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Fine Mapping Reveals Sex Bias in Quantitative Trait Loci Affecting Growth, Skeletal Size and Obesity-Related Traits on Mouse Chromosomes 2 and 11

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ABSTRACT

Previous speed congenic analysis has suggested that the expression of growth and obesity quantitative trait loci (QTL) on distal mouse chromosomes (MMU) 2 and 11, segregating between the CAST/EiJ (CAST) and C57BL/6J-*hg/hg* (HG) strains, is dependent on sex. To confirm, fine map, and further evaluate QTL × sex interactions, we constructed congenic by recipient F_2 crosses for the HG.CAST-(*D2Mit329-D2Mit457*)N(6) (HG2D) and HG.CAST-(*D11Mit260-D11Mit255*)N(6) (HG11) congenic strains. Over 700 F_2 mice were densely genotyped and phenotyped for a panel of 40 body and organ weight, skeletal length, and obesity-related traits at 9 weeks of age. Linkage analysis revealed 20 QTL affecting a representative subset of phenotypes in $HG2DF_2$ and $HG11F_2$ mice. The effect of sex was quantified by comparing two linear models: the first model included sex as an additive covariate and the second incorporated sex as an additive and an interactive covariate. Of the 20 QTL, 8 were sex biased, sex specific, or sex antagonistic. Most traits were regulated by single QTL; however, two closely linked loci were identified for five traits in $HG2DF_2$ mice. Additionally, the confidence intervals for most QTL were significantly reduced relative to the original mapping results, setting the stage for quantitative trait gene (QTG) discovery. These results highlight the importance of assessing the contribution of sex in complex trait analyses.

ONE approach to discovering genetic variation contributing to common human disease is the use of mouse models to identify quantitative trait loci (QTL) affecting traits of biomedical importance. There are several advantages to using mice for the analysis of complex traits such as a highly controlled environment, availability of inbred strains, and ability to produce genetically defined crosses, just to name a few (Abiola *et al.* 2003). More importantly, once identified, mouse QTL can be systemically fine mapped until the underlying gene(s) is discovered. Although a plethora of QTL have been discovered (Flint *et al.* 2005), the transition to the next more important step of identifying quantitative trait genes (QTG) has proven difficult. In spite of this, the number of QTG identified has steadily increased in recent years (Bodnar *et al.* 2002; Klein *et al.* 2004; Yalcin *et al.* 2004; Oliver *et al.* 2005; Wang *et al.* 2005; Clee *et al.* 2006).

In 2001, the identification of growth and carcass composition QTL was reported in an F_2 cross between the low-body-weight (mature male body weight of ~15 g) CAST/EiJ (CAST) and high-body-weight (mature male body weight of ~40 g) C57BL/6J-*hg/hg* (referred to as *high growth* or HG) mouse strains (Corva *et al.* 2001). The HG strain lacks expression of the *Socs2* gene (a negative inhibitor of growth hormone signaling) due to a spontaneously arising 500-kbp deletion on mouse chromosome (MMU) 10 (Horvat and Medrano 2001; Wong *et al.* 2002). In the above cross, MMU2 and MMU11 harbored QTL with significant affects on weight gain (*Wg2* on MMU2 and *Wg4* on MMU11), carcass ash (*Cara1* on MMU2 and *Cara2* on MMU11), and carcass protein (*Carp1* on MMU2 and *Carp2* on MMU11). Interestingly, the expression of *Wg2* and *Carp2* were altered in mutant (lacking *Socs2*) relative to wild-type (possessing a functional *Socs2*) F_2 mice, indicating that these loci were modifiers of the high-growth (*hg*) phenotype. Specifically, *Wg2* influenced weight gain in an overdominant fashion and was located at 60 cM in mutant mice, while in wild-type mice *Wg2* was additive and peaked at 80 cM (Corva *et al.* 2001). On the other hand, CAST alleles at *Carp2* decreased carcass protein in mutant but not wild-type F_2 mice (Corva *et al.* 2001).

Congenic strains possess alleles within a defined chromosomal segment from an inbred donor strain on the genetic background of a second recipient strain (Silver 1995). To decrease the time required to generate congenics, the "speed congenic approach" utilizes genetic

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markers to increase the rate at which unlinked recipient strain alleles are eliminated (Markel *et al.* 1997; Wakeland *et al.* 1997). We have recently used this approach to isolate the aforementioned MMU2 and MMU11 QTL (Farber *et al.* 2006). Two speed congenic strains, HG.CAST-(*D2Mit329-D2Mit457*)N(6) (HG2D) and HG.CAST-(*D11Mit260-D11Mit255*)N(6) (HG11), were developed by introgressing CAST donor regions on an HG background. HG2D mice possessed CAST alleles on MMU2 from 74.9 to 181.2 Mbp and were developed, but not characterized due to reduced fertility in homozygous congenic mice. However, a second strain, HG.CAST-(*D2Mit329-D2Mit490*)N(6) (HG2M), nested within HG2D and extending from 74.9 to 138.6 Mbp, was characterized and displayed sex-specific alterations in adiposity, among other growth and skeletal length phenotypes (Farber *et al.* 2006). Similarly, the HG11 congenic (CAST alleles on MMU11 from 61.6 to 114.0 Mbp) displayed sex-specific differences in growth, body length, adiposity, and carcass composition (Farber *et al.* 2006).

To further dissect QTL × sex interactions apparent on both chromosomes, we chose to pursue the HG2D and HG11 strains in parallel using congenic-by-recipient F_2 intercrosses. One of the advantages of this approach is the elimination of any phenotypic contribution of unlinked contaminating alleles, since these regions segregate randomly with respect to donor region genotype in the F_2. Additionally, the power to resolve QTL and detect QTL × sex interactions is increased relative to a traditional whole-genome F_2, since only QTL within the donor regions are segregating. Therefore, we have implemented this strategy with the objectives of (1) confirming that QTL effects are due to alleles within each donor region, (2) refining the genomic interval harboring each QTL, and (3) characterizing the effect of sex on QTL expression. Our results demonstrate a highly complex inheritance pattern for QTL affecting growth, skeletal size, and obesity-related traits on both chromosomes. This complexity involves multiple QTL affecting the same trait and varying degrees of QTL × sex interactions. These data provide insight into the sexually dimorphic genetic architecture of biomedically important traits and serve as the platform for QTG discovery.

MATERIALS AND METHODS

Mice: The creation of the HG2D and HG11 speed congenic strains has been previously described (Farber *et al.* 2006). For this study, congenic-by-recipient F_2 crosses were generated by mating a single congenic male from each strain (HG2D and HG11) to multiple recipient C57BL/6J-*hg/hg* (HG) females. The resulting F_1 mice were intermated to generate $HG2DF_2$ ($n = 278$) and $HG11F_2$ ($n = 454$) mice. The average litter size was 7.4 ± 0.3 pups for $HG2DF_1$ and 6.9 ± 0.3 pups for $HG11F_1$ females. F_2 mice were provided with a normal chow diet (Purina 5008; 23.5% protein, 6.5% fat, 3.3 kcal/g) and water *ad libitum*. All mice were housed in polycarbonate cages under controlled conditions of temperature ($21° \pm 2°$), humidity (40–70%), and lighting (14 hr light/10 hr dark, lights on at 7 am), and managed according to the guidelines of the American Association for Accreditation of Laboratory Animal Care.

Phenotypic analysis: F_2 mice were weighed at 2, 3, 6, and 9 weeks of age to the nearest 0.1 g. At the time of sacrifice (9 weeks ± 5 days), nonfasted mice were anesthetized under isoflurane and nasal–anal length, nasal–tail length, and tail length (nasal–tail − nasal–anal = tail length) were measured to the nearest millimeter. Blood was collected via the retroorbital sinus from anesthetized mice in separator tubes (Microtainer, Becton Dickinson) containing 8 μl of 0.5 m EDTA. Plasma was isolated by centrifugation for 5 min at $8000 \times g$ and stored at −70° until analysis. Anesthetized mice were sacrificed by decapitation and exsanguination. Fat pads (femoral, gonadal, mesenteric, and retroperitoneal), whole brain, liver, spleen, heart, and kidney were removed and weighed to the nearest milligram. Femurs were also removed from all $HG11F_2$ carcasses, cleaned, and measured to the nearest 0.5 mm using calipers.

QTL influencing plasma lipid levels have been identified on distal MMU2 between B6 and CAST mice (Mehrabian *et al.* 1998, 2000; Estrada-Smith *et al.* 2004). Therefore, to identify QTL for these traits in our cross, HG2D plasma profiles for total cholesterol (TC), high-density lipoprotein cholesterol (HDL), cholesterol esters, unesterified cholesterol, free fatty acids, and triglycerides were assayed as described in Mehrabian *et al.* (1993). Very-low-density and low-density lipoprotein cholesterol levels were calculated by subtracting HDL from TC. A Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA) was used to measure plasma glucose by the glucose oxidase reaction.

Genotyping: $HG2DF_2$ and $HG11F_2$ mice were genotyped using published microsatellite markers (Table 1) (Dietrich *et al.* 1996). DNA was isolated from 1.0- to 2.0-mm tail clips by digesting with proteinase K (Fisher) at 55° in a buffer composed of 0.45% NP40 (Sigma, St. Louis), 0.45% Tween 20 (Fisher), and 1× PCR buffer (Promega, Madison, WI). The product of this digestion was diluted (1:10) in sterile H_2O and used for genotyping without further purification. Microsatellite genotyping was performed using standard PCR and gel electrophoresis protocols. Reaction conditions for each marker are listed in Table 1.

Statistical analysis: Basic statistical analysis was preformed using the Rcmdr package of R (Ihaka and Gentleman 1996; Fox 2005). Standard descriptive statistics were computed for all traits to test normality assumptions and to identify putative data entry errors. A simple linear regression model was used to determine the relationship between body weight and fat mass in $HG2DF_2$ mice. A χ^2 test was used to determine adherence to Mendelian segregation ratios for all markers.

QTL analysis: QTL scans within each cross were implemented using the R/qtl package of R (Broman *et al.* 2003). Initially, sex-averaged genetic maps (using a Kosambi map function) were created using the "est.map" function. Conditional genotype probabilities were then calculated using the "calc.genoprob" function across each congenic donor region at 0.5-cM intervals. As a first approximation, maximum-likelihood (EM algorithm) interval mapping was used to screen for QTL in each sex separately via the "scanone" function, with age at sacrifice and litter size as additive covariates (data not shown) (Lander and Botstein 1989). These analyses, along with trait means for nonrecombinant mice (data not shown), suggested strong sex effects for many traits. Therefore, to account for the effect of sex, we reanalyzed each trait using methods outlined in Solberg *et al.* (2004). The approach consisted of fitting two separate models using

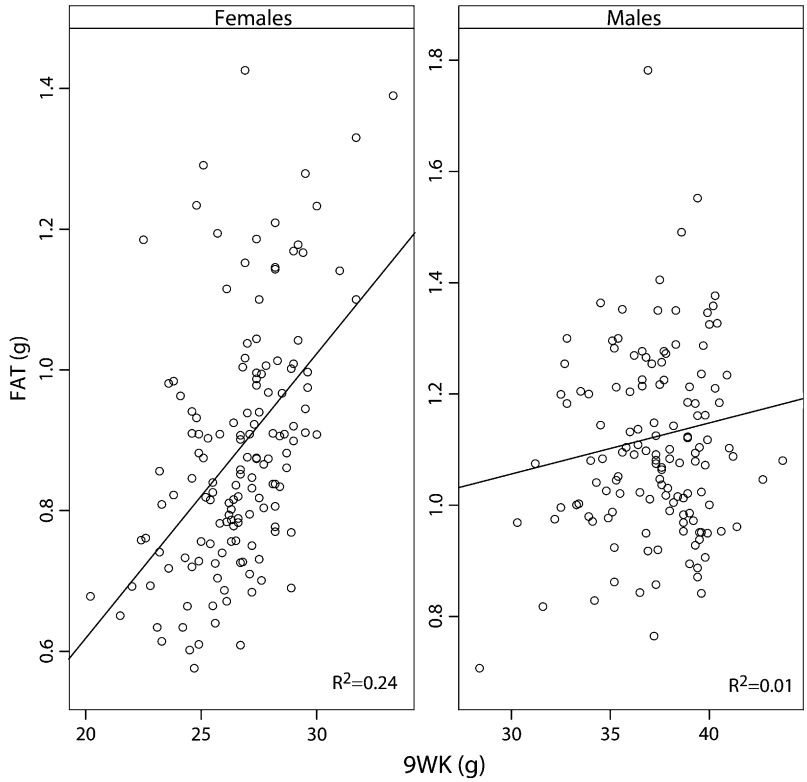

FIGURE 1.—Linear regression of FAT on 9WK weight illustrates the strong relationship between fat and body weight in female but not male $HG2DF_2$ mice. R^2 is the coefficient of determination and indicates the percentage of variance in FAT explained by 9WK.

the complete data set; Model_Additive (Model_A) was a linear model with sex, age at sacrifice, and litter size as additive covariates; Model_SexInteraction (Model_SI) used the same additive covariates but included sex as a covariate interacting with QTL genotype. Model_SI accounted for QTL × sex interactions arising from loci that are sex specific (QTL influencing a trait in only one sex), sex biased (QTL with an allelic effect that is more pronounced in one sex), or sex antagonistic (QTL with allelic effects going in opposite directions between the sexes). The difference in LOD score (ΔLOD) between the models (LOD Model_SI–LOD Model_A) was calculated using the "arithscan" function and used to estimate the magnitude of QTL × sex interactions. A small ΔLOD may be biologically meaningful; however, the overall threshold for declaring a significant QTL was higher using Model_SI, due to an increase in the number of estimated parameters. Therefore, we arbitrarily chose ΔLOD ≥ 1 as the cutoff for declaring a QTL to be sex biased. To declare sex specificity, we followed an approach similar to the one used in SOLBERG *et al.* (2004). They required the ΔLOD to exceed a nominal $P < 0.05$ threshold for QTL significance. In our case, the average significance threshold for all traits was LOD = 3.0 ($P < 0.05$). Therefore, QTL with a ΔLOD ≥ 3.0 were deemed sex specific or sex antagonistic. Significance thresholds for all models were calculated by permuting the observed data 1000 times using the "n.perm" function (CHURCHILL and DOERGE 1994). Additionally, 1.5-LOD confidence intervals (C.I.) were calculated using the "lodint" function.

We used linear regression to address the relationship between total fat pad mass (FAT) and weight at 9 weeks of age (9WK). FAT was highly correlated with 9WK in females but not in males (Figure 1), illustrating the sex-dependent relationship between these two traits. Several recent studies have suggested that due to the lack of equality in this relationship between the sexes (STYLIANOU *et al.* 2006) and because of induced spurious correlation between the traits (LANG *et al.* 2005), the use of ratios like adiposity index (fat pad mass/body weight × 100) in QTL analysis may result in statistical artifacts. Therefore, to circumvent potential problems, we chose to adjust FAT (in the $HG2DF_2$ cross) and gonadal fat pad weight (GFP) (in the $HG11F_2$ cross) by including weight at sacrifice (WSAC) and WSAC × sex terms as additive covariates in both Model_A and Model_SI. We also used this same approach to identify QTL affecting organ weight independent of body size.

A number of traits, primarily in the HG2D cross, appeared to be regulated by multiple QTL. To test this statistically, we used the "fitqtl" function to evaluate two-QTL models. Model fitness was evaluated after adjusting the data for the effects of sex. The two-QTL model was accepted if both QTL terms were significant at the $P < 0.05$ level.

RESULTS

Development of the $HG2DF_2$ and $HG11F_2$ crosses: Over 700 congenic-derived F_2 mice ($n = 278$ for $HG2DF_2$ and $n = 454$ for $HG11F_2$) were produced to characterize QTL on MMU2 and MMU11. In each cross, only CAST donor regions on MMU2 ($HG2DF_2$) and MMU11 ($HG11F_2$) were segregating and each represented <3.5% of the genome. The inheritance of HG and CAST alleles was followed using microsatellite markers. A total of 19 and 11 markers were genotyped in the $HG2DF_2$ and $HG11F_2$ crosses, respectively (Table 1). The average marker spacing was 2.7 cM for $HG2DF_2$ and 3.0 cM for $HG11F_2$. The $HG2DF_2$ congenic spanned 104.4 Mbp or 51.5 cM and the $HG11F_2$ congenic spanned 52.1 Mbp or 33.8 cM (Table 1). *P*-values for tests of Mendelian segregation are presented in Table 1.

TABLE 1

Microsatellite markers typed in $HG2DF_2$ and $HG11F_2$ mice

Marker	MMU	PCR conditions	cM	Mbp	Genotype counts (C/H/B/missing)	χ^2
D2Mit329	2	55/2.0	0.0	74.9	71/133/74/0	0.75
D2Mit93	2	55/1.5	0.0	76.7	69/131/73/5	0.76
D2Mit94	2	55/1.5	1.7	80.0	72/130/76/0	0.53
D2Mit160	2	55/1.5	3.0	84.8	69/133/76/0	0.65
D2Mit439	2	55/2.0	5.0	92.0	69/133/75/1	0.71
D2Mit130	2	55/1.5	5.8	97.4	69/134/75/0	0.73
D2Mit389	2	55/1.5	7.4	103.1	68/133/77/0	0.58
D2Mit207	2	55/1.5	9.1	112.0	69/130/79/0	0.39
D2Mit17	2	55/1.5	13.2	122.6	63/127/71/17	0.71
D2Mit224	2	55/2.0	16.5	129.0	65/139/74/0	0.75
D2Mit223	2	55/2.0	19.7	134.7	63/146/69/0	0.62
D2Mit212	2	55/1.5	23.3	141.7	68/145/65/0	0.75
D2Mit260	2	55/1.5	26.3	148.9	66/147/65/0	0.63
D2Mit262	2	55/2.0	30.1	155.6	66/141/71/0	0.89
D2Mit196	2	55/2.0	33.5	160.2	62/147/69/0	0.53
D2Mit454	2	55/2.0	36.8	164.0	57/150/70/1	0.21
D2Mit456	2	55/1.5	41.4	168.7	56/155/64/3	0.09
D2Mit213	2	55/2.0	50.1	174.3	61/145/71/1	0.51
D2Mit148	2	55/1.5	51.5	179.3	57/147/73/1	0.24
D11Mit261	11	55/2.0	0.0	62.2	93/243/114/4	0.09
D11Mit5	11	55/1.5	1.2	67.3	92/244/116/2	0.07
D11Mit90	11	55/2.0	2.9	70.6	95/237/120/2	0.15
D11Mit280	11	55/2.0	6.0	77.1	95/240/117/2	0.14
D11Mit36	11	55/1.5	9.0	83.9	93/242/117/2	0.09
D11Mit285	11	58/2.0	13.4	90.0	92/245/112/5	0.06
D11Mit67	11	58/1.5	17.2	97.1	91/245/116/2	0.05
D11Mit330	11	55/2.0	19.2	99.6	91/248/114/1	0.04
D11Mit10	11	55/2.0	22.6	104.7	93/245/113/3	0.08
D11Mit224	11	55/2.0	25.5	108.6	98/239/109/8	0.24
D11Mit255	11	55/3.0	33.8	114.3	110/213/114/17	0.84

PCR conditions, annealing temperature/$MgCl_2$ concentration; cM, relative genetic position of each marker within each congenic donor region calculated using cross data; Mbp, genomic position according to the August 2005 mm7 UCSC genome assembly (NCBI Build 35); genotype counts, number of mice with C (CAST/CAST), H (HG/CAST), B (HG/HG), or missing genotype at each marker; χ^2, *P*-value for χ^2 test of Mendelian segregation. Markers did not significantly deviate from Mendelian segregation after Bonferroni correction for multiple comparisons.

All MMU2 markers were inherited in the expected 1:2:1 ratio. In contrast, nearly all $HG11F_2$ markers showed evidence (nominal *P*-values <0.10) suggestive of skewed inheritance, although none were significant after correcting for multiple comparisons. The underlying cause was a deficiency of CAST/CAST homozygotes in both sexes and may indicate a slight prenatal selection against these mice, although a small number of mice that were not genotyped did not live to sacrifice and may have contributed to the observed deficiency.

QTL analysis: Measurements on >40 traits were collected in both crosses. Linkage analysis was preformed on all traits; however, we report only results for the traits with the most significant LOD scores. We used this strategy since many of the traits within the four trait categories [growth (body weight), organ weight, skeletal length, and obesity] were highly correlated (data not shown) and reflect pleiotropic effects of a single QTL. For example, in $HG2DF_2$ mice, weight at 6 weeks of age (6WK) and at 9WK and weight gain from 2–6, 2–9, 3–6, 3–9, and 6–9 weeks were affected by the same QTL; therefore, to eliminate redundancy we have presented only results for the traits (6WK and 9WK) with the most significant LOD score peaks. Table 2 lists the statistics for all reported QTL. We have provided the approximate physical location of each QTL by cross-referencing the centimorgan and megabase-pair position of each marker in Table 1. QTL have been named according to Mouse Genome Informatics guidelines as determined by the International Committee on Standardized Genetic Nomenclature for Mice (http://www.informatics.jax.org/mgihome/nomen/index.shtml).

In $HG2DF_2$ mice, the LOD score profiles for 6WK, 9WK, kidney weight (KID), FAT, and TC suggested a minimum of two QTL. To gain statistical support for the presence of distinct loci, we analyzed two-QTL models. Both QTL terms were significant ($P < 0.05$) in models for 6WK and TC (data not shown), but not for the remaining

TABLE 2

Growth, organ weight, skeletal length, and obesity-related QTL identified in $HG2DF_2$ and $HG11F_2$ mice

Trait	QTL	Peak LOD		ΔLOD	Sex	Position (cM)	Position (Mbp)	C.I. (cM)	Allelic effects				%V
		Model_SI	Model_A						*a* ♂	*d* ♂	*a* ♀	*d* ♀	
						$HG2DF_2$							
6WK (g)	*Wg5*	9.0***	8.7	0.3		9.0	112	2.0–29.5	−0.82	0.99	−0.99	0.43	13.8
	Wg6	7.5***	6.0	1.5	M	27.5	150		−1.04	1.50	−0.53	0.36	11.7
9WK (g)	*Wg5*	10.8***	10.3	0.5		9.0	112	2.0–13.0	−1.41	0.93	−0.99	0.39	16.4
	Wg6	6.0***	4.0	2.0	M	27.5	150		−1.24	1.44	−0.37	0.24	9.4
SPL (mg)	*Swq6*	11.0***	10.9	0.1		30.0	155	26.0–42.0	5.88	5.82	8.59	−0.63	16.7
KID (mg)	*Kwq7*	9.8***	8.5	1.4	M	12.5	120	8.5–13.8	0.59	20.25	8.59	−0.63	15.0
	Kwq8	7.9***	7.9	0.0		41.0	168		15.92	7.12	8.69	1.29	12.3
TAIL (cm)	*Tailq7*	6.7***	6.5	0.2		12.5	120	2.5–22.5	−0.09	−0.02	−0.13	0.04	10.5
NA (cm)	*Bdlnq7*	5.3***	4.6	0.7		33.0	159	0.5–37.5	−0.03	−0.02	−0.05	0.21	8.4
FAT (g)	*Fatq1*	8.7***	6.5	2.2	F	20.3	136	9.7–25.0	−0.04	0.02	−0.11	−0.01	13.5
	Fatq2	5.3***	4.9	0.4		37.0	164		−0.05	−0.01	−0.08	0.02	8.5
TC (mg/dliter)	*Tcq1*	5.8***	5.6	0.2		16.5	129		−3.78	1.81	−4.77	0.35	9.2
	Tcq2	6.8***	6.7	0.1		32.0	158	12.5–44.5	−5.35	0.21	−4.21	0.27	10.7
						$HG11F_2$							
9WK (g)	*Wg7*	3.3*	0.3	3.0	I	18.0	98	0.0–28.5	0.66	0.15	−0.49	0.16	5.2
LIV (g)	*Lwq9*	7.0***	2.6	4.5	M	10.5	85	7.0–16.0	0.06	−0.04	−0.02	−0.00	11.0
KID (mg)	*Kwq9*	8.0***	7.2	0.8		11.5	88	7.0–17.0	−10.13	2.26	−12.71	2.73	12.4
BRN (mg)	*Brwq1*	6.0***	5.4	0.6		18.5	99	7.0–22.5	−13.02	−1.96	−7.99	−5.62	9.5
TAIL (cm)	*Tailq8*	8.5***	6.1	2.3	M	19.0	99	10.0–34.5	0.12	0.03	0.03	0.01	13.1
FEM (mm)	*Feml4*	16.5***	13.8	2.7	F	5.0	75	1.5–8.5	−0.11	0.07	−0.26	0.07	24.0
GFP (mg)	*Gfpq1*	3.5*	3.4	0.1		32.5	113	0.0–35.0	25.12	31.35	38.59	13.64	5.6

Model_SI and Model_A are described in detail in MATERIALS AND METHODS. ΔLOD is the difference in LOD between the two models. Sex indicates QTL that are either sex biased (ΔLOD > 1) or sex specific/sex antagonistic (ΔLOD > 3); M, QTL with more significant effects in males; F, QTL with more significant effects in females; I, sex-antagonistic QTL. Position of QTL peak is given in centimorgans and in approximate megabase pairs. C.I., 1.5-LOD confidence interval. Sex-specific additive (*a*) and dominance (*d*) effects were calculated for each sex separately using unadjusted genotypic means at each marker nearest the QTL peak (*a*, the average difference in trait means between homozygous genotypes; *d*, the deviation from the average difference between homozygotes; positive and negative *a* values indicate an increase and a decrease, respectively, in the trait mean due to replacing B6 alleles with CAST alleles). %V, the percentage of phenotypic variance explained by each QTL, calculated as %V = 100(1 − 10^(−2LOD(*n*))), where *n* is the number of F_2 mice and LOD is the peak LOD at each QTL. $^*P < 0.05$; $^{***}P < 0.001$.

traits (although the rest were suggestive with $P < 0.10$). The lack of support for the other traits could be due to the tight linkage between the putative QTL. In light of this, we still chose to report the second QTL for all traits. Our decision is supported by the observation that in all cases, except for the second TC QTL, either the second QTL was sex biased when the first was not or the second QTL was not sex biased when the first was (Table 2). We also have evidence from a separate analysis of B6.CAST MMU2 subcongenics on a wild-type (pure C57BL/6J) background, clearly demonstrating the presence of multiple QTL for weight and adiposity within the 2D donor region (C. R. FARBER and J. F. MEDRANO, unpublished results).

The 1.5-LOD C.I., in centimorgans, is reported for the QTL with the most significant LOD score per trait (Table 2). In addition, it should be noted that C.I.'s for traits with multiple putative QTL may be inflated due to closely linked QTL with similar peak LOD scores [as an example, see LOD score plot for 6WK in $HG2DF_2$ mice (Table 2; Figure 2A)]. In the following sections and figures, the three genotypes are abbreviated as C (CAST/CAST), H (HG/CAST), and B (HG/HG).

$HG2DF_2$ growth traits: Two QTL, weight gain QTL 5 (*Wg5*) and weight gain QTL 6 (*Wg6*), influencing both 6WK and 9WK, were identified in $HG2DF_2$ mice (Table 2; Figure 2A). *Wg5* and *Wg6* altered only postnatal growth without influencing weight at 2 and 3 weeks of age (data not shown). *Wg5* and *Wg6* had peak LOD scores for both traits at 9.0 and 27.5 cM, respectively. *Wg5* was unaffected by sex (Table 2; Figure 2B); in contrast, *Wg6* displayed male-biased expression (6WK ΔLOD = 1.5; 9WK ΔLOD = 2.0) (Table 2; Figure 2C). Both QTL, especially *Wg6*, demonstrated a higher dominance effect in males than in females (Table 2). This is presented graphically in Figure 2C, which shows genotypic means for the marker (*D2Mit260*) most closely linked to *Wg6*. These results are concordant with data from the original mapping experiment in which weight gain 2 (*Wg2*) demonstrated a high level of dominance in HG but not in B6 mice (CORVA *et al.* 2001). The additive effects for both QTL were negative, indicating that C alleles decreased body weight.

$HG11F_2$ growth traits: A single QTL, weight gain QTL 7 (*Wg7*), was localized in $HG11F_2$ mice (Table 2;

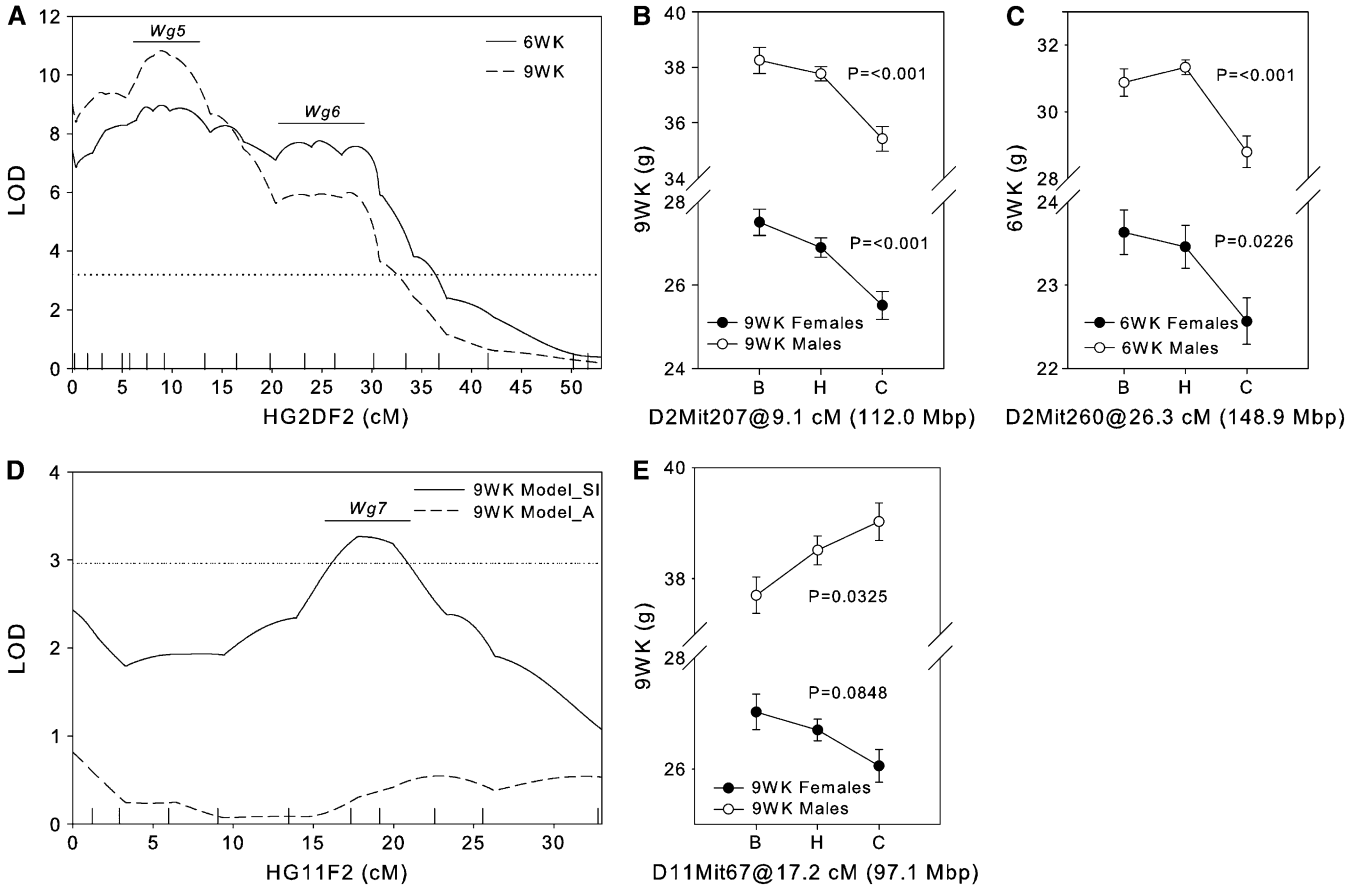

FIGURE 2.—LOD score profiles and genotypic contrasts for body weight in $HG2DF_2$ and $HG11F_2$ mice. LOD score plots are shown with peak position delineated by a horizontal solid bar underneath the QTL name. A dotted line represents the significant LOD threshold for each trait (or the highest if multiple traits are plotted). Genotypic contrasts for the marker most closely linked to QTL are plotted as mean ± SEM for each genotype (B, HG/HG; H, HG/CAST; C, CAST/CAST) along with a *P*-value for the effect of genotype within each sex as determined by a one-way ANOVA. Genotype effects were deemed significant at $P < 0.05$. (A) LOD scores for 6WK and 9WK in $HG2DF_2$ mice. (B) Effect of *D2Mit207* on 9WK in $HG2DF_2$ mice. (C) Effect of *D2Mit260* on 6WK in $HG2DF_2$ mice. (D) LOD scores for 9WK using sex as an additive and interactive covariate (9WK Model_SI) or as an additive covariate only (9WK Model_A) in $HG11F_2$ mice. (E) Effect of *D11Mit67* on 9WK in $HG11F_2$ mice.

Figure 2D). This QTL was the only locus demonstrating sex-antagonistic expression in either cross (ΔLOD = 3.0). *Wg7* increased 9WK by 1.3 g in male mice and decreased 9WK by 1.0 g in female C mice (Table 2; Figure 2E). Linkage was not detected using a strictly additive model (Model_A); however, when a QTL × sex interaction term was included (Model_SI), a significant LOD of 3.3 ($P < 0.05$) was obtained for 9WK (Figure 2D). 6WK and most weight gain periods also showed similar LOD score profiles but did not reach statistical significance (data not shown). *Wg7* was located at 18.0 cM and explained 5.2% of the phenotypic variance in 9WK.

$HG2DF_2$ organ weights: To identify QTL influencing growth independent of systemic changes in body weight or adiposity, we measured weights of the five major organs: liver (LIV), spleen (SPL), heart (HRT), KID, and brain (BRN). In each case, organ weights were adjusted for differences in WSAC by including WSAC and WSAC × sex terms as additive covariates. In $HG2DF_2$ mice, highly significant QTL for SPL (spleen weight QTL 6; *Swg6*) and KID (kidney weight QTL 7 and 8; *Kwq7* and *Kwq8*) weight were discovered (Table 2; Figure 3A). *Swq6* had a peak LOD score of 11.0 at 30.0 cM and explained 16.7% of the variance in SPL. Interestingly, it affected SPL in an additive fashion in males, but displayed a large dominance effect in females (Table 2; Figure 3B). *Kwq7* and *Kwq8* had peak LOD scores of 9.8 and 7.9 at 12.5 and 41.0 cM, respectively. *Kwq8* was expressed equally in both sexes; however, *Kwq7* was male biased (ΔLOD = 1.4). In females, both loci were inherited additively; however, male mice heterozygous for *Kwq7* had the highest KID weight (Table 2; Figure 3C).

$HG11F_2$ organ weights: Significant QTL regulating the size of the BRN, LIV, and KID, independent of body mass, were localized in $HG11F_2$ mice (Table 2; Figure 3D). Brain-weight QTL 1 (*Brwq1*) was located at 18.5 cM and accounted for 9.5% of the phenotypic variance in BRN (Table 2). Female mice with C alleles at *Brwq1* had an ~25-mg reduction in brain weight, relative to B

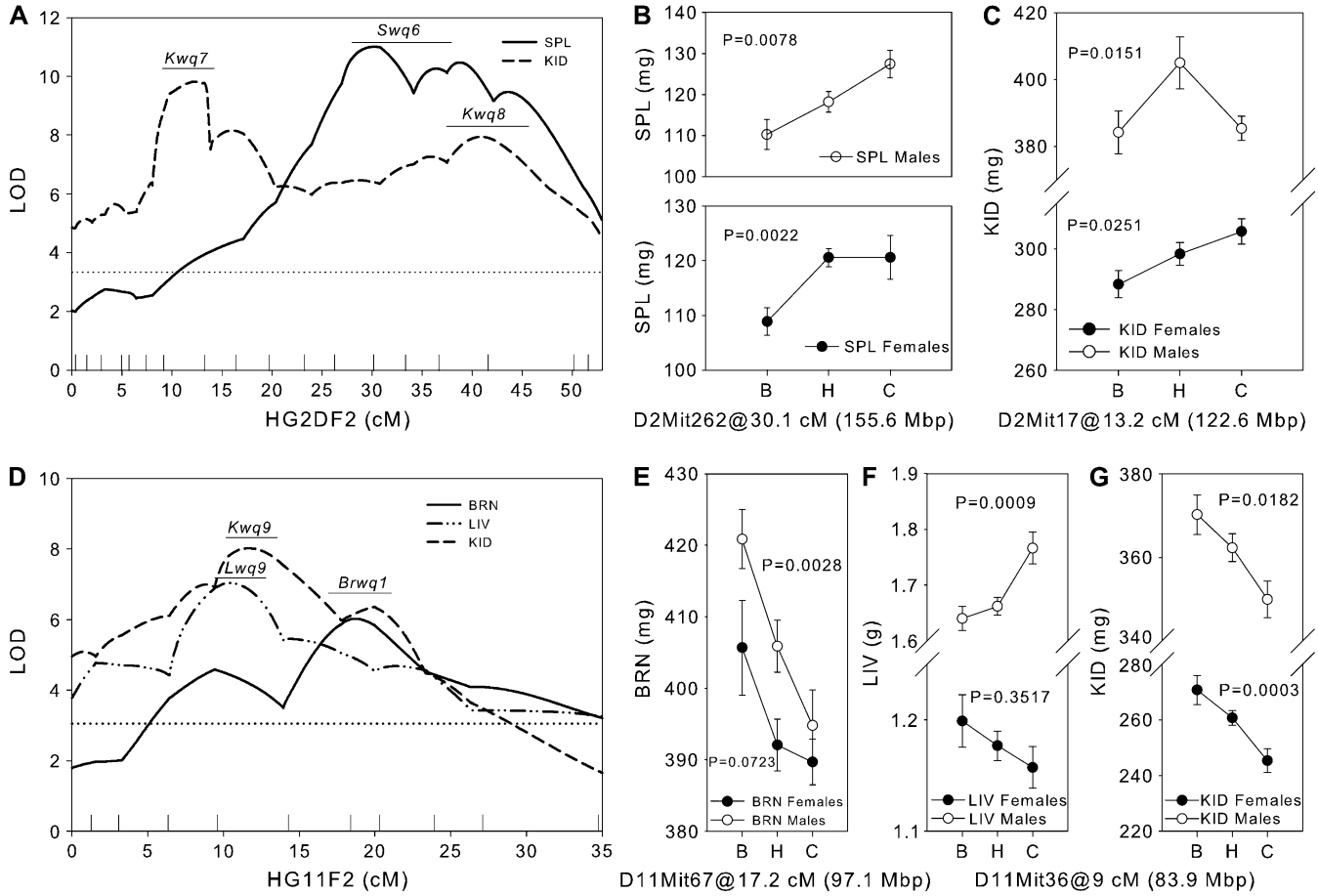

FIGURE 3.—LOD score profiles and genotypic contrasts for organ weights in $HG2DF_2$ and $HG11F_2$ mice. See the legend of Figure 2 for a detailed description of the figure. (A) LOD scores for SPL and KID in $HG2DF_2$ mice. (B) Effect of *D2Mit262* on SPL in $HG2DF_2$ mice. (C) Effect of *D2Mit17* on KID in $HG2DF_2$ mice. (D) LOD scores for BRN, LIV, and KID in $HG11F_2$ mice. (E) effect of *D11Mit67* on BRN in $HG11F_2$ mice. (F and G). Effect of *D11Mit36* on LIV and KID in $HG11F_2$ mice.

females (Figure 3E). Liver-weight QTL 9 (*Lwq9*) was a male-specific locus ($\Delta LOD = 4.5$) (Figure 3F) located at 10.5 cM with a LOD of 7.0 and explained 11.0% of the phenotypic variance in LIV. LIV was unaffected in females but C males had significantly elevated LIV, relative to B and H mice, which did not differ. Kidney-weight QTL 9 (*Kwq9*) displayed a similar LOD score profile, relative to *Lwq9*, with a peak LOD score of 8.0 at 11.5 cM, possibly suggesting a common QTL (Figure 3D). However, instead of increasing KID in C mice, it elicited a decrease (Figure 3G), arguing for two distinct loci. In further support for separate genes, the expression of *Kwq9* was independent of sex.

$HG2DF_2$ skeletal length traits: In the same manner as described for organ weights, we identified QTL influencing skeletal length independent of changes in body weight. However, unlike organ weights, QTL for skeletal length traits were relatively unaffected by body weight. The LOD score profiles for nasal–anal body length (NA) and tail length (TAIL) in $HG2DF_2$ mice and TAIL and femur length (FEM) in $HG11F_2$ mice were nearly identical whether or not WSAC and WSAC × sex interactions were included as additive covariates (data not shown). Therefore, due to lower significance thresholds, we report QTL only for measures of skeletal and body length unadjusted for body weight.

Tail-length QTL 7 (*Tailq7*) mapped with a peak LOD score of 6.7 at 12.5 cM in $HG2DF_2$ mice (Table 2; Figure 4A). Although *Tailq7* overlaps with *Wg5*, the decrease in TAIL is not likely a pleiotropic effect of weight, since, as indicated above, weight was a minor contributor to the differences in TAIL. In addition, *Wg5* displayed a high level of apparent dominance and *Tailq7* was additive (Table 2; Figure 4B).

Interestingly, TAIL and NA were not controlled by the same QTL in $HG2DF_2$ mice. In contrast to *Tailq7*, body-length QTL 7 (*Bdlnq7*) mapped at 33.0 cM with a peak LOD score of 5.3 (Table 2; Figure 4A). The ΔLOD for *Bdlnq7* was only 0.7, providing insufficient statistical evidence to classify it as sex biased on the basis of our criteria (Table 2). *Bdlnq7* also displayed a large dominance effect in females (Table 2; Figure 4C). *Bdlnq7* accounted for 8.4% of the variance in NA (Table 2).

$HG11F_2$ skeletal length traits: The tail-length QTL 8 (*Tailq8*) mapped with a peak LOD score of 8.5 at 19.0 cM (Table 2; Figure 4D). *Tailq8* was male biased

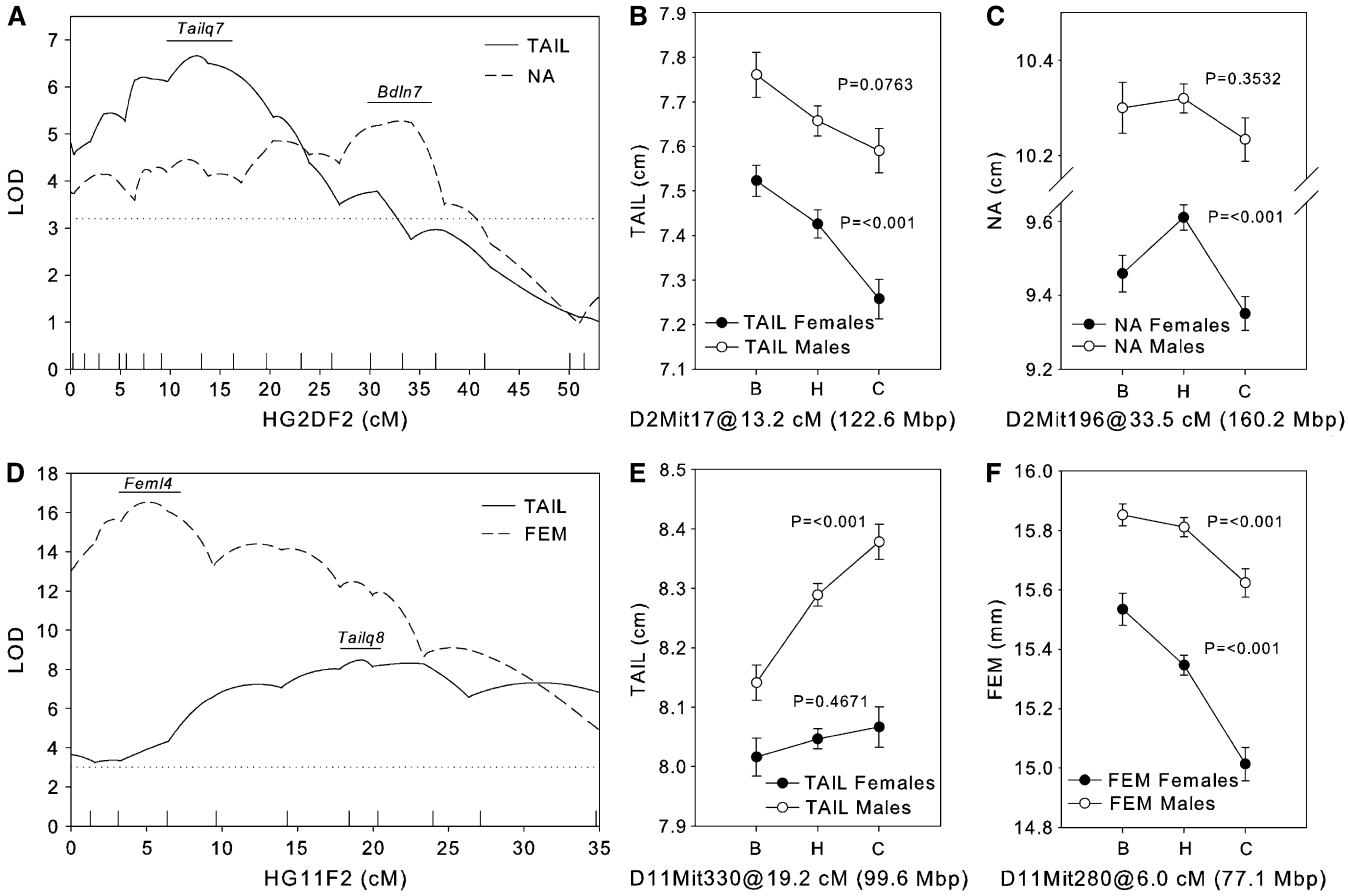

FIGURE 4.—LOD score profiles and genotypic contrasts for skeletal length in $HG2DF_2$ and $HG11F_2$ mice. See the legend of Figure 2 for a detailed description of the figure. (A) LOD scores for TAIL and NA in $HG2DF_2$ mice. (B) Effect of *D2Mit17* on TAIL in $HG2DF_2$ mice. (C) Effect of *D2Mit196* on NA in $HG2DF_2$ mice. (D) LOD scores for TAIL and FEM in $HG11F_2$ mice. (E) Effect of *D11Mit330* on TAIL in $HG11F_2$ mice. (F) Effect of *D11Mit280* on FEM in $HG11F_2$ mice.

(ΔLOD = 2.3), having only a minor, nonsignificant effect on TAIL in females (Figure 4E). *Tailq8* was additive and explained 13.1% of the variance in TAIL. The most significant QTL in either cross was femur-length QTL 4 (*Feml4*), a female-biased QTL (ΔLOD = 2.7) with a peak LOD of 16.5 at 5.0 cM (Table 2; Figure 4D). *Feml4* explained 24.0% of the variance in FEM. *Feml4* decreased FEM by nearly 0.6 mm (4%) in C genotype females (Figure 4F).

$HG2DF_2$ obesity traits: One of the most clearly sexually dimorphic traits in either cross was obesity in $HG2DF_2$ mice (Table 2; Figure 5A). Two QTL, total fat pad mass QTL 1 and 2 (*Fatq1* and *Fatq2*), influenced FAT (adjusted for WSAC) with peaks at 20.3 and 37.0 cM. *Fatq1* was female biased (ΔLOD = 2.2), decreasing FAT by 28% in C females compared to only a 7% decrease in C males (Figure 5B). *Fatq2* decreased FAT in both sexes, although its effect size was much smaller.

Although the peak locations were not identical, considerable overlap was observed among *Fatq1*, *Fatq2*, and the two QTL controlling TC, suggesting pleiotropy (Table 2; Figure 5D). Total plasma cholesterol QTL 1 and 2 (*Tcq1* and *Tcq2*) were located at 16.5 and 32.0 cM and regulated TC equally in both sexes. *Tcq2*, the more significant of the two loci, decreased TC by 12% in C mice of either sex (Figure 5C).

$HG11F_2$ obesity traits: Only small changes in adipose mass were observed in $HG11F_2$ mice. A minor QTL primarily altering GFP mass, GFP QTL 1 (*Gfpq1*), was located at 32.5 cM with a peak LOD of 3.5 (Table 2; Figure 5D). This was the location of the peak; however, the LOD score profile suggests that GFP may be influenced by more than one gene, each with very small effects (Figure 5D). Given its low peak LOD scores, we elected not to consider each peak a distinct QTL. All other fat pad weights and FAT also demonstrated similar peak LOD scores but did not reach statistical significance. *Gfpq1* was expressed in both sexes and increased GFP mass in C mice (Figure 5E).

DISCUSSION

Genetic fine mapping is a critical step in the progression from QTL to QTG. In this study we used congenic-derived F_2 intercrosses to dissect two regions of the mouse genome, MMU2 and MMU11, harboring

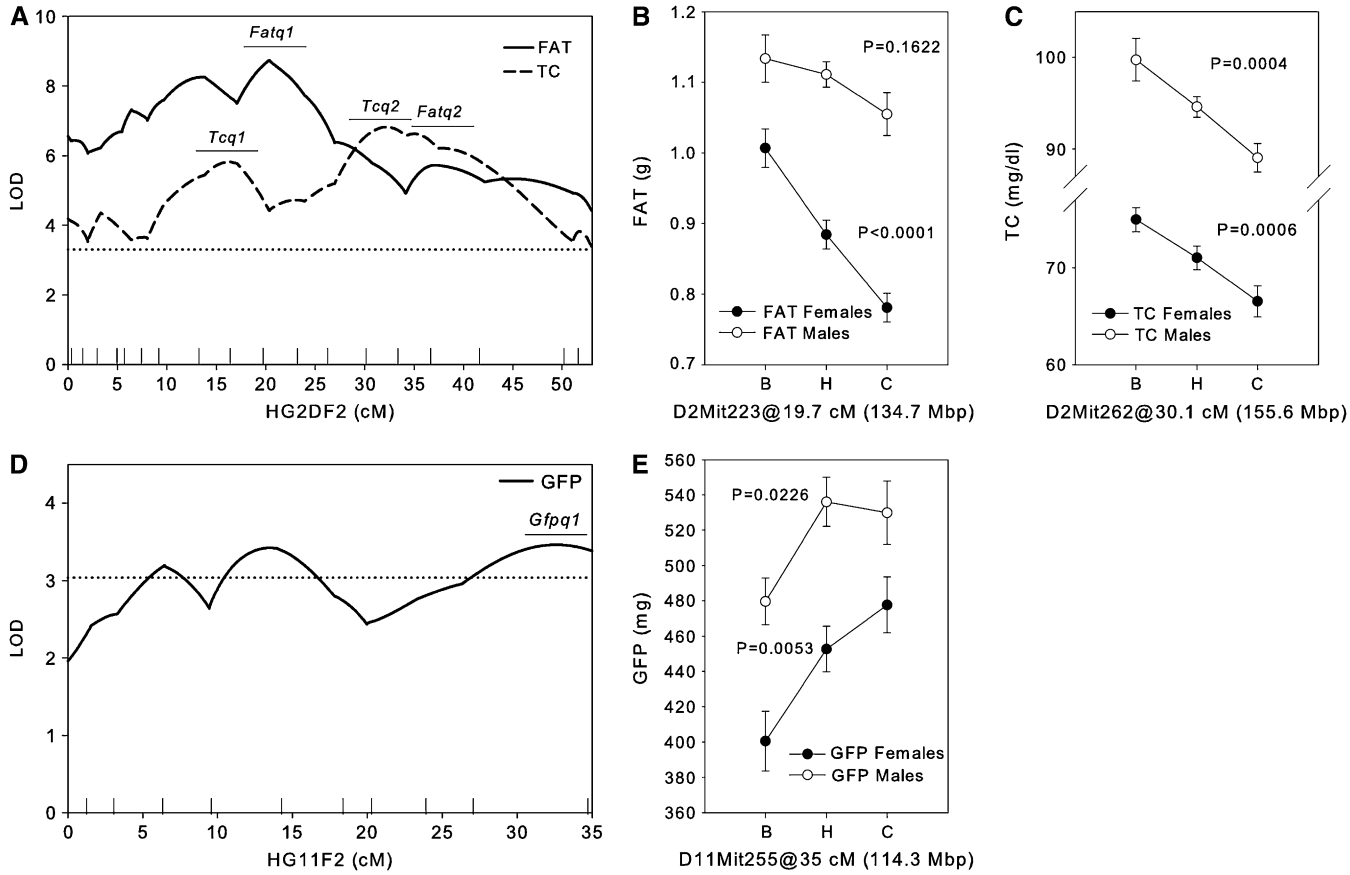

FIGURE 5.—LOD score profiles and genotypic contrasts for obesity-related traits in $HG2DF_2$ and $HG11F_2$ mice. See the legend of Figure 2 for a detailed description of the figure. (A) LOD scores for FAT and TC in $HG2DF_2$ mice. (B) Effect of *D2Mit223* on FAT in $HG2DF_2$ mice. (C) Effect of *D2Mit262* on TC in $HG2DF_2$ mice. (D) LOD scores for GFP in $HG11F_2$ mice. (E) Effect of *D11Mit255* on GFP in $HG11F_2$ mice.

growth, skeletal size, and obesity-related QTL. Previous studies have implicated sex as a modifier of growth and adiposity in these two chromosomal regions (FARBER *et al.* 2006); therefore, an important objective of our study was to clarify the nature and the extent of QTL × sex interactions. To our surprise, the interactions were more widespread than originally expected and, of the 20 total QTL identified, 40% were influenced by sex. Moderate sex differences were seen for most of the remaining QTL; however, on the basis of our criteria QTL × sex interactions could not be statistically established. In addition to the influence of sex, multiple QTL affecting the same trait and nonadditive gene action added to the genetic complexities of the MMU2 and MMU11 regions.

In $HG2DF_2$ mice, significant decreases in body weight were observed in mice with C alleles at *Wg5* and *Wg6*. This was exclusively due to a slower rate of postnatal gain (data not shown). The difference in body weight was also reflected by concordant decreases in HRT and LIV (data not shown). In contrast, three QTL were identified, independent of body weight, which increased SPL (*Swq6*) and KID (*Kwq7* and *Kwq8*) weight in C mice. This indicates the existence of genes on MMU2 that influence organ size through pathways independent of systemic changes in weight.

Two QTL were detected for both FAT and TC in $HG2DF_2$ mice. Although the peaks were not perfectly coincident, there is substantial overlap between the loci, suggesting pleiotropy. A similar study of B6 and CAST identified similar QTL on MMU2 for subcutaneous fat mass and percentage of body lipids, as well as HDL cholesterol (MEHRABIAN *et al.* 1998). We did not detect significant linkage with HDL, although suggestive peaks were observed. It should be noted that plasma lipids in our study were measured in nonfasted mice.

In $HG11F_2$ mice, 9WK (and most other growth traits, albeit to a lesser degree) was regulated by *Wg7*, the only sexually antagonistic QTL identified in either cross. *Wg7* increased 9WK in C genotype male mice while decreasing weight in females of the same genotype. The phenomenon of sexually antagonistic QTL has also been recently described for fat mass in a separate mouse cross (WANG *et al.* 2006).

The trait affected most significantly in $HG11F_2$ mice was skeletal length. Both *Tailq8* and *Feml4* accounted for substantial portions of the variance in their respective traits. Both loci were complex; *Tailq8* was male biased

while *Feml4* was female biased. Although both QTL control measures of skeletal dimension, they were linked in repulsion. Male mice with C alleles at *Tailq8* possessed a longer tail; however, if the same mice also possessed C alleles at *Feml4*, femur length was decreased (Figure 4, E and F).

MMU2 is a hotspot for growth and obesity QTL. Over 30 QTL have been identified (Corva and Medrano 2001; Perusse *et al.* 2005), of which several have been subsequently fine mapped using congenic strains and congenic-derived crosses (Diament *et al.* 2004; Estrada-Smith *et al.* 2004; Warden *et al.* 2004; Jerez-Timaure *et al.* 2005). A high percentage of previously identified MMU2 QTL and those uncovered in this study overlap and may represent the same QTG. More importantly, three human chromosomal regions share significant intervals of synteny with the HG2D donor region. MMU2 from 84 to 110 Mbp is syntenic with human chromosome (HSA) 11 from 26 to 57 Mbp, MMU2 from 111 to 126 Mbp is syntenic with HSA15 from 30 to 49 Mbp, and MMU2 from 128 to 181 Mbp is syntenic with the entire HSA20. Genetic linkage and SNP association studies have identified correlations between genotype and obesity [mainly body mass index (BMI); BMI was analyzed in the $HG2DF_2$ cross and its LOD score profile was nearly identical to WK9 and therefore was not reported] in each of these human regions, supporting the potential correspondence between human and mouse QTG (Perusse *et al.* 2005).

Of particular interest is HSA20, which, like its MMU2 syntenic region, is an obesity hotspot. Numerous linkages between HSA20 and obesity-related traits have been identified (Lembertas *et al.* 1997; Hunt *et al.* 2001; Dong *et al.* 2003; Gorlova *et al.* 2003; Collaku *et al.* 2004). Additionally, the Mendelian syndromes Bardet–Biedl syndrome 6 (Katsanis *et al.* 2001) and Albright hereditary osteodystrophy (Patten *et al.* 1990), which are associated with obese states, are located on HSA20. To the best of our knowledge, no evidence of sex-specific obesity QTL have been found on HSA20. *Fatq1*, *Fatq2*, *Tcq1*, and *Tcq2* will be our top priority for future investigations, given the important implications that their identification may have.

To identify traits modified by sex, we first analyzed nonrecombinant $HG2DF_2$ and $HG11F_2$ mice and preformed linkage analysis in each sex separately (data not shown). From this analysis most traits demonstrated a dependency on sex. Our next step was to quantify these effects by evaluating the fit of two linear models relative to a null model. The approach that we used was similar to the one described in Solberg *et al.* (2004). The first model, Model_A, included sex as an additive covariate and the second, Model_SI, was equivalent to Model_A plus a QTL × sex interaction term. The use of multiple linear models proved effective, most importantly because QTL that were not identified in the sex-specific analysis were detected [*e.g.*, *Wg7* was detected only when Model_SI was used (Table 2; Figure 2D)]. Therefore, this is an effective strategy for evaluating QTL × covariate interactions (in our case sex was included as a covariate, but other covariates could also be used) and for disentangling complex interactions critical to the proper assessment of complex traits.

The sex-specific genetic architecture of human obesity and lipid levels (Weiss *et al.* 2006) as well as femoral bone structure (Peacock *et al.* 2005) have been reported. Similarly, several studies in mice have also demonstrated the effect of sex on the segregation of QTL influencing complex traits (Turner *et al.* 2003; Wang *et al.* 2005). In development of the HG2D and HG11 congenic strains, sex was not expected to alter QTL expression, since QTL × sex interactions were not detected in the original mapping experiment (Corva *et al.* 2001). However, the loci captured in congenic strains did exhibit differences between the sexes (Farber *et al.* 2006). In our previous congenic analysis, MMU2 was comprehensively examined by generating strains with identical donor regions on both B6 and HG backgrounds with the aim of evaluating QTL × *hg*, but not QTL × sex interactions. One critical finding was the observation that QTL expression was sexually dimorphic in some HG strains, but not in the corresponding B6 strains (Farber *et al.* 2006). Sex effects specific to the *hg* locus have been reported; *hg* is recessive in females and partially additive in males, with respect to body weight (*i.e.*, heterozygous +/*hg* males display an intermediate weight between +/+ and *hg/hg* males) (Horvat and Medrano 1995). Therefore, it is likely that the observed sex × QTL interaction effects are a result of the *hg* mutation.

One explanation for the finding that CAST MMU2 and MMU11 chromosomes on an HG background harbor sex-biased/-specific QTL, but not other chromosomes, may be the result of the clustering of genes involved in growth hormone (*Gh*) signaling. HG mice lack *Socs2* gene expression (Horvat and Medrano 2001) and SOCS2 protein is a negative regulator of growth hormone (*Gh*) signaling both *in vitro* and *in vivo* (Greenhalgh *et al.* 2005). QTL on MMU2 and MMU11 have been reported to interact with *hg* (Corva *et al.* 2001) and we have recently described the identification of *Gh* pathway genes located within these QTL and their sequence in CAST mice (Farber *et al.* 2006). Interestingly, the HG11 region near *Wg7* (18.0 cM or near 98 Mbp), *Brwq1* (18.5 cM or near 99 Mbp), and *Tailq8* (19.0 cM or near 99 Mbp) is in close proximity to a region of MMU11, which is saturated with genes involved in the central intracellular pathway regulating the response to *Gh*, such as signal transducer and activator of transcription 5a and 5b (*Stat5b* at 100.5 Mbp and *Stat5a* at 100.6 Mbp) and the structual *Gh* gene itself at 106.1 Mbp. In addition, functional candidates responsive to *Gh* or known to be involved in *Gh* signaling, such as *Stat3* (100.3 Mbp), somatostatin receptor 2 (*Sstr2* at 113.4

Mbp), hepatic transcription factor 3 (*Tcf2* at 83.6 Mbp), Solute carrier family 2, facilitated glucose transporter, member 4 (*Slc2a4*, also known as *Glut4* at 69.7 Mbp), and insulin-like growth factor binding protein 4 (*Igfbp4* at 98.9 Mbp), are all located within the confines of the HG11 strain. The sexually dimorphic nature of *Gh* signaling is well documented; therefore, the QTL × sex interactions may be regulated by *Gh*-signaling genes. Future studies will address this by characterizing the sequence and expression levels (in male and female mice) of these genes in congenic mice.

Our results also suggest that *hg* increases the degree of dominance, particularly for body and organ weight QTL. In the original intercross between HG and CAST (Corva *et al.* 2001), *Wg2* was additive (with respect to body weight and growth rate) in wild-type F_2 mice, but overdominant in *hg/hg* F_2 mice. Similarly, in $HG2DF_2$ heterozygous mice, most growth and organ weight QTL appeared not to be inherited in a strictly additive fashion. Thus, *hg* also appears to modulate gene action, which may also be mediated via *Gh* pathway genes.

Congenic-derived intercrosses are advantageous over other QTL fine-mapping methods such as subcongenic analysis for a number of reasons. First, they provide a quick confirmation of effects discovered in homozygous congenic strains. One criticism of congenic models is the lack of replication in fine-mapping experiments, after observing a phenotype in the initial characterization of the congenic model. In some (maybe most) cases, this is due to phenotypic contributions from unlinked contaminating alleles, which exist in the congenic initially but are eliminated during the backcrossing required for fine mapping. These effects are quickly identified in congenic-derived crosses since unlinked alleles segregate randomly with respect to donor region genotype. Second, relative to a whole-genome scan, the statistical power to detect and resolve QTL, not to mention to characterize environment effects (such as sex), is substantially increased. This is primarily due to the elimination of background genetic noise, consisting of the direct and interactive effects of tens or hundreds of QTL. This approach has been used to successfully fine map growth and obesity QTL within the MB2 congenic (Jerez-Timaure *et al.* 2005).

Originally, the 1.5-LOD C.I.'s for the MMU2 and MMU11 QTL were between 30 and 35 cM (Corva *et al.* 2001). In this study, the C.I.'s ranged from 5.3 to 37 cM, with a median C.I. of 15.5. Due to this reduction, we can now focus on each C.I. using targeted subcongenic strains and gene expression analysis to ultimately identify the underlying QTG. It is also worth noting that a number of QTL that we detected were not seen in the original study, highlighting the added power of detection afforded by the congenic-derived crosses.

In summary, we have gained considerable insight into the genetic architecture of growth, skeletal size, and obesity QTL segregating in two well-defined regions of the mouse genome. Our results highlight the importance of assessing the extent of QTL × sex interaction in linkage analyses. Additionally, it is likely that the extensive sex effects are a direct result of *hg*, which implicates genes involved in *Gh* function as putative QTG. These data serve as the platform for future QTG discovery and provide significant insight into the molecular mechanisms governing differences in growth and obesity between the genders.

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