Genetic interaction between a maternal factor and the zygotic genome controls the intestine length in PRM/Alf mice

Geneviève Aubin-Houzelstein Aubin Houzelstein, Nelly R. da Silva, Sylvain S. Bellier, Pierrick Salaun, Xavier Montagutelli, Jean-Jacques J.-J. Panthier

► To cite this version:

Geneviève Aubin-Houzelstein Aubin Houzelstein, Nelly R. da Silva, Sylvain S. Bellier, Pierrick Salaun, Xavier Montagutelli, et al.. Genetic interaction between a maternal factor and the zygotic genome controls the intestine length in PRM/Alf mice. *Physiological Genomics*, 2003, 16, pp.82-89. hal-02676147

HAL Id: hal-02676147

<https://hal.inrae.fr/hal-02676147>

Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Genetic interaction between a maternal factor and the zygotic genome controls the intestine length in PRM/Alf mice

Geneviève Aubin-Houzelstein,^{1*} Nelly R. Da Silva,^{1*} Sylvain Bellier,¹ Pierrick Salaün,¹ Xavier Montagutelli,² and Jean-Jacques Panthier^{1,2}

¹UMR 955 INRA-ENVA de Génétique Moléculaire et Cellulaire, Ecole Nationale Vétérinaire d'Alfort, 94704 Maisons-Alfort cedex, France; and ²Unité de Génétique des Mammifères, Institut Pasteur, 75724 Paris cedex 15, France

Submitted 30 June 2003; accepted in final form 30 October 2003

Aubin-Houzelstein, Geneviève, Nelly R. Da Silva, Sylvain Bellier, Pierrick Salaün, Xavier Montagutelli, and Jean-Jacques Panthier. Genetic interaction between a maternal factor and the zygotic genome controls the intestine length in PRM/Alf mice. *Physiol Genomics* 16: 82–89, 2003. First published October 14, 2003; 10.1152/physiolgenomics.00106.2003.—Postoperative management of small and large bowel resections would be helped by use of intestinotrophic molecules. Here, we present a mouse inbred strain called PRM/Alf that is characterized by a selective intestinal lengthening. We show that PRM/Alf intestine is one-third longer compared with other inbred strains. The phenotype is acquired mostly during the postnatal period, before weaning. Its genetic determinism is polygenic, and involves a strong maternal effect. Cross-fostering experiments revealed that the dam's genotype acts synergistically with the offspring's genotype to confer the longest intestine. Moreover, genes in the offspring have a direct effect on intestine length. Possible involvement of milk growth factors and identification of candidate genes are discussed.

inbred strains; mouse; genotype-environment interaction; organ development; gut adaptation

MANY GENES ARE KNOWN TO CONTROL total body size, most of them belonging to the growth hormone (GH) and insulin-like growth factor (IGF) pathways. However, very few are identified as tissue- or organ-specific growth control genes.

Regarding the intestine, the known trophic factors include peptide growth factors and cytokines. The intestinotrophic peptide growth factors are epidermal growth factor (EGF), transforming growth factor- α and - β (TGF- α and TGF- β), GH, IGF-I and IGF-II, insulin, IGF binding proteins (IGFBPs), keratinocyte growth factor (KGF), gastrin, peptide YY, neurotensin, bombesin, and glucagon-like peptide-2 (GLP2). The cytokines are interleukins 11, 3, and 15 (3, 11, 13, 25). Most of the intestinotrophic factors act on intestinal epithelium growth and maturation via control of epithelial cell proliferation, differentiation, and apoptosis. Nevertheless, whereas all these factors are able to increase intestine weight to some extent, only IGF-I and GLP2 are documented to have an impact on intestine length among other effects (8, 23).

To our knowledge, no gene is known to selectively control the length of the digestive tract. In this report, we present a mouse inbred strain called PRM/Alf characterized by a con-

siderable intestinal lengthening. We show that this trait is polygenic and that it is subjected to a maternal effect. This model is a unique tool to identify intestinotrophic molecules. Identification of factors controlling bowel longitudinal growth could be of major interest in noninvasive, postoperative management of small and large bowel resections.

MATERIAL AND METHODS

Mice. All mice were bred and maintained under identical conditions in our animal facility at the Alfort Medical Veterinary School. The mice were kept at uniform temperature (21°C) with regulated humidity and fed with standardized diets (formula A03; Usine d'Alimentation Rationnelle, Rennes, France). The PRM/Alf inbred strain of mice was initiated with breeding pairs carrying the coat color patchwork mutation obtained from Karen Moore. These mice were subjected to successive brother-sister matings (F ? + 30 and over). The corresponding inbred strain is registered PRM/Alf. C57BL/6J, C3H/He, and (C57BL/6J $\times$ CBA/J)F1 mice were obtained from the INRA (Jouy-en-Josas, France), and DBA/2J mice were from Charles River Laboratories (Saint-Aubin-les-Elbeuf, France).

PRM/Alf mice were crossed with DBA/2J mice to produce F1 hybrid progeny. Then three crosses were undertaken: 1) a backcross on PRM/Alf, [(DBA/2J $\times$ PRM/Alf)F1 $\times$ PRM/Alf]BC1; 2) a backcross on DBA/2J, [(DBA/2J $\times$ PRM/Alf)F1 $\times$ DBA/2J]BC1; and 3) an intercross, F2.

Animal care and use were approved by the Alfort Veterinary School ethical council in accordance with the European Community Standards.

Adiposity index determination. Eleven males and eleven females from both PRM/Alf and DBA/2J strains were euthanized at 4 mo of age. They were weighed before and after disembowelment, and their intra-abdominal fat, composed of retroperitoneal, mesenteric, inguinal, and gonadic fat pads, was dissected and weighed. Their adiposity index, defined as the intra-abdominal fat weight:postdisembowelment weight ratio was calculated.

Measure of body size, body weight, and intestine length. The mice were weaned at 1 mo of age. They were euthanized at 3 mo of age. Their sex was recorded. Their body length was measured. They were weighed before and after disembowelment with a digital balance. The intestine was dissected from the cardia to the anus. Pylorus-anus, pylorus-cecum, and cecum-anus distances were measured to obtain intestine total length, small intestine length, and large intestine length, respectively. Relationships were tested between intestinal length and sex, body length, postdisembowelment weight, and postnatal age.

Adoptions. PRM/Alf and DBA/2J entire litters were exchanged at birth so that PRM/Alf offspring were fostered by a DBA/2J foster mother and vice versa. The pups were then raised and proceeded as before. Controls were PRM/Alf and DBA/2J mice bred by their own mother.

Statistics. Statistical analyses were done with the StatView F-4.51.3.PPC software from Abacus Concepts (Berkeley). Data are expressed as means $\pm$ standard deviation. Distribution normality was tested by comparing the observed distribution to a normal distribution

*G. Aubin-Houzelstein and N. R. Da Silva contributed equally to this work.
Article published online before print. See web site for date of publication (<http://physiolgenomics.physiology.org>).

Address for reprint requests and other correspondence: J.-J. Panthier, UMR 955 INRA-ENVA de Génétique Moléculaire et Cellulaire, Ecole Nationale Vétérinaire d'Alfort, 7 Ave. du Général de Gaulle, 94704 Maisons-Alfort cedex, France (E-mail jjpanthier@vet-alfort.fr).

with the same mean and standard deviation and use of a Kolmogorov-Smirnov test. Variances were compared with an F-test. Means were compared with a Student's *t*-test for normally distributed values with equal variance and with a Mann-Whitney U-test otherwise. Correlations were sought by calculating the Pearson correlation coefficients between pairs of variables and use of a Fisher *z* transformation.

RESULTS

PRM/Alf mice exhibit an elongated intestine. During the study of the PRM/Alf strain that carry the patchwork mutation (1, 2), we discovered serendipitously that the mice had a distended abdomen (Fig. 1A). To test whether they were fatter than mice from another inbred strain, we measured and compared the adiposity index of PRM/Alf and DBA/2J mice. We found no increase in the adiposity index (AI) of PRM/Alf females compared with DBA/2J (AI = 3.9 ± 0.7 in 11 PRM/Alf females and AI = 3.5 ± 0.6 in 11 DBA/2J females; Student's *t*-test, $P > 0.05$). PRM/Alf males were even leaner than DBA/2J males (AI = 2.3 ± 0.4 in 11 PRM/Alf males and AI = 3.7 ± 0.8 in 11 DBA/2J males; Student's *t*-test, $P < 0.001$).

To test whether the abdominal distension was due to an elongated digestive tract, we compared the intestine length of

3-mo-old mice from PRM/Alf, DBA/2J, C57BL/6J, and C3H/He strains. We found that the intestine of PRM/Alf was significantly longer than in the other strains (74.8 ± 5.3 cm in 42 PRM/Alf mice vs. 54.1 ± 3.1 cm in 45 DBA/2J mice, 49.7 ± 2.5 cm in 48 C57BL/6J mice, and 49.0 ± 3.7 cm in 39 C3H/He mice; Student's *t*-test, $P < 0.001$ between PRM/Alf and the three other strains; Figs. 1 and 2). In all strains but PRM/Alf, the intestine length was significantly different between males and females (Table 1; Student's *t*-test, $P < 0.01$). However, as the difference between sexes was very small compared with the difference between strains, we chose to pool the males and females data. To test whether the lengthening was homogenous in all parts of the intestine, we measured small intestine length (SIL) and large intestine length (LIL) in the same animals as above. Both SIL and LIL were significantly greater in PRM/Alf mice compared with the other strains (Table 2; Student's *t*-test, $P < 0.001$). The relative lengths of the small and large intestines remained constant in all populations tested (80–83.5% and 16.5–20%, respectively; Table 2). To test whether intestine lengthening in PRM/Alf mice was linked to an increase in body length and/or body weight, we measured and weighed the mice when dissecting

Fig. 1. Intestine lengthening in the PRM/Alf strain. A: (from left to right) 5-mo-old females from DBA/2J, C57BL/6J, C3H/He, and PRM/Alf inbred strains, respectively. Note the overall larger abdomen of the PRM/Alf female. B: intra-abdominal part of the digestive tract of the females shown in A, in the same order. The PRM/Alf intestine was 75.5 cm in length, compared with 47.5, 58.0, and 47.0 cm in DBA/2J, C57BL/6J, and C3H/He controls, respectively. The salt-and-pepper coat color in the PRM/Alf mouse is due to the patchwork recessive mutation specific to the PRM/Alf strain.

Fig. 2. Intestine lengthening is specific to the PRM/Alf inbred strain. DBA/2J, C57BL/6J, C3H/He, and PRM/Alf mice were weaned at 1 mo of age. They were euthanized at 3 mo of age, and their intestine length was measured. The number of mice analyzed in each population is given in parentheses. Error bars correspond to the standard errors of means.

their intestine. As both body length and body weight are influenced by sex, we considered each sex separately. We found that the body length (BL) and the postdisembowelment weight (PDW) of PRM/Alf mice were higher compared with mice from the other strains (Table 3; Student's *t*-test, $P < 0.001$). Thus we calculated the intestine length:body length (IL:BL) and intestine length:postdisembowelment weight (IL:PDW) ratios in all strains tested. IL:BL ratios were significantly higher in PRM/Alf compared with DBA/2J, C57BL/6J, and C3H/He in both sexes (Table 3; Student's *t*-test, $P < 0.001$ in all strain pairs). IL:PDW was significantly higher in PRM/Alf compared with the other strains, except for DBA/2J-PRM/Alf (Table 3; Student's *t*-test, $P < 0.003$ at least in all strain pairs, but DBA/2J-PRM/Alf, where $P = 0.04$ in females and $P > 0.05$ in males).

Intestine lengthening occurs postnatally in the PRM/Alf strain. To determine whether the intestine lengthening occurs during embryogenesis or during postnatal development, we measured the intestine length on PRM/Alf and DBA/2J mice euthanized at birth (*P0*) and at postnatal days 15 (*P15*), *P30*, and *P90* (Fig. 3). As we found no influence of sex on intestine length at any time point, except at *P90* in DBA/2J (Table 1), we pooled the results from males and females. There was no difference in intestine length between PRM/Alf and DBA/2J newborn (IL = 10.5 ± 1.1 cm in 42 PRM/Alf mice; IL =

10.1 ± 1.2 cm in 44 DBA/2J mice, Student's *t*-test, $P > 0.05$). At *P15*, the intestine of PRM/Alf mice was significantly longer compared with DBA/2J mice (IL = 30.1 ± 3.4 cm in 39 PRM/Alf mice; IL = 22.3 ± 3.0 cm in 44 DBA/2J mice, Student's *t*-test, $P < 0.001$). The difference was even increased at *P30* (IL = 59.0 ± 5.2 cm in 44 PRM/Alf mice; IL = 36.7 ± 3.2 cm in 39 DBA/2J mice, Student's *t*-test, $P < 0.001$). The difference was maintained throughout adulthood, with the intestine of PRM/Alf mice being one-third longer than the intestine of DBA/2J. There was no correlation between intestine length and litter size in either PRM/Alf or DBA/2J strains; for instance, at *P30*, Pearson's correlation coefficient values were: $r = 0.05$ in 39 DBA/2J mice belonging to 7 litters of 3–9 pups (Fisher's *z*-test, $P > 0.05$) and $r = -0.001$ in 44 PRM/Alf mice belonging to 7 litters of 3–13 pups (Fisher's *z*-test, $P > 0.05$).

To determine when the increase in body weight and body length happened in PRM/Alf, the same animals were also weighed and measured at *P0*, *P15*, *P30*, and *P90*. We found that both postdisembowelment weight and body length started being significantly increased in PRM/Alf at *P30* in both sexes, whereas difference in intestine length between PRM/Alf and DBA/2J was already highly significant at *P15* (Fig. 3).

Determinism of intestine lengthening in PRM/Alf mice. To assess the basic inheritance of intestine lengthening in this model, we analyzed the expression of the trait in segregating mice. We produced the parental strains, PRM/Alf, DBA/2J, their F1 hybrids, (PRM/Alf $\times$ DBA/2J)F1 and (DBA/2J $\times$ PRM/Alf)F1, the first backcross progeny, [(DBA/2J $\times$ PRM/Alf)F1 $\times$ PRM/Alf]BC1 and [(DBA/2J $\times$ PRM/Alf)F1 $\times$ DBA/2J]BC1, and the (F1 $\times$ F1)F2 generation (in crosses females are noted first). Means of intestine length in 3-mo-old mice from the different generations are shown in Fig. 4. Aspects of intestine length distributions in males are shown in Fig. 5.

The means of intestine length in F1 and F2 mice were intermediate between the means of intestinal length in DBA/2J and PRM/Alf mice (Fig. 4). Furthermore, the means of intestinal length in BC1 progeny were intermediate between the means of intestinal length in F1 mice and in the parental strain used for the backcross. In females, means for hybrids of first and second generations were closer to DBA/2J means than in males (Fig. 4). Distributions of intestine length in all generations were normal (Fig. 5; Kolmogorov-Smirnov test). Such distributions are expected for a quantitative trait inherited in a polygenic way.

The variance of the trait was higher in PRM/Alf than in DBA/2J ($s^2 = 51.0$ and $s^2 = 16.0$ in 16 PRM/Alf females and 26 PRM/Alf males, respectively, whereas $s^2 = 8.8$ and $s^2 =$

Table 1. Sex influence on intestine length in different inbred strains

Sex	Strain											
	PRM/Alf			DBA/2J			C57BL/6J			C3H/He		
	IL	n	P	IL	n	P	IL	n	P	IL	n	P
Female	74.8 $\pm$ 7.1	16	>0.05	55.3 $\pm$ 3.0	29	0.0002	48.8 $\pm$ 1.9	25	0.008	47.0 $\pm$ 3.0	14	0.007
Male	74.8 $\pm$ 4.0	26		51.8 $\pm$ 2.0	16		50.7 $\pm$ 2.9	23		50.2 $\pm$ 3.6	25	

Values are means for intestine length (IL) $\pm$ SD in cm; n, number of mice tested; P value is for the Student's *t*-test. In all strains tested except PRM/Alf, there was a significant difference in intestine length between males and females.

Table 2. Both small intestine and large intestine are lengthened in PRM/Alf mice

Strain	n	IL	P	SIL	P	LIL	P
PRM/Alf	42	74.8±5.3		61.7±5.1		13.2±1.1	
DBA/2J	45	54.1±3.1	<0.0001	43.8±02.7	<0.0001	10.2±0.9	<0.0001
C57BL/6J	48	49.7±2.5	<0.0001	41.5±2.2	<0.0001	8.2±0.9	<0.0001
C3H/He	39	49.0±3.7	<0.0001	39.3±3.5	<0.0001	9.7±0.9	<0.0001

Values are means ± SD in cm; n, number of mice tested. Small intestine length (SIL) and large intestine length (LIL) were measured in the same animals as in Fig. 2. P value is for the Student's *t*-test between PRM/Alf and the indicated strain. IL, but also SIL and LIL, were significantly greater in PRM/Alf.

3.9 in 29 DBA/2J females and 16 DBA/2J males). In the F1 female progeny, the variance was intermediate between the variances in the parental strains ($s^2 = 27.4$ in 39 F1 females). In the F1 male population, it was even higher than in both parental strains ($s^2 = 25.9$ in 74 F1 males).

To test whether the high variance in F1 progeny could be linked to the direction of the cross, we split the F1 hybrid population according to the mother. We found that the variances in F1 subpopulations were not significantly different from variances in the complete F1 population, except for F1 males born from PRM/Alf females (in females, $s^2 = 27.3$ for 21 F1 born from PRM/Alf females and $s^2 = 21.4$ for 18 F1 born from DBA/2J females; in males, $s^2 = 13.5$ for 29 F1 born from PRM/Alf females and $s^2 = 26.6$ for 45 F1 born from DBA/2J females, respectively; F-test, $P > 0.05$, except for F1 males born from PRM/Alf females compared with the entire F1 male population, $P = 0.04$). Nevertheless, F1 hybrids born from PRM/Alf females had a longer intestine than F1 hybrids born from DBA/2J females (means of 62.4 ± 5.2 vs. 58.7 ± 4.6 cm in females, respectively; 64.3 ± 3.7 vs. 59.9 ± 5.2 cm in males, respectively; Student's *t*-test, $P = 0.03$ in females, $P < 0.001$ in males; Fig. 6).

Maternal effects are a source of genetic variance of the intestinal length. Lengthening of the digestive tract occurred during the suckling period. Moreover, (PRM/Alf × DBA/2J)F1 mice had intestine significantly longer than (DBA/2J × PRM/Alf)F1 mice. Therefore, we tested whether the mother's genotype could modify intestine length in the offspring. For this purpose, we performed cross-fostering experiments. PRM/Alf and DBA/2J inbred pups were exchanged at birth so that PRM/Alf pups were raised by DBA/2J foster mothers and vice versa.

The sizes of the litters are highly variable in both PRM/Alf and DBA/2J inbred lines. Hence, the number of pups transferred was variable between litters (from 3 to 7 PRM/Alf pups transferred to DBA/2J females; from 3 to 9 DBA/2J pups

transferred to PRM/Alf females). However, the mean number of pups transferred was not statistically different between DBA/2J and PRM/Alf females ($n = 5.3 \pm 1.4$ in 43 DBA/2J pups raised by PRM/Alf females; $n = 5.4 \pm 2.4$ in 38 PRM/Alf pups raised by DBA/2J; Mann-Whitney U test, $U = 749.5$, $P > 0.05$). For practical reasons, it was rarely possible to exchange two litters of the same size. However, there was no systematic bias in the number of pups exchanged between both inbred lines (data not shown).

PRM/Alf mice raised by DBA/2J females had an intestine significantly shorter than nonadopted PRM/Alf mice (Fig. 7, left, Student's *t*-test, $P < 0.001$). When raised by PRM/Alf nurse-dams, the intestine length of DBA/2J mice was also significantly, although moderately, shortened (Fig. 7, right, Student's *t*-test, $P < 0.001$). We found similar results in cross-fostering experiments between PRM/Alf and C3H/He mice (data not shown). Thus the genotype of the fostering mother can influence intestine length of suckling mice. In other words, a maternal effect contributed to the intestine lengthening in PRM/Alf mice.

To assess the mode of transmission of the genes responsible for this maternal effect, we further tested whether a maternal effect could be found with (PRM/Alf × DBA/2J)F1 nurse-dams. For this purpose, PRM/Alf pups were raised by (PRM/Alf × DBA/2J)F1, PRM/Alf, and DBA/2J nurse-dams. We measured the intestine length of the resulting PRM/Alf mice. PRM/Alf mice raised by PRM/Alf females had an intestine significantly longer than PRM/Alf mice nursed by either (PRM/Alf × DBA/2J)F1 or DBA/2J nurse-dams (Fig. 7, left, compare dark-gray squares to either white or light-gray squares; Student's *t*-test, $P < 0.001$). When raised by an F1 nurse-dam, PRM/Alf mice had an even shorter intestine than when raised by a DBA/2J nurse-dam (Fig. 7, left, compare light-gray and white squares; Mann-Whitney U test, $U = 857.5$, $P = 0.01$). Thus a (PRM/Alf × DBA/2J)F1 nurse-dam

Table 3. Increase in body length and body weight alone cannot explain all intestine lengthening in 3-mo-old PRM/Alf mice

Sex	Strain	n	BL, cm	P	PDW, g	P	IL/BL	P	IL/PDW	P
Females	PRM/Alf	16	11.3±0.5		26.2±2.8		6.6±0.6		2.9±0.2	
	DBA/2J	29	9.6±0.5	<0.0001	20.3±1.3	<0.0001	5.8±0.4	<0.0001	2.7±0.2	0.04
	C3H/He	14	10.3±0.2	<0.0001	19.9±1.3	<0.0001	4.6±0.3	<0.0001	2.4±0.2	<0.0001
	C57BL/6J	25	9.8±0.3	<0.0001	19.1±0.9	<0.0001	5.0±0.2	<0.0001	2.5±0.1	<0.0001
Males	PRM/Alf	19, 26*	10.8±0.6		32.1±3.3		6.9±0.4		2.4±0.2	
	DBA/2J	16	10.5±0.3	>0.05	23.1±1.5	<0.0001	5.0±0.2	<0.0001	2.2±0.2	>0.05
	C3H/He	25	10.7±0.3	>0.05	24.7±2.2	<0.0001	4.7±0.3	<0.0001	2.1±0.4	0.0033
	C57BL/6J	23	10.4±0.2	0.0102	26.8±1.4	<0.0001	4.9±0.3	<0.0001	1.9±0.1	<0.0001

Values are means ± SD; n, number of mice tested. Body length (BL) and postdisembowelment weight (PDW) were measured on the same animals as in Table 2 and Fig. 2: IL/BL, intestine length:body length ratio; IL:PDW, intestine length:postdisembowelment weight ratio. P value is for the Student's *t*-test between PRM/Alf and the indicated strain: *Of 26 PRM/Alf males dissected, 7 data were missing for BL, but all 26 data were recorded for PDW. IL/BL ratio in PRM/Alf was significantly higher than in all other strains in both sexes. It was also true for IL/PDW, except for DBA/2J-PRM/Alf males.

Fig. 3. Intestine lengthening in PRM/Alf mice occurs during the early postnatal period and precedes increase in body weight and body size. Intestine length (*top*) and postdisembowelment weight (*middle*) were measured on PRM/Alf and DBA/2J mice euthanized at birth (*P0*) and at postnatal days 15 (*P15*), 30, and 90. Body length (*bottom*) was not recorded at *P0* as it could not be measured accurately. The numbers of mice analyzed were 22, 19, 24, and 16 PRM/Alf females and 22, 24, 19, and 14 DBA/2J females at *P0*, *P15*, *P30*, and *P90*, respectively. Results in males were qualitatively the same, but quantitatively different for postdisembowelment weight and body length. Each point represents the mean for the given day in the given population. The error bars are the standard errors of means. Asterisks indicate the statistical significance of the Student's *t*-test between PRM/Alf and DBA/2J mice at the given day: * $0.01 \leq P < 0.05$; *** $P < 0.001$; NS, not significant. Although no difference was found in intestine length between DBA/2J and PRM/Alf newborn entire population, a significant difference was found in newborn females only (Student's *t*-test, $P = 0.04$).

is unable to confer the maternal effect provided by a PRM/Alf nurse-dam.

Interaction between the nurse-dam's and the pup's genotypes. Cross-fostering experiments revealed that DBA/2J mice raised by PRM/Alf nurse-dams exhibited a shorter intestine

than PRM/Alf pups raised by PRM/Alf mothers (Fig. 7, compare dark-gray symbols on the *right* and *left*). This result suggests that the genotype of the progeny could interact with the nurse-dam's genotype in the maternal effect. To investigate further the importance of the progeny genotype, we compared the intestine lengths of PRM/Alf, (PRM/Alf $\times$ DBA/2J)F1, and DBA/2J mice raised by PRM/Alf nurse-dams (Fig. 7, dark-gray symbols). We found that F1 mice raised by PRM/Alf nurse-dams exhibited an intestine length that was intermediate between the intestine length of PRM/Alf and DBA/2J mice raised by PRM/Alf nurse-dams (Student's *t*-test, $P < 0.001$). This was also true, although to a lesser extent, for mice raised by DBA/2J nurse-dams (Fig. 7, white symbols, Student's *t*-test, $P < 0.001$). Thus some genes in the offspring's genome interact with the maternal effect. Moreover, the increase in intestine length conferred by the PRM/Alf genome in the offspring is greater with a PRM/Alf nurse-dam than with a DBA/2J nurse-dam (Fig. 7). Thus, in the maternal effect

Fig. 4. Intestine length means of 3-mo-old mice in PRM/Alf, DBA/2J parental strains and in F1, F2, and BC1 generations. To the *left* are the values in females, and to the *right* are the values in males. Next to each point are given the means of intestine length in centimeters, with the number of mice analyzed in parentheses. The error bars are the standard errors of means. BC1 on PRM/Alf and BC1 on DBA/2J stand for [(DBA/2J $\times$ PRM/Alf)F1 $\times$ PRM/Alf]BC1 and [(DBA/2J $\times$ PRM/Alf)F1 $\times$ DBA/2J]BC1, respectively. We found a significant influence of sex on intestine length in the DBA/2J parental strain, F2 generation, and [(DBA/2J $\times$ PRM/Alf)F1 $\times$ PRM/Alf]BC1 generation (Student's *t*-test, $P < 0.001$, $P = 0.002$, and $P < 0.001$, respectively).

Fig. 5. Intestine length distributions in PRM/Alf, DBA/2J parental strains and in F1, F2, and BC1 generations in males. A: parental strains. B: first and second generations of intercrosses. C: backcrosses. Numbers of mice analyzed are given in parentheses. Vertical dotted lines represent intestine length means in the parental populations. Note the normal aspect of every distribution (Kolmogorov-Smirnov test).

leading to a longer intestine, there is a positive genetic interaction between the PRM/Alf nurse-dam's and pup's genomes.

Genes in PRM/Alf progeny directly account for the intestine lengthening. Cross-fostering experiments revealed that the intestine of PRM/Alf mice fostered by DBA/2J nurse-dams was

longer compared with DBA/2J mice fostered by their own DBA/2J mothers (Fig. 7, compare white symbols in *right* and *left*; Student's *t*-test, $P < 0.001$). Thus, even when a PRM/Alf pup is raised by a non-PRM/Alf nurse-dam that is unable to confer the PRM/Alf maternal effect, the PRM/Alf genotype is still associated with intestine lengthening.

DISCUSSION

We show here that the PRM/Alf strain is characterized by a considerable intestine lengthening that affects both the small and the large intestines. The genetic determinism of the trait is polygenic. It also depends on the pup's genotype and its maternal environment during the suckling period.

Intestine lengthening phenotype in PRM/Alf mice is subjected to a postnatal maternal effect Maternal effects belong to the indirect genetic effects, where the genotype of one individual influences the expression of the phenotype of another individual, as opposed to direct genetic effects, where the genotype of the individual has direct effects on its own phenotype. Maternal effects can have genetic and environmental components. Nevertheless, from the standpoint of the offspring, both environmental and maternal genetic effects are an environmental source of variance (17). Maternal genetic effects can account for more than 50% of the phenotypic variance (14). In mammals, they have been found to have the strongest weight on early developmental characters such as early growth (14). Prenatal maternal effects, reflecting uterine environment, can influence birth weight and may still influence postnatal growth during the first week (9). Postnatal maternal effects are especially important among mammals, where the offspring are fostered for a prolonged time. Two recent quantitative trait loci (QTL) studies on early growth and on "diabesity" in mice have shown that maternal genetic effects accounted for a greater part of phenotypic variance than direct

Fig. 6. Influence of cross direction in intestine length in the F1 population. The F1 population was split according to the mother's strain. For clarity purpose, only male distributions are shown. Nevertheless, qualitatively similar results were found in females. F1 males born from a PRM/Alf female had a significantly longer intestine than F1 males born from a DBA/2J female (Student's *t*-test, $P < 0.001$).

Fig. 7. Intestine length of PRM/Alf, DBA/2J, and (PRM/Alf $\times$ DBA/2J)F1 mice in cross-fostering experiments. From left to right are given the means of intestine length in PRM/Alf, F1, and DBA/2J offspring, respectively. For a given offspring's genotype, the female that nursed the offspring is indicated by the color code. Next to each point is the mean for the given population. In parentheses is given the number of mice analyzed. The error bars are the standard errors of means. Asterisks indicate the statistical significance of the Student's *t*-test within a given offspring's genotype population: * $0.01 \leq P < 0.05$; *** $P < 0.001$. Influence of the offspring's genotype on intestine length is shown by comparing means for populations of a same color along the horizontal axis, by following the dotted lines; for each point pair, means were statistically different (Student's *t*-test, $P < 0.001$). Influence of the nurse-dam's genotype is shown by comparing means for populations of a given offspring's genotype along the vertical axis.

genetic effects (19). The contribution of maternal effects to phenotypic variance can be partitioned in a dam, a nurse-dam, and a dam-by-nurse-dam interaction parts (20, 24). The dam contribution evaluates the direct genetic effects together with the prenatal maternal effect. The nurse contribution evaluates the postnatal maternal effect. The dam-by-nurse-dam term evaluates the interaction between the dam and the nurse-dam contributions to phenotypic variance. In our study, we show that the PRM/Alf foster mother influenced intestine lengthening in her offspring mostly during the suckling period. In other words, the maternal effect in PRM/Alf intestine lengthening is mostly postnatal. It probably involves the milk composition. Cross-fostering experiments showed that, for a PRM/Alf or a (PRM/Alf $\times$ DBA/2J)F1 offspring, the intestine of the offspring is longer when nursed by a PRM/Alf dam; thus the postnatal maternal effect involves the genotype of the mother. Furthermore, for a dam of a given genotype, the intestine is longer when the offspring belongs to the PRM/Alf strain; thus the postnatal maternal effect involves the genotype of the offspring. The longest intestine is recovered when both the dam and the offspring belong to the PRM/Alf strain. Thus both the mother's and the offspring's genotypes interact in the postnatal maternal effect conferring intestine lengthening.

Candidate genes for intestine lengthening. Whereas many genes are known to influence postnatal growth in mice, very few have an effect on intestine length. Among the known intestinotrophic factors, only IGF-I, GLP2, and its receptor (GLP2R) are identified as positive regulators of intestine length (4, 10, 12, 16, 18, 22).

In addition to their considerable intestine lengthening, PRM/Alf mice are slightly heavier and longer than DBA/2J, C3H/He, and C57BL/6J mice. Candidate genes for PRM/Alf intestine phenotype must have a strong trophic effect on the intestine and may have a slight effect on body growth. According to PRM/Alf intestine growth curves, the candidates must act mostly during the early postnatal period, before weaning. Candidates for the maternal effect in intestine lengthening may

be growth factors secreted in the milk. Their receptors may be expressed in the digestive tract to account for the synergistic effect between the dam's and the offspring's genotypes in intestine lengthening. Importantly, the intestinal lengthening in PRM/Alf affects both the small intestine (SI) and the large intestine (LI) up to the same extent. Concentration of bioactive milk growth factors diminishes as a result of protein hydrolysis as the chyme moves down the digestive tract. If growth factor(s) present in PRM/Alf milk and responsible for intestine lengthening acted directly on intestine length, then one should assume that they escape digestion and pass on to the colon. Alternatively, they could be absorbed in the SI as small peptides, pass across the basolateral membrane to the bloodstream, and act systemically to trigger the expression of specific gene networks in cells within the intestine. Interestingly, PRM/Alf breeder females have a longer intestine than PRM/Alf virgins (data not shown). This suggests that the intestinotrophic factor(s) may be present not only in the milk but also in the lactating dam's bloodstream, while absent or in limited amount in the nonlactating female's bloodstream.

Colostrum- and milk-borne factors that are known to affect intestine growth include EGF, IGF-I and IGF-II, insulin, IGF-BPs, TGF- β , and lactoferrin. These factors are involved in stimulating intestinal mucosa growth and maturation and play a role in facilitating postnatal adaptation of the gastrointestinal tract in neonates (5, 6, 7, 15, 21, 25). These factors do not seem to be absorbed in the small intestine so that they are present in an active form in the intestinal lumen. However, apart from IGF-I (22) and to a smaller extent lactoferrin (26), none of them is known to affect intestinal length.

To identify loci contributing to intestine lengthening either directly or via the maternal effect, a QTL analysis is needed. Protein analysis of PRM/Alf milk should also help identify growth factors involved in the maternal effect.

Altogether, the PRM/Alf inbred strain constitutes a unique rodent model for intestine lengthening. By use of rodent

genetics methods, it should help finding intestinotrophic factors of clinical relevance in humans.

ACKNOWLEDGMENTS

Present address of N. R. Da Silva: Human Genetics Unit, Medical Research Council, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, UK.

GRANTS

This work was supported by the Association pour la Recherche Contre le Cancer (Grants 4332 and 99/7468). N. R. Da Silva was funded by the Ministère de la Recherche et Nouvelles Technologies and by the Association pour la Recherche Contre le Cancer.

REFERENCES

1. Aubin-Houzelstein G, Bernex F, Elbaz C, and Panthier JJ. Survival of patchwork melanoblasts is dependent upon their number in the hair follicle at the end of embryogenesis. *Dev Biol* 198; 266–276, 1998.
2. Aubin-Houzelstein G and Panthier JJ. The patchwork mouse phenotype: implication for melanocyte replacement in the hair follicle. *Pigment Cell Res* 12: 181–186, 1999.
3. Baksheev L and Fuller PJ. Humoral factors in intestinal adaptation. *Trends Endocrinol Metab* 11: 401–405, 2000.
4. Bjerknes M and Cheng H. Modulation of specific intestinal epithelial progenitors by enteric neurons. *Proc Natl Acad Sci USA* 98: 12497–12502, 2001.
5. Blum JW and Baumrucker CR. Colostral and milk insulin-like growth factors and related substances: mammary gland and neonatal (intestinal and systemic) targets. *Domest Anim Endocrinol* 23: 101–110, 2002.
6. Blum JW and Hammon H. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. *Livest Prod Sci* 66: 151–159, 2000.
7. Burrin DG, Davis TA, Fiorotto ML, and Reeds PJ. Role of milk-borne vs endogenous insulin-like growth factor I in neonatal growth. *J Anim Sci* 75: 2739–2743, 1997.
8. Christl SU and Scheppach W. Metabolic consequences of total colectomy. *Scand J Gastroenterol Suppl* 222: 20–24, 1997.
9. Cowley DE, Pomp D, Atchley WR, Eisen EJ, and Hawkins-Brown D. The impact of maternal uterine genotype on postnatal growth and adult body size in mice. *Genetics* 122: 193–203, 1989.
10. Donovan SM, Hintz RL, Wilson DM, and Rosenfeld RG. Insulin-like growth factors I and II and their binding proteins in rat milk. *Pediatr Res* 29: 50–55, 1991.
11. Drucker DJ. Epithelial cell growth and differentiation. I. Intestinal growth factors. *Am J Physiol Gastrointest Liver Physiol* 273: G3–G6, 1997.
12. Drucker DJ. Gut adaptation and the glucagon-like peptides. *Gut* 50: 428–435, 2002.
13. Drucker DJ, DeForest L, and Brubaker PL. Intestinal response to growth factors administered alone or in combination with human [Gly2]glucagon-like peptide 2. *Am J Physiol Gastrointest Liver Physiol* 273: G1252–G1262, 1997.
14. el-Oksh HA, Sutherland TM, and Williams JS. Prenatal and postnatal maternal influence on growth in mice. *Genetics* 57: 79–94, 1967.
15. Laerke HN, Lesniewska V, Hedemann MS, Jensen BB, and Pierzynowski SG. Aspects of gastrointestinal motility in relation to the development of digestive function in neonates. *Livest Prod Sci* 66: 133–139, 2000.
16. Lovshin J, Yusta B, Iliopoulos I, Migirdicyan A, Dableh L, Brubaker PL, and Drucker DJ. Ontogeny of the glucagon-like peptide-2 receptor axis in the developing rat intestine. *Endocrinology* 141: 4194–4201, 2000.
17. Lynch M and Walsh B. Maternal effects. In: *Genetics and Analysis of Quantitative Traits*. Sunderland: Sinauer Associates, 1998, p. 687–714.
18. Ohneda K, Ulshen MH, Fuller CR, D'Ercole AJ, and Lund PK. Enhanced growth of small bowel in transgenic mice expressing human insulin-like growth factor I. *Gastroenterology* 112: 444–454, 1997.
19. Reifsnnyder PC, Churchill G, and Leiter EH. Maternal environment and genotype interact to establish diabetes in mice. *Genome Res* 10: 1568–1578, 2000.
20. Riska B, Rutledge JJ, and Atchley WR. Covariance between direct and maternal genetic effects in mice, with a model of persistent environmental influences. *Genet Res* 45: 287–297, 1985.
21. Schanbacher FL, Talhouk RS, Murray FA, Gherman LI, and Willett LB. Milk-borne bioactive peptides. *Int Dairy J* 8: 393–403, 1998.
22. Steeb CB, Trahair JF, Tomas FM, and Read LC. Prolonged administration of IGF peptides enhances growth of gastrointestinal tissues in normal rats. *Am J Physiol Gastrointest Liver Physiol* 266: G1090–G1098, 1994.
23. Vanderhoof JA and Langnas AN. Short-bowel syndrome in children and adults. *Gastroenterology* 113: 1767–1778, 1997.
24. Wolf JB, Vaughn TT, Pletscher LS, and Cheverud JM. Contribution of maternal effect QTL to genetic architecture of early growth in mice. *Heredity* 89: 300–310, 2002.
25. Xu RJ, Wanga F, and Zhang SH. Postnatal adaptation of the gastrointestinal tract in neonatal pigs: a possible role of milk-borne growth factors. *Livest Prod Sci* 66: 95–107, 2000.
26. Zhang P, Sawicki V, Lewis A, Hanson L, Nuijens JH, and Neville MC. Human lactoferrin in the milk of transgenic mice increases intestinal growth in ten-day-old suckling neonates. *Adv Exp Med Biol* 501: 107–113, 2001.