*k* or *b*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*b*	(*b*)	*b*	*b*	*b*	*b*	L
B10.0L	*d* or *b*	*d*	*d*	*d*	*d*	*k*	*k*	*k*	*k*	(*k*)	*k*	*k*	*a*	*b*	L
B10.A(1R)	*k* or *b*	*k*	*k*	*k*	*k*	*d*	*d*	*b*	*b*	(*b*)	*b*	*b*	*b*	*b*	M
B10.BR	*k* or *b*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	(*k*)	*k*	*k*	*a*	*b*	M
B10	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	M
B10.A(18R)	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*d*	*d*	*d*	*a*	*b*	M
B10.A(2R)[c]	*k* or *b*	*k*	*k*	*k*	*k*	*d*	*d/b*	*d/b*	*d/b*	b	b	b	b	b	M
B10.D2	*d* or *b*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	(*d*)	*d*	*d*	*a*	*b*	M
B10.A(5R)	*b*	*b*	*b*	*b/k*	*k*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*a*	*b*	H

[a] See Refs. 4–7; Alleles in parentheses, presumed.
[b] Relative frequency: L, low; M, intermediate; H, high.
[c] Evidence suggests that B10.A(2R) is the result of an unequal crossover in this region (Ref. 5).

These examinations were performed by three individuals in the period 1981–1983 and by one person thereafter. The experiments reported here extended over a period of 5 years (1985–1990).

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 contained 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. 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 dams were started on the vitamin-A-supplemented diets on the day the vaginal plug was detected.

The sampling unit in these studies is the individual mouse. Frequencies were compared by means of the Fisher exact test and by log linear analyses (8) using NCSS (Kaysville, UT) statistical programs.

Results

The frequency of DVS observed in the 10 congenic strains examined between 1985 and 1990 are listed in Table II, and they are compared with data obtained in 1981–1983, which has been reported previously (2, 3). It was found that with the exception of the B10 strain (19.6% vs 11.8%, $P = 0.02$), the present results are in good agreement with those noted earlier. In addition, in the case of B10.A mice, the frequency of DVS did not differ significantly from that observed in the production colony of Jackson Laboratory in 1990 (4.3% vs 8.0%); however, the Jackson Laboratory did report a lower frequency among their B10.A(5R) mice (17% vs 29.1%, $P = 0.046$; the results obtained in this laboratory over time were in close agreement, i.e., 24.7% vs 29.1%).

Log linear analyses of the data indicate that B10.A, B10.A(15R), B10.OL and B10.A(1R), B10.BR, B10, B10.A(18R), B10.D2, and B10.A(2R) are members of distinct homogeneous groups which differ from each other ($P < 0.0001$) and from B10.A(5R), the strain with the highest frequency ($P < 0.00000$). A more detailed examination of the data revealed that although the frequency of DVS in B10.A(1R) differed significantly from that in the B10.A, B10.OL, and B10.A(15R) groups ($P = 0.005$), the difference between B10.A(1R) and B10.A(15R), two very similar recombinant strains (recombination sites between C4 and B144), is problematic ($P = 0.054$). Therefore, the assignment of B10.A(1R) to the group with an intermediate frequency of DVS should be viewed as tentative, and final assignment will depend upon further studies with this strain. The addition of vitamin A to the diet of the dams had no effect on the frequency of DVS in B10, B10.A(18R), B10.A(1R), B10.A(5R), or B10.A(15R) mice.

A reciprocal cross between B10.A(15R) mice, a strain with a low frequency of DVS, and B10.A(5R), the strain with the highest frequency, resulted in progeny with intermediate or high frequencies of the anomaly (Table III). This agrees with the results obtained in crosses between B10.A (low frequency) and B10 (intermediate frequency) mice, where approximately 17% of the female progeny had DVS (2). These results suggest that the trait is dominant or codominant for high frequency. However, when B10.A(15R) mice were crossed with B10.A(18R), a strain with an intermediate frequency of DVS, the progeny clearly had a low incidence of DVS, suggesting either that the trait is recessive (B10.A(5R) and B10.A(15R) mice share *k* and *d* alleles between Eβ and C4, whereas B10.A(18R) mice have *b* alleles in this region) or that there is a complex cis/trans interaction between the H-2-associated genes which contributes to the occurrence of DVS.

Discussion

These data confirm previous reports (2, 3) which showed that two loci associated with the murine major

Table II. Effects of MHC, Time, and Dietary Vitamin A on Frequency of DVS in Congenic Strains of Mice

Strain	1981–1983			1985–1990[a]			*P* Fisher exact	Added vitamin A[b] (200 IU/day)		
	DVS$^+$	DVS$^-$	%	DVS$^+$	DVS$^-$	%		DVS$^+$	DVS$^-$	%
B10.A[c]	18	269	6.3	8	177	4.3	.28	ND	ND	
B10.A(15R)	ND	ND		13	228	5.4		6	97	5.8
B10.0L	ND	ND		6	98	5.8		ND	ND	
B10.A(1R)	ND	ND		23	193	10.6		14	84	14.3
B10.BR	22	139	13.7	29	248	10.5	.35	ND	ND	
B10	52	213	19.6	27	202	11.8	.02	8	92	8.0
B10.A(18R)	34	167	16.9	38	232	14.1	.37	10	59	14.5
B10.A(2R)	36	146	19.8	43	213	16.8	.45	ND	ND	
B10.D2	20	89	18.3	14	73	16.1	.70	ND	ND	
B10.A(5R)[c]	41	125	24.7	59	144	29.1	.41	24	57	29.6

[a] B10.A, B10.A(15R), B10.0L and B10.A(1R), B10.BR, B10, B10.A(18R), B10.A(2R), and B10.D2 are homogeneous groups that differ from each other and B10.A(5R), according to log linear analysis, $P < 0.0001$.
[b] No significant differences were noted when compared with 1985–1990 results.
[c] Frequency in Jackson Laboratory production colony: B10.A, 8^+, 92^- (NS compared with data presented here); B10.A(5R), 17^+, 83^- ($P = 0.046$, when compared with 1985–1990 data).

Table III. Frequency of DVS among Progeny of Crosses between Congenic Strains with Low, Intermediate, and High Frequencies

Strain (female × male)	No. examined	Percentage DVS	*P* (Fisher exact)
B10.A(18R)	270	14.1	
			0.0005
B10.A(18R) × B10.A(15R)	87	8.0	
			0.40
B10.A(15R) × B10.A(18R)	118	5.1	
			1.00
B10.A(15R)[a]	241	5.4	
			0.004
B10.A(15R) × B10.A(5R)	106	14.1	
			0.11
B10.A(5R) × B10.A(15R)	102	23.9	
			0.34
B10.A(5R)[b]	203	29.1	

[a] Versus B10.A(18R) × B10.A(15R), $P = 0.43$.
[b] Versus B10.A(15R) × B10.A(5R), $P = 0.003$.

histocompatibility complex (MHC) have a significant influence on the incidence of DVS. The first locus (*DVS-1*) maps centromeric to Eα (compare B10.A and B10.D2, which differ only in this region; Table I) and the second gene (*DVS-2*) is located distal to Eα (compare B10.A with B10.BR). A detailed examination of the data indicates that with one exception (B10.A(1R)), strains with *d*:*k* or *k*:*d* alleles at these loci have low frequencies of DVS, those with *b*:*b*, *d*:*d*, *k*:*k*, or *k*:*b* (B10.A(2R)) alleles have intermediate values, and B10.A(5R), with *b*:*d*, has a high frequency (B10.A(18R), which differs from B10.A(5R) in the Eβ:B144 interval, has an intermediate frequency).

The observation that B10.A, B10.A(15R), and B10.0L mice have similar low frequencies of DVS indicates that (i) *DVS-2* is centromeric to B144 (compare B10.A and B10.A(15R) with B10.A(2R), which has *b* alleles in this region and a higher frequency of DVS; also compare B10.A(18R) with B10.A(5R)), (ii) *DVS-2* is centromeric to *Dcp-2* (B10.A(15R) has a low frequency of glucocorticosteroid-induced cleft palate and thus is *Dcp-2*^{b}; see Ref. 5), (iii) *DVS-2* maps between *Acp* and *Dcp-2* if the B10.A(1R) group has an intermediate frequency of DVS as the data suggest (if further studies show that the B10.A(1R) group has a low frequency of DVS, *DVS-2* is located in the C4:*Dcp-2* interval), and (iv), as the data suggest in the case of *Dcp-1* and *Dcp-2* (9), the two MHC-associated DVS genes interact to produce the phenotype (compare B10.A with B10.D2 and B10.BR); unfortunately no data have been generated on the frequency of DVS in crosses between B10.D2 and B10.BR, which would

indicate whether the genes are trans- or cis-acting, as is the case with *Dcp-1* and *Dcp-2* (9).

The highest incidence of DVS was observed in B10.A(5R) females. This could be the result of (i) a mutation in an MHC-associated or nonassociated gene that occurred at the Jackson Laboratory prior to 1981, when the first of several shipments of B10.A(5R) mice was obtained; (ii) the crossover event within the β_1:β_2 intron of the Eβ gene (10) resulted in altered function of *DVS-1*; or (iii) *b*:*d* DVS alleles interact to produce a high frequency of DVS. If the last possibility is correct, it would place *DVS-1* centromeric to Eβ.

When the progeny of reciprocal crosses between B10.A(15R), a strain with a low frequency of DVS, and B10.A(18R) (intermediate) and B10.A(5R) (high) were examined, (i) no maternal effect was noted and (ii) the frequency of DVS was low in the first cross (B10.A(15R) × B10.A(18R) and high or intermediate in the second (B10.A(15R) × B10.A(5R)). In the original study (2), reciprocal crosses between B10.A and B10 produced a 17% incidence of DVS in the progeny, which is consistent with the results obtained in the B10.A(15R) × B10.A(5R) cross and suggests that the trait is dominant for high frequency. The results from the B10.A(15R) × B10.A(18R) crosses, however, suggest dominance for low frequency and are difficult to reconcile with the results from the other crosses without invoking recessive genes, complex cis/trans interactions, experimental error, significant environmental effects, or non-H-2 differences that may exist (11, 12).

With the exception of the B10 and B10.A(5R) strains, variations in the frequency of DVS within a given strain when measured over time in this laboratory or when compared with observations at the Jackson Laboratory were relatively minor. These differences also may be due to variations in ascertainment, unidentified environmental factors, or non-H-2 differences that might possibly exist between these congenic strains. The studies reported here suggest that at the dose given, dietary vitamin A has no effect on the incidence of DVS. This stands in contrast to the effect of vitamin A on susceptibility to cleft palate (4), microphthalmia (13), and micrognathia (14).

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