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Quantitative trait loci affecting growth in *high growth* (*hg*) mice

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Abstract. A genome-wide scan was performed in order to identify Quantitative Trait Loci (QTL) associated with growth in a population segregating *high growth* (*hg*), a partially recessive mutation that enhances growth rate and body size in the mouse. A sample of 262 *hg*/*hg* mice was selected from a C57BL/6J-*hg*/*hg* × CAST/EiJ F_2 cross and typed with 79 SSLP markers distributed across the genome. Eight significant loci were identified through interval mapping. Loci on Chromosomes (Chrs) 2 and 8 affected the growth rate of F_2 mice. Loci on Chr 2 and 11 affected growth rate and carcass lean mass (protein and ash). A locus on Chr 9 modified femur length and another one in Chr 17 affected both carcass lean mass and femur length, but none of these had significant effects on growth rate. Loci on Chrs 5 and 9 modified carcass fat content. Additive effects were positive for C57BL/6J alleles, except for the two loci affecting carcass fatness. Typing of selected markers in 274 +/+ F_2 mice revealed significant interactions between *hg* and other growth QTL, which were detected as changes in gene action (additive or dominant) and in allele substitution effects. Knowledge about interactions between loci, especially when major genes are involved, will help in the identification of positional candidate genes and in the understanding of the complex genetic regulation of growth rate and body size in mammals.

Introduction

The high growth locus (*hg*) is an autosomal, partially recessive mutation that enhances weight gain and body size by 30–50% in the mouse (Bradford and Famula 1984; Horvat and Medrano 1995). Despite the drastic change in growth rate, *hg*/*hg* mice are proportionate in the size of tissues and organs (Famula et al. 1988). This unique phenotype distinguishes *hg* from other known spontaneous mutations that affect body weight by causing either obesity (*Lep*^{ob}, *Lepr*^{db}, *Cpe*^{fat}, *A*^{y}, *tub*) or dwarfism (*Pit1*^{dw}, *Prop1*^{df}, *Ghrhr*^{lit}, *Hmgic*^{pg}, *mn*, *dm*; Lyon et al. 1996). Genetic and physical mapping have determined that a deletion in Chr 10 causes the high growth (HG) phenotype (Horvat and Medrano 1996, 1998). Recently, the *hg* phenotype has been identified as resulting from a lack of expression of the suppressor of cytokine signaling 2 (*Socs2* or *Cish2*; Horvat and Medrano 2001).

A spontaneous mutation enhancing growth rate and body size is a valuable model for studying the genetics of growth in mammals. Changes in body size are usually achieved through an altered pattern of cell proliferation (Raff 1996). It has been demonstrated that at least in the muscle, HG mice have a larger number of fibers owing to enhanced cell proliferation and delayed fusion of myoblasts (Summers and Medrano 1994, 1997). However, there is no evidence of any abnormalities in tissue development as observed in other mouse models of enhanced growth, such as the $p27^{Kip}$ gene knockout (Nakayama et al. 1996).

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One of the potential limitations to the extension to other species of discoveries from major gene mutations in the mouse is the confounding effect of other genetic and nongenetic factors on the phenotype under study, and *hg* is not an exception to that limitation. We have demonstrated that the nutritional environment has a profound effect on the HG phenotype (Corva and Medrano 2000). Also, other lines of evidence suggest that the genetic background could modulate the effects of *hg* on growth. The *hg* locus was found in a strain of mice selected for high 3- to 6-week weight gain (Bradford and Famula 1984). In that genetic background, weight gain data followed a bimodal distribution, and it was possible to identify most *hg*/*hg* mice based on their phenotype. A C57BL/6J–*hg*/*hg* × CAST/EiJ F_2 cross (Horvat and Medrano 1995) was used to map *hg* to Chr 10, and in this population weight gain data followed a normal distribution, and it was no longer possible to identify *hg*/*hg* individuals without knowledge of their genotypes. This information, together with the fact that *hg* was discovered in a line selected for high weight gain, led us to hypothesize that the expressivity of *hg* was modulated by other genes associated with growth regulation.

In this paper we present the results of a genome-wide scan on the *hg*/*hg* individuals of a C57BL/6J-*hg*/*hg* × CAST/EiJ F_2 cross to identify QTL affecting growth rate, body size, and carcass composition. After identifying QTL in the *hg*/*hg* individuals, we examined the effects of these QTL also in +/+ F_2 mice, for identifying genetic modifier loci of *hg*. We considered as unique modifiers of *hg* all the QTL that were detected in the *hg*/*hg* background, but not in the +/+ background and those that had effects in both backgrounds but displayed significant differences in gene action.

Materials and methods

Mouse crosses. The *hg* locus has been introgressed into the C57BL/6J (C57) background by nine backcrosses to create the congenic line C57BL/6J-*hg*/*hg* (HG). In this experiment, congenic mice from the seventh generation of inbreeding were used. CAST/EiJ (CAST) males were mated to HG females to create the mapping cross (Horvat and Medrano 1995). CAST mice are smaller and much leaner than C57 mice, even when the mice are on a high-fat diet (York et al. 1996). Therefore, the cross is suitable to detect specific alleles interacting with *hg* that modify body size and composition.

A total of 75 F_1 and 1132 F_2 mice were produced. The F_2 cross was genotyped for *hg* by using the linked marker *D10Mit41* and a marker that maps to the *hg* deletion, *D10Mit69* (Horvat and Medrano 1996). Mice homozygous for HG alleles at *D10Mit41* and without a PCR amplification product for *D10Mit69* (indicating homozygosity for the *hg* deletion) were considered to be of *hg*/*hg* genotype, and mice homozygous for CAST alleles at *D10Mit41* and amplifying for *D10Mit69* were regarded as being of +/+ genotype. Using such a screen, we determined that the cross was composed of 274 +/+ mice, 596 +/*hg* mice, and 262 *hg*/*hg* mice, which is in agreement with Mendelian segregation ratios in the F_2 population.

A second experimental cross was created by mating C57 and CAST mice. Sixty F_1 mice and 330 F_2 mice were produced. This F_2 cross was used to confirm the significance of linkage of markers identified on Chr 2 of the F_2 cross segregating *hg*, by means of selective genotyping.

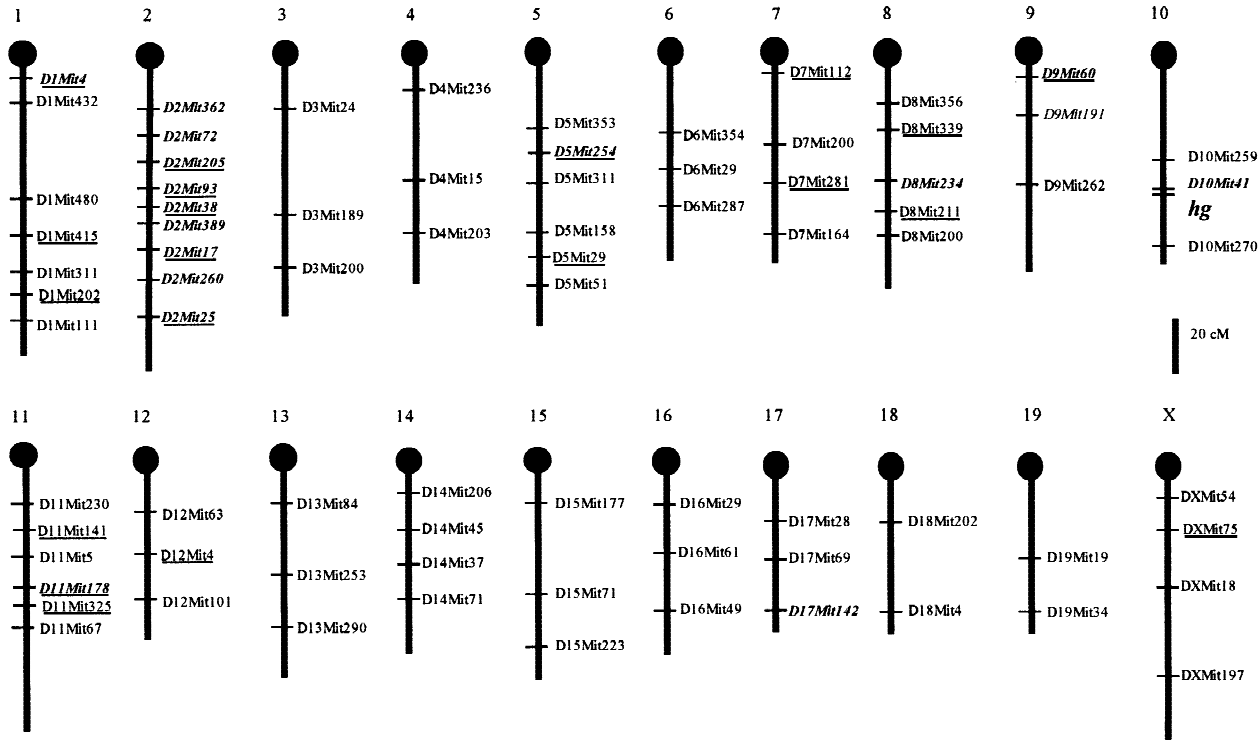

Fig. 1. Map of markers typed in the C57-*hg*/*hg* × CAST F_2 cross. Underlined markers were added in significant chromosomal regions identified through ANOVA. Markers in bold italics were also typed in the +/+ mice of the F_2 cross.

Husbandry and phenotype determinations. Mice were weaned at 3 weeks of age. Feed (Purina 5008; 23.5% protein, 6.5% fat, 3.3 Kcal/g) and water were offered ad libitum. Mice were weighed to the nearest 0.1 g at 2, 3, 6, and 9 weeks of age and sacrificed after 9 weeks of age by cervical dislocation. Liver, spleen, and skin were removed, and the carcass was frozen. To perform the chemical analysis, carcasses were thawed at room temperature. Carcass water content was determined by freeze-drying the carcasses to a constant weight. Lipid content was estimated by the carcass weight change after extraction with ether for 7 days, followed by acetone for 5 days in a Soxhlet apparatus. Body ash content was determined by incinerating the carcass in a muffle furnace at 575°C for 16 h. One femur bone was removed from the partially ashed carcass and measured to 0.1 mm. Only live weights at the same ages mentioned above were recorded in the C57 × CAST cross.

We measured protein mass because the dry matter is considered a better estimator of dynamics of cell populations of an organ or body than their fresh weight (Graham et al. 1998). We also included ash mass and femur length to have an estimation of differences in skeletal mass and size, respectively. Although we did not measure body length of the F_2 mice, Famula et al. (1988) demonstrated that femur length is a good predictor of body length (regression $R^2 \geq 0.83$; uniform regression slopes between *hg*/*hg* and control mice).

Genotyping and linkage analysis. In order to find QTL, we followed a hierarchical search approach (Brown et al. 1994). In the first step, the *hg*/*hg* mice from the F_2 cross were genotyped with a set of 59 SSLP markers (Research Genetics, Huntsville, Ala.) covering the 19 autosomal chromosomes and Chr X (Fig.1). Some of these markers were chosen on the basis of their proximity to known genes and previously identified growth QTL. The typing of markers was performed according to conventional PCR and agarose gel electrophoresis methods. The linkage of these markers to loci affecting weight gain and body composition was evaluated through ANOVA, by using the GLM procedure of SAS (SAS 1998). The model included marker genotype information and the fixed effects of sex, parity, litter size, and two-way interactions. The analyzed traits were body weight at 2, 3, 6, and 9 weeks of age; weight gain from 2 to 6, 6 to 9, and 2 to 9 weeks of age (G26, G69 and G29, respectively); carcass protein; carcass ash; femur length; and carcass fat percentage. The analysis of body weight at 2 and 3 weeks of age and G69 produced no significant linkage results. The results for body weight at 6 and 9 weeks of age and for G26 were almost identical to those for G29. In addition, evaluation of phenotypic data from the F_2 cross suggested that *hg* had a more noticeable effect on growth rates than on live weight differences (Table 1). Therefore, we report here the results corresponding to G29 and carcass composition traits.

The threshold to declare significant linkage in the ANOVA was established by choosing a genome-wide P value of 0.10 and applying the Bonferroni correction for multiple comparisons (SAS 1998). Therefore, a nominal value of $P \leq 0.006$ was considered indicative of linkage in the single-marker analyses. More markers were added in those chromosomes showing significant linkage in the ANOVA, for a total of 79 markers (Fig. 1). The GGT (Graphical Genotypes) software (van Berloo 1999) was used to create a graphical display of the genotyped chromosomes in each individual in order to assist in error checking and genotyping quality control.

In order to determine the location of a locus on a given chromosome, interval mapping was performed by using regression analysis (Haley and Knott 1992). The appropriate programs were written and run with the SAS software (SAS 1998). Before performing the final linkage analysis, the regression programs were tested with the same data set used when *hg* was mapped (Horvat and Medrano 1995). The regression analysis produced results almost identical to those with Mapmaker (Lander et al. 1987) in the earlier report.

Before proceeding to the analysis, we also evaluated positions and orders of our markers by using Mapmaker. The estimated distances between markers that we obtained with Mapmaker (Lander et al. 1987) were in agreement with the mouse consensus map (MGD 2000); therefore, we used the information from the consensus map for the analysis.

The regression models included additive (a) and dominance (d) terms, together with the effects of sex and age. Conditioning markers were included in the models to account for background genetic effects (Zeng 1994). These markers were selected for each trait by backward regression analysis with a probability of $P < 0.05$. In a first step, single chromosomes were analyzed. In a second step, all significant markers were evaluated together, and only those remaining after the backward selection were included in the models. Conditioning markers were omitted from the model when their corresponding chromosomes were analyzed.

The regression analysis between two markers was performed at 2-cM intervals. The results were expressed as LOD scores, $LOD = 4.605 \times$ likelihood ratio test (LR), where $LR = n \times \log_e (RSS_{reduced}/RSS_{full})$ [n is the sample size, and RSS_{full} and $RSS_{reduced}$ are the Residual Sum of Squares of the complete regression model (full) and the model with the additive (a)

Table 1. Means and standard deviations (SD) of traits measured in the C57-*hg/hg* × CAST F_2 cross.

	Females						
	+/+ Mice			*hg/hg* Mice			
	n	Mean	SD	n	Mean	SD	Prob.
Wt2 g	138	8.8	1.3	130	8.6	1.5	N.S.
Wt3 g	139	11.5	1.7	130	11.5	1.9	N.S.
Wt6 g	134	17.5	2.0	129	20.2	3.1	<0.0001
Wt9 g	130	19.2	2.3	123	23.4	3.3	<0.0001
G29 g	131	10.4	2.2	123	14.8	3.1	<0.0001
Carcass weight g	129	12.42	1.86	122	15.47	2.78	<0.0001
Carcass protein g	128	2.41	0.34	121	2.91	0.52	<0.0001
Carcass ash g	128	0.65	0.10	122	0.81	0.16	<0.0001
Carcass fat %	128	10.4	4.0	122	12.8	5.2	<0.0001
Femur length mm	127	14.5	0.6	122	15.4	0.9	<0.0001
	Males						
	+/+ Mice			*hg/hg* Mice			
	n	Mean	SD	n	Mean	SD	Prob.
Wt2 g	135	9.3	1.4	130	8.6	1.4	<0.0001
Wt3 g	135	12.7	1.9	130	12.1	2.2	<0.05
Wt6 g	131	21.2	2.5	127	24.4	4.4	<0.0001
Wt9 g	124	23.8	3.2	123	28.7	4.7	<0.0001
G29 g	124	14.6	2.9	123	20.1	4.2	<0.0001
Carcass weight g	130	14.99	2.13	123	18.36	3.33	<0.0001
Carcass protein g	130	2.97	0.40	123	3.53	0.64	<0.0001
Carcass ash g	130	0.71	0.09	123	0.85	0.15	<0.0001
Carcass fat %	130	9.6	4.5	123	12.4	5.6	<0.0001
Femur length mm	130	14.9	0.6	119	15.6	0.8	<0.0001

Wt2–Wt9: live weights at 2, 3, 6, and 9 weeks of age, respectively. G29: Weight gain from 2 to 9 weeks of age.

and dominance (d) terms omitted (reduced), respectively (Haley and Knott 1992)].

Empirical significance thresholds were calculated by using a permutation method (Churchill and Doerge 1994). Phenotypes were permuted against genotypes and conditioning markers, and the regression analyses were repeated 1000 times. The experiment-wise significance threshold, $P < 0.05$ or $P < 0.01$, for each trait was established by choosing the 50th or the 10th highest LOD score across all chromosomes, respectively. The estimated experiment-wise thresholds for the interval mapping had similar values among traits and chromosomes, with extremes of 1.80 for femur length and 2.47 for carcass protein ($P < 0.05$), and 2.65 for carcass protein and 3.25 for carcass ash ($P < 0.01$).

The most significant markers in the *hg*/*hg* subpopulation were also typed in the +/+ subpopulation in order to verify whether the same loci were detected as QTL in the genetic background carrying the wild-type allele at the *hg* locus. Chr 2 seemed to harbor genes with very strong effect on growth, both in the +/+ and *hg*/*hg* subpopulations. Therefore, we genotyped the +/+ mice with the same set of markers that we used on *hg*/*hg* mice. Interval mapping was performed as described above.

To confirm the linkage of markers *D2Mit389* and *D2Mit260* to QTL in Chr 2 in an independent population, we performed selective genotyping in the F_2 cross (N = 330) that did not segregate *hg*. Data on weight gain from 3 to 6 weeks of age was corrected for the effects of dam, litter, and parity with the GLM procedure of SAS (SAS 1998). The mice were ranked based on the adjusted data, and 24 mice (12 from each sex) from the extreme ends of the F_2 distribution were typed. The means of the high and low weight gain groups were 1.83 standard deviations above and 1.68 standard deviations below the population mean respectively. For each marker, a chi-square test was performed with the FREQ procedure of SAS (SAS 1998) to compare allele frequencies between the two groups.

Results

As a relative comparison of the size of +/+ and *hg*/*hg* mice, the phenotypic means of traits recorded in our F_2 population are presented in Table 1. At 2 and 3 weeks of age, there were no significant differences in weight between +/+ and *hg*/*hg* females. However, *hg*/*hg* males were smaller than +/+ mice at the same ages. At 6 and 9 weeks of age, *hg*/*hg* mice of both sexes were significantly heavier than the wild-type mice. These results show that *hg*/*hg* mice grew faster than +/+ mice, especially after weaning. In fact, a significant genotype × sex interaction was detected for weight gain between 2 and 9 weeks of age. Among females, *hg*/*hg* mice gained 42% more weight than +/+ mice, whereas the difference in weight gain for males was 38%. The increased growth rate of *hg*/*hg* mice is typical of the effect of this locus (Bradford and Famula 1984).

At sacrifice, *hg*/*hg* mice had heavier carcasses than +/+ mice. The carcasses of *hg*/*hg* mice had more protein and ash, a longer femur, and a higher fat content than the carcasses of +/+ mice (Table 1).

Eight chromosomes harboring significant markers where identified with ANOVA and then analyzed by interval mapping. Eight loci were identified through interval mapping, which were designated *Q2Ucd1*, *Q2Ucd2*, *Q5Ucd1*, *Q8Ucd1*, *Q9Ucd1*, *Q9Ucd2*, *Q11Ucd1*, and *Q17Ucd1* (Table 2). Markers on Chrs 13 and X were identified as significant in the ANOVA, but did not reach the minimum significance thresholds by interval mapping. No significant sex-specific effects were detected in our scan.

Significant loci identified in *hg*/*hg* mice can be arbitrarily placed in four groups. Group I: loci in Chrs 2 and 8 (*Q2Ucd1* and *Q8Ucd1*), which affected only the growth rate of F_2 mice. Group II: loci on Chrs 2 and 11 (*Q2Ucd2* and *Q11Ucd1*) that affected growth rate and carcass lean mass (protein and ash). Although we conducted independent analyses for each trait, the consistency in the location of these two loci in the different analyses made us consider that the same locus was affecting more than one trait, which is highly suggestive of pleiotropy. Group III: Loci on Chr 9 (*Q9Ucd2*), that modified femur length and Chr 17 (*Q17Ucd1*) that modified carcass lean mass (protein and ash) and femur length. These two loci had no effect on growth rate. For Chr 17, we also assumed that a single locus was affecting two different traits. Group IV: Loci on Chrs 5 and 9 (*Q5Ucd1* and *Q9Ucd1*) that modified carcass fat content. QTL displayed various gene action modes (Table 2) from additivity (e.g., *Q17Ucd1-p*), dominance (e.g., *Q2Ucd1-femur*), partial dominance (e.g., *Q11Ucd1-p*) to overdominance (e.g., *Q2Ucd2-wg29*).

Table 2. Most likely locations and effects of loci detected in the *hg/hg* subpopulation.

Trait	Locus[a]	Chrom.	Location (cM)	LOD[b] Score	Additivity (SE)	Dominance (SE)	% Vp[c]
Weight gain	*Q2Ucd1-wg29*	2	31	3.75	1.174 (0.327)	0.841 (0.463)	4.2
2–9 wk (g)	*Q2Ucd2-wg29*	2	61	7.43	1.309 (0.319)	2.286 (0.509)	10.4
	Q8Ucd1-wg29	8	45	3.01	0.892 (0.328)	1.397 (0.512)	4.3
	Q11Ucd1-wg29	11	46	3.41	1.283 (0.324)	0.320 (0.491)	4.1
Carcass	*Q2Ucd2-p*	2	63	4.89	0.165 (0.043)	0.272 (0.079)	7.6
Protein (g)	*Q11Ucd1-p*	11	46	5.01	0.206 (0.042)	0.111 (0.064)	5.7
	Q17Ucd1-p	17	46	4.77	0.231 (0.048)	−0.054 (0.069)	6.5
Carcass	*Q2Ucd2-ash*	2	63	4.34	0.037 (0.011)	0.062 (0.017)	6.9
Ash (g)	*Q11Ucd1-ash*	11	50	3.18	0.039 (0.010)	0.026 (0.015)	3.9
	Q17Ucd1-ash	17	48	3.67	0.048 (0.011)	−0.009 (0.015)	4.9
Carcass	*Q5Ucd1-fp*	5	38	2.46	−1.642 (0.492)	0.270 (0.766)	6.2
Fat (%)	*Q9Ucd1-fp*	9	10	5.83	−2.347 (0.454)	0.562 (0.744)	12.5
Femur	*Q2UCd2-femur*	2	59	2.72	0.202 (0.068)	0.207 (0.107)	4.2
length (mm)	*Q9Ucd2-femur*	9	20	6.34	0.365 (0.072)	0.224 (0.112)	10.7
	Q17Ucd1-femur	17	48	3.51	0.305 (0.071)	−0.087 (0.096)	6.6

[a]Locus nomenclature: Q for QTL, chromosome #, *Ucd* for University of California, a consecutive number for each QTL on a chromosome, and a letter code referring to the phenotype. QTL affecting more than one trait are distinguished by the phenotype code.
[b]LOD scores were significant at $P < 0.01$, with the exception of LODs for *Q5Ucd1-fp* and *Q2Ucd2-femur,* which were significant at $P < 0.05$.
[c]% V_P, % of Phenotypic Variance explained by the QTL. %Vp = V_G *100/Vp, where $V_G = \frac{1}{2} a^2 + \frac{1}{4} d^2$.

The loci affecting growth rate and carcass lean mass individually explained 4.1% to 10.4% of the phenotypic variance on weight gain in the *hg*/*hg* subpopulation. The locus *Q2Ucd2* had very significant effects on all the studied traits with the exception of fat percentage. On the other hand, the two significant loci *Q5Ucd1* and *Q9Ucd1* explained 6.2% and 12.5% of the phenotypic variance for fat content in the *hg*/*hg* subpopulation respectively (Table 2). Interestingly, the C57 alleles had a positive additive effect for all the identified loci with the exception of loci *Q5Ucd1* and *Q9Ucd1,* for which the CAST alleles were responsible for increasing carcass fat percentage.

In order to identify modifiers of *hg,* we genotyped the marker that was closest to the significant QTL in the *hg*/*hg* group, ALSO in the +/+ mice of the F_2 cross. Four markers showed statistically significant interactions in the ANOVA (nominal $P < 0.05$); (Fig. 2). Interestingly, these markers are examples of three different types of genetic interaction. Marker *D2Mit389* on Chr 2 (close to *Q2Ucd2*) had an additive effect on weight gain on +/+ mice, but became over dominant in the presence of *hg.* The locus on Chr 17 (*Q17Ucd1*) modifying femur length, which had an additive effect in *hg*/*hg* mice, tended to be dominant in +/+ mice. Loci on Chrs 9 and 11 (*Q9Ucd1* and *Q11Ucd1*) that affected carcass fatness and carcass protein mass in an additive manner in the *hg*/*hg* mice had no significant effects in +/+ mice.

In view of the importance of the effect of *Q2Ucd2* on growth rate and carcass lean mass of *hg*/*hg* mice, we genotyped the +/+ mice with the same markers of Chr 2 and performed the interval mapping for growth rate (G29) and carcass composition traits (Fig. 3). It can be seen that the LOD curves have distinctive patterns in each subpopulation and that the highest LOD scores in each case corresponded to different positions on the chromosome. In +/+ mice, a very significant locus affecting growth rate and carcass lean mass was detected distal to the location of *Q2Ucd2.*

In order to confirm the existence of the two important loci found on Chr 2 of *hg*/*hg* and +/+ mice, we selectively genotyped a C57 × CAST F_2 cross with markers *D2Mit389* and *D2Mit260.* For *D2Mit389,* the frequency of the C57 allele in the groups of high and low weight gain were 0.65 and 0.33, respectively ($\chi^2 = 10.3$, $P < 0.0013$, $n = 24$), whereas the corresponding frequencies for *D2Mit260* were 0.65 and 0.29, respectively ($\chi^2 = 12.9$, $P < 0.0003$, $n = 24$). These results indicate that both markers were linked to QTL and that it was not an artifact of the F_2 cross segregating *hg.*

Discussion

It has been suggested that in terms of genetic analysis, our knowledge about the genome is progressing faster than our understanding of the phenotype a situation that has been addressed as a "phenotype gap" (Graham et al. 1998). Given the complexity of the regulation of animal growth, a good characterization of the phenotype and novel strategies for genetic analysis would help in understanding the regulation of growth and body size. In this study, we have contributed to that understanding by describing the influence of the genetic background on a major locus affecting growth.

Our genome-wide scan identified eight loci responsible for the differences in growth among *hg*/*hg* mice. The traits that we measured allowed us to distinguish loci that affected growth rate from those affecting body size and carcass composition. Growth rate and body size have a positive genetic correlation (Bishop and Hill 1985); however, we found loci that affected both traits independently. Although we were primarily interested in the influence of *hg* on linear growth, we included the analysis of body composition based on previous knowledge about the variability between CAST and C57, not only in size but also in body composition (York et al. 1996). These distinguishing features between lines made the C57 × CAST cross an ideal material for evaluating the effects of a major locus affecting growth.

In our search for modifiers, we detected significant two-way interactions between four loci and *hg (Q2Ucd2, Q9Ucd1, Q11Ucd1, Q17Ucd1).* The comparison of means between the *hg*/*hg* and +/+ subpopulations for selected markers proved that not all the loci that modulated the effects of *hg* on growth were present in the +/+ F_2 mice. Additionally, some loci that were significant in both backgrounds had a different type of genetic action depending on the background. This result emphasizes the relevance of epistasis in the genetic regulation of growth in mammals (Routman and Cheverud 1997).

A large number of growth QTL have been reported in the literature. We found agreement in the position of QTL in *hg*/*hg* mice and other QTL affecting growth and body composition, which could imply that the same genes are being detected. Although we list below the comparisons of QTL positions between our cross and other crosses, we would like to make a cautionary note that such comparisons are problematic and may be questionable. It has been shown (Keightley and Knott 1999) that getting a

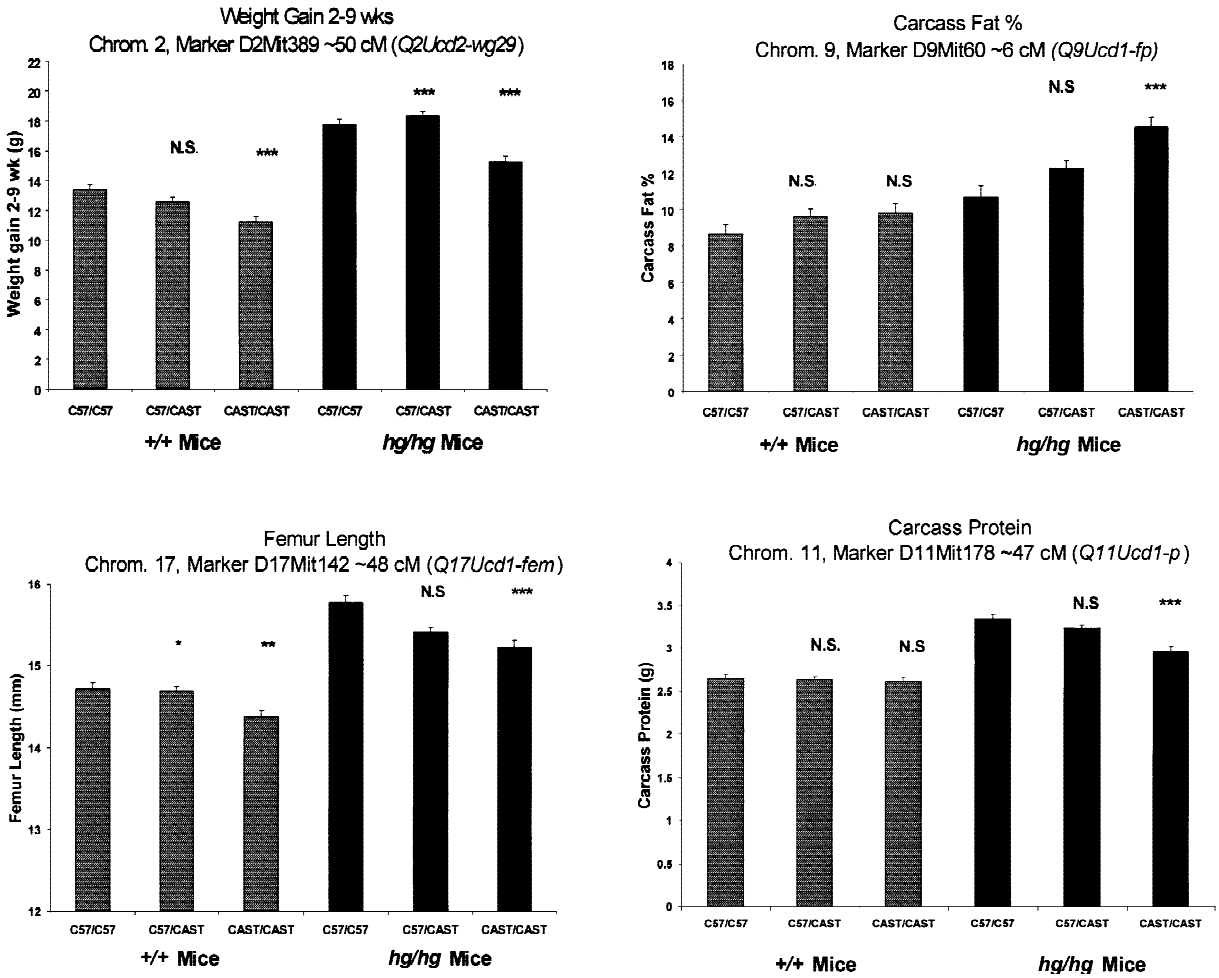

Fig. 2. Sex-adjusted means and standard errors for selected traits and markers that showed significant two-way interactions with *hg*. Mice were classified on the basis of their genotype at the markers closest to a detected QTL. Symbols above the C57/CAST and CAST/CAST bars indicate the significance of the contrasts: [C57/CAST − ½ × (C57/C57 + CAST/CAST)] and [C57/C57 − CAST/CAST], respectively (NS: not significant, *:$P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

statistically significant correlation between two QTL mapping experiments (i.e., that the same QTL segregates in two crosses) would be unlikely especially for cases where the variation in the trait is explained by a large number of QTL and where experimental populations are not closely related, which is the case with the experiments compared here.

Cheverud et al. (1996) reported a QTL for early weight gain (1 to 3 weeks of age) on Chr 2 in the LG/J × SM/J cross, in a position similar to *Q2Ucd2*. Loci affecting 6-week weight on Chr 2 and 11 were reported by Brockmann et al. (1998) in the DUK × DU6 cross, in the corresponding regions to where we mapped *Q2Ucd2* and *Q11Ucd1* respectively. In addition, a significant locus in the vicinity of *Q11Ucd1* was identified by Keightley et al. (1996) in a line selected for high 6-week weight. We were not able to find information on other growth QTL mapping to the locations of *Q2Ucd1*, *Q8Ucd1*, or *Q17Ucd1*.

A QTL affecting adult body weight in a C57 × CAST cross has been mapped to the same region in Chr 2 where we found a very significant QTL in +/+ mice (region around 80–90 cM; Mehrabian et al. 1998). A very large confidence interval reported for the location of that locus, together with the pattern of LOD scores along the chromosome, suggests that a second locus, probably at the same location of *Q2Ucd2*, could have affected body weight in that experiment. Also, an adult body weight QTL mapping to the distal region of Chr 2 has been identified in the NZB/BINJ × SM/J cross (Lembertas et al. 1997).

Results of genomic scans for obesity QTL on C57 × CAST crosses have been reported (York et al. 1996; Mehrabian et al. 1998). Our loci *Q5Ucd1* and *Q9Ucd1* were not detected in those experiments. Because high-fat diets were used in the two cited experiments, their results are not strictly comparable to ours. However, we did not detect those two loci as significant in the +/+ subpopulation either. There is evidence in the literature of loci affecting carcass composition in locations similar to *Q5Ucd1* and *Q9Ucd1*. Taylor and Phillips (1996) suggested the existence of a putative obesity QTL in the central portion of Chr 5 in a 129/Sv × EL/Suz cross. Also, the locus *Obq5* identified in a C57 × KK/H1Lt cross, which affected several adiposity-related traits (Taylor et al. 1999), mapped close to *Q9Ucd1*. Together, these results indicate that *hg* produced metabolic changes beyond the effects on growth rate and body size. Interestingly, *hg*/*hg* F_2 mice were on average fatter than their wild-type counterparts (Table 1). Previous experiments comparing the body composition of *hg*/*hg* and control mice found differences in carcass composition of the magnitude reported here (Calvert et al. 1984). The CAST alleles at *Q5Ucd1* and *Q9Ucd1* increased carcass fat content, but only in *hg*/*hg* mice (Table 2). Therefore, this particular effect on body composition could involve specific alleles from CAST origin interacting with

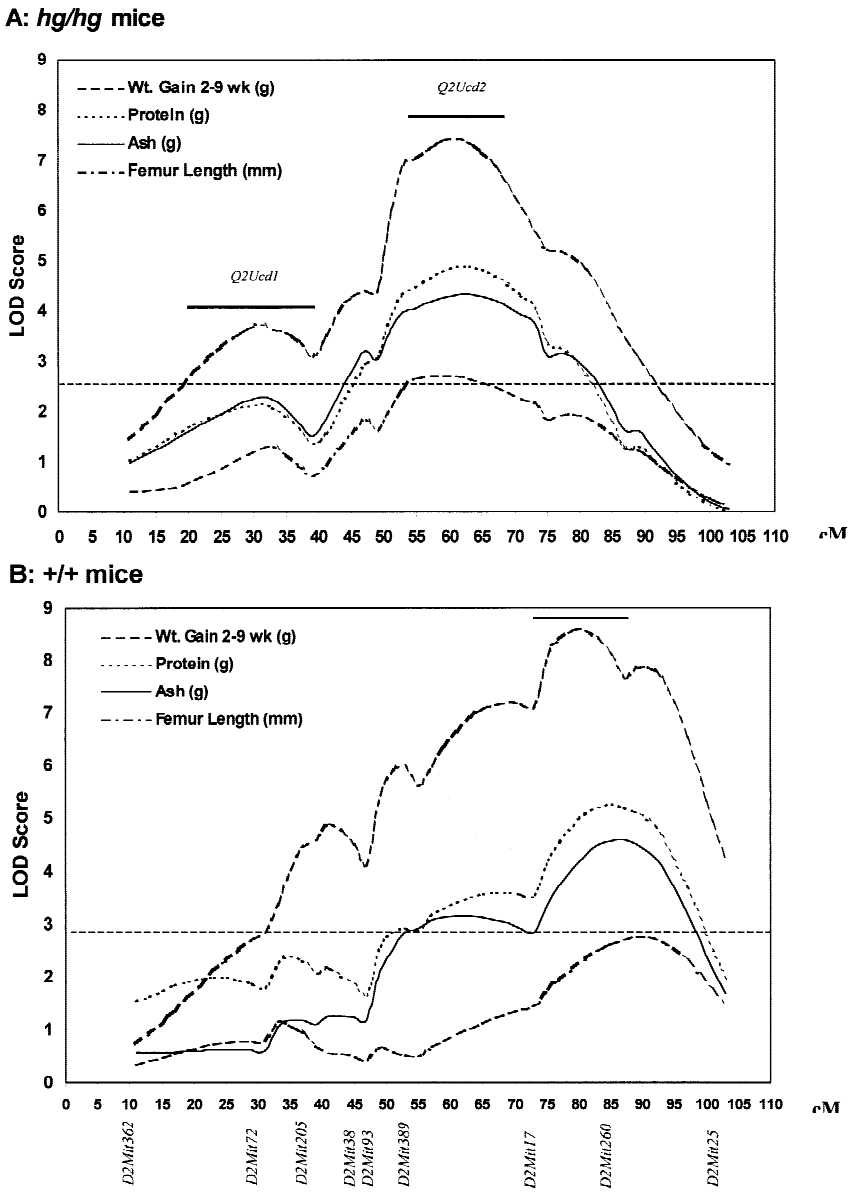

Fig. 3. LOD score plots for G29, carcass protein, carcass ash, and femur length adjusted for the effects of sex and age, on Chr 2 of *hg*/*h*, **(A)** and +/+ **(B)** F_2 mice. The genome-wide and chromosome significance thresholds are shown as dotted lines for *hg*/*hg* and +/+ mice respectively. Markers used in the analysis are shown below the horizontal axis. Peak LOD scores for QTL are identified with a horizontal line.

hg. This result is not surprising. It has been confirmed for both body weight (Cheverud et al. 1996) and body composition (Mehrabian et al. 1998) that alleles that increase the phenotypic mean of a trait in a mapping cross may come from the parental inbred line with the lower phenotypic mean for that trait.

Among the QTL detected in *hg*/*hg* mice there are loci that influenced both growth rate and final body size, and loci that affected each trait independently (Table 2). The final body size of an animal is mostly a function of its cell mass, which in turn results from the product of cell number and cell size (Conlon and Raff 1999). Although there is agreement about the existence of a genetic regulation of those parameters, little is known about how the changes in cell number and size that lead to a change in body mass are coordinated with time (Su and O'Farrell 1998; Conlon and Raff 1999). Our results confirm that, although there are genetic factors underlying a connection between final body size and the time taken to achieve it (Webster 1989), there are genes that act independently on each of those variables, probably through alterations of the dynamics of cell proliferation, cell enlargement, or both (Su and O'Farrell 1998; Conlon and Raff 1999).

The regulation of growth rate and ultimately body size can be regarded as the result of the balance between growth promoting and growth inhibiting factors (Efstratiadis 1998). The recent discovery of myostatin, a negative regulator of muscle mass, is consequent with the existence of growth inhibitors (McPherron et al. 1997; Lee and McPherron 1999). Moreover, the *hg* phenotype has been identified as resulting from a lack of expression of the suppressor of cytokine signaling 2 (*Socs2* or *Cish2*; Horvat and Medrano 2001). The identification of modifiers of *hg*, detected here, will be of value in identifying the genes involved in the functional pathways leading to variation in body size due to the influence of putative growth inhibiting factors. Also, high levels of IGF-I have been consistently reported in *hg*/*hg* mice (Medrano et al. 1991; Reiser et al. 1996). At least some of the modifiers that we report here could ultimately be genes involved in pathways influenced by IGF-I. It appears that interactions between the *hg* allele (lack of *Socs2*) and modifiers of *hg* are responsible for some of the reproductive problems that have been documented in this mutation (Cargill et al. 1999). It would be useful, therefore, to fine map modifiers of *hg* and ultimately positionally clone them.

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References

Bishop SC, Hill WG (1985) Effects of selection on growth, body composition, and food intake in mice. III. Correlated responses: growth, body composition, food intake and efficiency and catabolism. Genet Res 46, 57–74

Bradford GE, Famula TR (1984) Evidence for a major gene for rapid postweaning growth in mice. Genet Res 44, 293–308

Brockmann GA, Haley CS, Renne U, Knott SA, Schwerin M (1998) Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth. Genetics 150, 369–381

Brown DL, Gorin MB, Weeks DE (1994) Efficient strategies for genomic searching using the affected-pedigree-member method of linkage analysis. Am J Hum Genet 54, 544–552

Calvert CC, Famula TR, Bradford GE (1984) Composition of growth in mice with a major gene for rapid postweaning gain. J Anim Sci 59, 361–365

Cargill SL, Medrano JF, Anderson GB (1999) Infertility in a line of mice with the *high growth* mutation is due to luteal insufficiency resulting from disruption at the hypothalamic-pituitary axis. Biol Reprod 61, 283–287

Cheverud JM, Routman EJ, Duarte FAM, van Swinderen B, Cothran K, Perel C (1996) Quantitative trait loci for murine growth. Genetics 142, 1305–1319

Churchill GA, Doerge RW (1994) Empirical threshold value for quantitative trait mapping. Genetics 138, 963–971

Conlon I, Raff M (1999) Size control in animal development. Cell 96, 235–244

Corva PM, Medrano JF (2000) Diet effects on growth and body composition in *high growth* (*hg*/*hg*) mice. Physiol Genomics 3, 17–23

Efstratiadis A (1998) Genetics of mouse growth. Int J Dev Biol 42, 955–976

Famula TR, Calvert CC, Luna E, Bradford GE (1988) Organ and skeletal growth in mice with a major gene for rapid postweaning growth. Growth Dev Aging 52, 145–150

Graham CF, Lund G, Zaina S (1998) Growth and the distal tip of mouse chromosome 7. Genet Res 72, 247–253

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69, 315–324

Horvat S, Medrano JF (1995) Interval mapping of *high growth* (*hg*), a major locus that increases weight gain in mice. Genetics 139, 1737–1748

Horvat S, Medrano JF (1996) The *high growth* (*hg*) locus maps to a deletion in mouse Chromosome 10. Genomics 36, 546–549

Horvat S, Medrano JF (1998) A 550-kb YAC and BAC contig encompassing the high growth deletion in mouse chromosome 10 and identification of the murine Raidd/Cradd gene in the candidate region. Genomics 54, 159–164

Horvat S, Medrano JF (2001) Lack of *Socs2* expression causes the *high growth* phenotype in mice. Genomics 72, 209–212

Keightley PD, Knott SA (1999) Testing the correspondence between map positions of quantitative trait loci. Genet Res 74, 323–328

Keightley PD, Hardge T, May L, Bulfield G (1996) A genetic map of quantitative trait loci for body weight in the mouse. Genetics 142, 227–235

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ et al. (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1, 174–181

Lee SJ, McPherron AC (1999) Myostatin and the control of skeletal muscle mass. Curr Opin Genet Dev 9, 604–607

Lembertas AV, Pérusse L, Chagnon YC, Fisler JS, Warden CH et al. (1997) Identification of an obesity quantitative trait locus on mouse chromosome 2 and evidence of linkage to body fat and insulin on the human homologous region 20q. J Clin Invest 100, 1240–1247

Lyon MF, Rastan S, Brown SDM (1996) *Genetic Variants and Strains of the Laboratory Mouse.* Oxford, New York: Oxford University Press

McPherron AC, Lawler AM, Lee SJ (1997) Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387, 83–90

Medrano JF, Pomp D, Sharrow L, Bradford GE, Downs TR et al (1991) Growth hormone and insulin-like growth factor-I measurements in high growth (*hg*) mice. Genet Res 58, 67–74

Mehrabian M, Wen PZ, Fisler J, Davis RC, Lusis AJ (1998) Genetic loci controlling body fat, lipoprotein metabolism, and insulin levels in a multifactorial mouse model. J Clin Invest 101, 2485–2496

MGD (2000) Mouse Genome Database, Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME. June 2000. URL: *http://www.informatics.jax.org/*

Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T et al (1996) Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell 85, 707–720

Raff MC (1996) Size control: the regulation of cell numbers in animal development. Cell 86, 173–175

Reiser K, Summers P, Medrano JF, Rucker R, Last J et al (1996) Effects of elevated circulating IGF-1 on the extracellular matrix in "high-growth" C57BL/6J mice. Am J Physiol 271, R696–R703

Routman EJ, Cheverud JM (1997) Gene effects on a quantitative trait: two-locus epistatic effects measured at microsatellite markers and at estimated QTL. Evolution 51, 1654–1662

SAS (1998) OnlineDoc (TM). SAS Institute Inc., Cary, NC

Su TT, O'Farrell PH (1998) Size control: cell proliferation does not equal growth. Curr Biol 8, R687–R689.

Summers PJ, Medrano JF (1994) Morphometric analysis of skeletal muscle growth in the high growth mouse. Growth Dev Aging 58, 135–148

Summers PJ, Medrano JF (1997) Delayed myogenesis associated with muscle hyperplasia in high-growth mice. Proc Soc Exp Biol Med 214, 380–385

Taylor BA, Phillips SJ (1996) Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics 34, 389–398

Taylor BA, Tarantino LM, Phillips SJ (1999) Gender-influenced obesity QTLs identified in a cross involving the KK type II diabetes-prone mouse strain. Mamm Genome 10, 963–968

van Berloo R (1999) GGT: Software for the display of graphical genotypes. J Hered 90, 328–329

Webster AJF (1989) Bioenergetics, bioengineering and growth. Anim Prod 48, 249–269

York B, Lei K, West DB (1996) Sensitivity to dietary obesity linked to a locus on Chromosome 15 in a CAST/Ei × C57BL/6J F_2 intercross. Mamm Genome 7, 677–681

Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136, 1457–1468