Fetal Weight at Term Influenced by H-2–Associated Loci (43682)

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Abstract. **Pregnant mice which in theory differ only in the region of the major histocompatibility complex (MHC) on chromosome 17 (C57BL/10, the inbred partner [host strain], and B10.D2, B10.BR, B10.A, B10.A[2R], B10.A[5R], B10.A[15R] and B10.A[18R]) were sacrificed on the 11th and 18th days of gestation, and the fetuses were sexed and weighed. Fetuses from reciprocal crosses between B10.A and B10.BR, B10.D2 and C57BL/10 were weighed and sexed on the 18th day of gestation. It was found that (i) fetal weights were not significantly different among the strains examined on day 11 (B10.BR, B10.A[15R] and B10.A[18R]), (ii) B10.BR fetuses of both sexes weighed significantly less than fetuses from the other strains on day 18, (iii) B10.D2 18-day-old male but not female fetuses were heavier than the males from the other strains (this difference was not present when corrections for litter size were made), (iv) the fetuses from the B10.A × B10.BR cross were the smallest, those from the B10.D2 × B10.A cross the largest, and those from the B10.A × C57BL/10 crosses intermediate, and (v) maternal effects were noted in the B10.A × B10.BR and B10.A × B10.D2 but not the B10.A × C57BL/10 crosses. The results suggest that there are two or more MHC associated loci that influence growth rate in late gestation. Among the candidate genes are Ped and Igfr II.** [P.S.E.B.M. 1994, Vol 205]

It has been estimated that from 19 to 54 genetic loci influence birth weight and neonatal growth in mice, and breeding studies have suggested that dominance is predominantly in the direction of large size (1 and references therein). More recent studies have shown that birth weights and neonatal growth of rodents are at least in part determined by genes associated with the major histocompatibility complex (MHC) (2–7): among these are loci that exert maternal effects on fetal weight through imprinting or other unknown mechanisms (3, 8).

Presented below are the results of studies in H-2 congenic strains of mice which suggest that (i) in the last third of gestation, fetal weight is influenced by two or more loci associated with the MHC, (ii) one gene effect is dominant for small size, and (iii) maternal and/or imprinting effects are significant factors in fetal growth. Weight was chosen as the index of growth because previous results have shown that it is an easily obtained, reproducible, and objective measure that correlates well with fetal development (9 and references therein).

Materials and Methods

The H-2 congenic strains C57BL/10, B10.D2, B10.BR, B10.A, B10.A(5R), B10.A(2R), B10.A(15R) and B10.A(18R) were maintained in this laboratory by brother × sister matings. The differences in H-2 haplotypes are shown in Table I (see also 10, 11). The mice were fed Purina Mouse Laboratory Chow (5001) and water *ad libitum*.

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 11th or 18th day of gestation, the pregnant mice were sacrificed. The fetuses were dissected free of maternal tissues and membranes, and examined for external defects and stage of development as previous described (9): all fetuses were weighed, and the abdomens of the 18-day-old fetuses were opened to determine the sex.

The data sets are presented as means and standard

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Table I. Alleles at Chromosome 17 Loci in Mouse Congenic Strains[a]

Congenic strain	3.02	3.30	8.35	9.02	18.44	18.66	18.70	18.80	19.00	19.09	19.19	19.82	23.90	27.10	32.20
	D17Tu52	D17LEH66EII	Igf2r	D17Tu50	H-2K	Eb	Ea	C4	BAT-5	H-2D	Ped	Grc	D17Tu16	D17Tu40	C3
C57BL/10	b	a	•	a	b	b	b	b	b	b	f	a	a	b	b
B10.D2	B	A	•	A	**D**	**D**	**D**	**D**	**D**	**D**	**f**	**C**	a	B	B
B10.BR	b	a	•	a	**K**	**K**	**K**	**K**	**K**	**K**	**S**	**F**	a	b	•
B10.A	B	A	•	A	**K**	**K**	**K**	**D**	**D**	**D**	f	**F**	a	B	B
B10.A(5R)	B	•	•	A	B	B/**K**	**K**	**D**	**D**	**D**	f	**F**	a	B	B
B10.A(15R)	B	A	•	A	**K**	**K**	**K**	**D**	B	B	•	A	a	B	B
B10.A(2R)	B	•	•	A	**K**	**K**	**K**	**D**	B	B	•	A	a	B	B
B10.A(18R)	B	•	•	A	B	B	B	B	**D**	**D**	•	•	•	•	B

[a] Capital letters in bold print, capital letters in normal print, and small letters indicate alleles derived from the donor strain, the inbred partner, and either the donor strain or the inbred partner, respectively. Bullets (•) indicate that the allele has not been determined. The regions underlined indicate the probable minimum donor contribution. The table was constructed with data from references 10 and 11. The site numbers are presented to two-decimal place resolution to indicate a relative gene order and not to imply that genetic mapping has been determined to this degree of accuracy.

deviations based on variations between litters. Comparisons between groups were made using analysis of variance, the Kruskal-Wallis Test, the unpaired T-test, the Mann-Whitney two-sample test and by multiple regression analysis (NCSS statistical program, NCSS, Kayville, UT). The most conservative P values are reported without correction for multiple determinations.

Results

On the 11th day of gestation, B10.BR fetuses were slightly heavier than B10.A(15R) and B10.A(18R) fetuses, but the differences were not significant (Table II).

On the 18th day, it was found that male and female B10.BR fetuses weighed significantly less than did fetuses from the other strains (Table III). The mean weight of B10.D2 male but not female fetuses was greater than that of males from other strains (vs B10.A[2R], $P = 0.077$; vs B10.A[5R], $P = 0.003$); however, this difference was not present when mean birth weights were adjusted for litter size using the appropriate regression coefficient (12). There were no significant correlations between fetal weight and litter size with the exceptions of B10.D2 males and females and B10.A(2R) males where negative correlations were found (-0.732, -0.685 and -0.349, respectively, Table III).

Mean fetal weights from the reciprocal crosses between B10.A and B10.BR, B10.D2 and C57BL/10 were lowest when the dam or sire was B10.BR, and highest when the dam was B10.D2 (Table IV). Maternal effects were noted in the B10.A × B10.D2 and B10.A × B10.BR but not the B10.A × C57BL/10 crosses: where this effect was present the smallest fetuses were obtained when the dam was B10.A.

No significant differences in overall or specific organ development were noted among the strains on day 11 (9) or 18 (data not reported).

Table II. Weights of H-2 Cogenic Fetuses on the 11th Day of Gestation

Congenic strain	Number of fetuses	Weight (mg ± S.D.)
B10.BR	60	23.8 ± 7.3
B10.A(15R)	23	21.5 ± 9.3
B10.A(18R)	64	21.6 ± 7.5

Discussion

Data presented here and previously (9) show that on the 11th day of gestation, fetuses of the H-2 congenic strains B10.BR, B10.A(15R) and B10.A(18R) do not differ significantly in weight or development; however, observations made seven days later clearly demonstrate that B10.BR fetuses are smaller and suggest that B10.D2 male fetuses may be larger than fetuses from six other congenic strains which, at least on the 18th day of gestation, appear to constitute a homogeneous group with regard to weight. This suggests that at some point between the 11th and 18th days of gestation an MHC-associated gene(s) that influences growth is activated or its function is modulated.

The most obvious candidate gene that could explain the differences in weight observed among these congenic strains is Ped (Qa-2) which has been mapped telomeric to H-2D in the MHC and which is known to influence preimplantation development (4, 13), birth weight, and litter size (14). B10.BR has been shown to possess the allele for slow (S) preimplantation development and the other congenic strains used in these studies have the fast (f) allele (4); at birth, mice congenic at the Ped locus were smaller when the slow allele was present (14). During the preimplantation phase of development the fast allele appears to be dominant, and at birth the alleles were codominant. No maternal or parent-of-origin effect has been noted at this locus. Two observations argue against Ped as the gene wholly responsible for the difference in weight noted in B10.BR: the reciprocal cross studies suggest that (a) small, not large, is dominant, and (b) a maternal effect is evident in the B10.A × B10.BR

Table III. Weights of H-2 Congenic Fetuses on the 18th Day of Gestation and Correlation of Weights with Litter Size

Strain	Litters		Weight (mg ± S.D.) (n)		Weight vs litter size (correlation coefficient) (p)	
	#	Mean size ± S.D.	Male[a]	Female[b]	Male	Female
B10.D2	7	6.0 ± 2.3	1143 ± 143 (27)	1048 ± 118 (19)	−0.732 (0.000)	−0.685 (0.001)
B10.A(2R)	15	5.7 ± 1.6	1082 ± 119 (39)	1011 ± 136 (46)	−0.349 (0.029)	−0.170
B10.A(5R)	13	7.2 ± 1.9	1059 ± 113 (44)	1023 ± 163 (48)	−0.012	−0.135
C57BL/10	12	6.3 ± 1.3	1048 ± 103 (37)	986 ± 114 (37)	−0.058	−0.091
B10.A(18R)	24	6.4 ± 1.9	1043 ± 94 (86)	981 ± 103 (75)	0.003	0.053
B10.A	12	6.7 ± 1.1	1039 ± 84 (48)	934 ± 96 (31)	0.254	−0.131
B10.A(15R)	7	7.9 ± 2.1	1020 ± 61 (22)	956 ± 92 (34)	−0.252	−0.287
B10.BR	9	5.9 ± 1.7	939 ± 104 (21)	886 ± 121 (32)	−0.025	−0.173

[a] C57BL/10, B10.A(18R), B10.A(2R), B10.A(5R), B10.A and B10.A(15R) are members of a homogeneous group, according to ANOVA. B10.D2 vs B10.A(2R), $P = 0.077$; B10.BR vs B10.A(15R), $P = 0.003$, according to Mann-Whitney two-sample test.
[b] C57BL/10, B10.A(18R), B10.A(2R), B10.A(5R) and B10.A(15R) are members of a homogeneous group. However, B10.D2 vs B10.A(5R), $P = 0.377$, vs B10.A(2R), $P = 0.283$, vs C57BL/10, $P = 0.077$, vs B10.A(18R), $P = 0.022$; B10.BR vs B10.A, $P = 0.051$, vs B10.A(15R), $P = 0.033$.

Table IV. Fetal Weights on the 18th Day of Gestation of Progeny from Crosses between Congenic Strains of Mice

Congenic strains (female × male)	Weight (mg ± S.D.) (N)	
	Male	Female
B10.D2 × B10.A	1117 ± 97[a] (24)	1053 ± 109[b] (31)
B10.A × B10.D2	1023 ± 81[a] (45)	977 ± 84[b] (37)
B10.A × C57BL/10	1039 ± 71[e] (40)	951 ± 61[f] (31)
C57BL/10 × B10.A	1042 ± 81[g] (29)	979 ± 99[h] (35)
B10.A × B10.BR	924 ± 147[c] (34)	864 ± 112[d] (48)
B10.BR × B10.A	967 ± 119[c] (49)	915 ± 109[d] (52)

[a] $P = 0.0003$.
[b] $P = 0.0004$.
[c] $P = 0.0751$.
[d] $P = 0.0124$.
[e] B10.A × C57BL/10 vs B10.A × B10.D2, $P = 0.2542$; vs B10.A × B10.BR, $P = 0.0000$.
[f] B10.A × C57BL/10 vs B10.A × B10.D2, $P = 0.3472$; vs B10.A × B10.BR, $P = 0.0002$.
[g] C57BL/10 × B10.A vs B10.D2 × B10.A, $P = 0.0037$; vs B10.BR × B10.A, $P = 0.0093$.
[h] C57BL/10 × B10.A vs B10.D2 × B10.A, $P = 0.0026$; vs B10.BR × B10.A, $P = 0.0088$.

crosses. The latter observation suggests that if Ped is the gene that defines the major differences in weight among these strains, a second locus needs to be postulated to explain the maternal effect noted in this and the B10.A × B10.D2 crosses.

When corrections are made for litter size the evidence suggests that B10.D2 male and female fetuses most likely do not differ significantly in weight from the strains which appear to constitute a homogeneous group (B10.A[2R], B10.A[5R], C57BL/10, B10.A[18R], B10.A and B10.A[15R]). However, even with this correction B10.D2 fetuses are significantly heavier than those from the B10.A and B10.A(15R) strains, $P < 0.001$ (both sexes, both strains); if real, this difference most likely is not caused by the Ped locus because these strains share the fast allele. However, the differences could be the result of variations in the D17Tu50:Eα interval or perhaps within the Grc complex (2). The Grc locus has been shown in the rat to be involved in neonatal growth and fertility, and to be associated with the MHC (2, 15). In the mouse the complex maps just telemeric to Ped (16). Grc has not been shown to influence intrauterine growth and development, and no maternal effect has been noted. Although B10.D2 differs from the other strains at this locus, the observations presented here are not consistent temporally or phenotypically with the changes noted when mutations are present within the Grc complex.

In the reciprocal crosses between B10.A and B10.BR, B10.D2 and C57BL/10, a maternal effect was noted in the B10.A × B10.BR, B10.A × B10.D2, but not the B10.A × C57BL/10 crosses. The smallest fe-

tuses in each cross where a maternal effect was observed were noted when the dam was B10.A; the largest fetuses were found when the sire was B10.D2. In order to explain this effect on the basis of a single locus, B10.A and C57BL/10 would need to share an allele, and B10.BR and B10.D2 would differ from each other and from B10.A and C57BL/10. A review of the loci in Table I and the more detailed data in Ref. 10 has not revealed a locus within the minimal length donor segments that fit these requirements. This suggests that the locus, if it does exist, is in the regions, central and distal, where host:donor recombination sites of B10.BR, B10.D2, and B10.A do not coincide. Alternatively, two or more loci are involved in producing the maternal effect.

If more than one gene is responsible for the maternal effect, a candidate gene in the B10.A × B10.BR cross is the receptor for Igf II which is maternally expressed, is involved in an unknown manner in fetal growth, and has been mapped centromeric to the MHC on chromosome 17 (17 and references therein). Of the strains studied, all but B10.BR appear to have Igfr II of host origin (C57BL/10); however, there is insufficient evidence at this time to state with any degree of certainty that this locus also is not of host origin in B10.BR. Further, to date polymorphism has not been demonstrated at this locus.

Others have noted MHC-associated maternal effects on fetal weight, although no specific loci have been identified (3, 8). These cited studies give support to the present data and make it less likely that the observations are the result of epigenetic factors or experimental error. A maternal effect locus (loci) which has an arbitrarily assigned positive effect on growth in the B10.D2 strain, a neutral effect in B10.BR, and a negative effect in B10.A and C57BL/10 (in this case they may both be of host origin) would explain the findings in the studies on reciprocal crosses and in the differences in weight noted between the homozygous B10.D2 and B10.A fetuses.

It is clear from these and other studies that birth weight is determined by multiple genetic and environmental factors: many of the genetic loci appear to be located on mouse chromosome 17 and several are closely associated with the MHC. Some, like Ped, have a relatively strong effect on weight, while others, such as those producing the maternal effects, have less of an effect. Some loci (e.g., Ped and Igf II/Igfr II) appear to be expressed to varying degrees throughout gestation while others (e.g., Grc) are more phase specific. Finally, weight is a gross measure of growth and development, and although in general there is a good correlation between weight and survival, it is not clear which of the identified loci contribute primarily to the acquisition of mass and which to differentiation and organ development.

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