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ORIGINAL CONTRIBUTIONS

Genetic resistance to diet-induced obesity in chromosome substitution strains of mice

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Abstract Discovery of genes that confer resistance to diseases such as diet-induced obesity could have tremendous therapeutic impact. We previously demonstrated that the C57BL/6J-Chr$^{A/J}$/NaJ panel of chromosome substitution strains (CSSs) is a unique model for studying resistance to diet-induced obesity. In the present study, three replicate CSS surveys showed remarkable consistency, with 13 A/J-derived chromosomes reproducibly conferring resistance to high-fat-diet-induced obesity. Twenty CSS intercrosses, one derived from each of the 19 autosomes and chromosome X, were used to determine the number and location of quantitative trait loci (QTLs) on individual chromosomes and localized six QTLs. However, analyses of mean body weight in intercross progeny versus C57BL/6J provided strong evidence that many QTLs discovered in the CSS surveys eluded detection in these CSS intercrosses. Studies of the temporal effects of these QTLs suggest that obesity resistance was dynamic, with QTLs acting at different ages or after different durations of diet exposure. Thus, these studies provide insight into the genetic architecture of complex traits such as resistance to diet-induced obesity in the C57BL/6J-Chr$^{A/J}$/NaJ CSSs. Because some of the QTLs detected in the CSS intercrosses were not detected using a traditional C57BL/6J × A/J intercross, our results demonstrate that surveys of CSSs and congenic strains derived from them are useful complementary tools for analyzing complex traits.

L. C. Burrage and A. E. Baskin-Hill contributed equally to this work.

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Introduction

The worldwide incidence of obesity among children, adolescents, and adults has risen dramatically in recent years (Kelly et al. 2008). Currently, 32% of U.S. adults are obese and 66% are overweight (Ogden et al. 2006; Wang et al. 2008). Increased body weight is a major public health concern because it is a component of metabolic syndrome, a constellation of medical conditions including high blood pressure, insulin resistance, and dyslipidemia. Recent evidence also suggests that increased body mass index (BMI), which is a measure of adiposity, is an independent risk factor for conditions such as cardiovascular disease, respiratory complications, sleep disorders, osteoarthritis, and several cancers (National Institutes of Health 1998; Renehan et al. 2008; Schelbert 2009; Yan et al. 2006).

Both genetic and environmental factors contribute to the development of obesity and other aspects of metabolic syndrome. Discovery of genes responsible for monogenic cases of obesity, such as leptin (*Lep*) (Montague et al. 1997; Zhang et al. 1994), melanocortin-4 receptor (Huszar et al. 1997; Loos et al. 2008; Vaisse et al. 1998; Yeo et al. 1998), and pro-opiomelanocortin (*Pomc1*) (Krude et al. 1998), provides insight into the physiologic control of satiety and energy metabolism. However, these and other single-gene variants account for only a small portion of the genetic variation in body weight in human populations (Walley et al. 2009). Instead, multiple genes together with environmental factors such as diet and activity contribute to common forms of obesity (Allison et al. 1996; Hinney et al. 2007; Lindgren et al. 2009; Maes et al. 1997; Willer et al. 2009). To date, few genes contributing to polygenic obesity have been identified in humans (Lindgren et al. 2009; Mutch and Clement 2006; Walley et al. 2009; Willer et al. 2009).

Laboratory animals provide important models for human obesity because both genetic and environmental factors can be controlled in ways that are often difficult in human studies. Studies of spontaneous and engineered mutant mice have led to the discovery of genes that are responsible for monogenic cases (Zhang et al. 1994). Inbred strains also provide models of multifactorial obesity (Brockmann and Bevova 2002). For example, C57BL/6J (B6) male mice are significantly more susceptible than A/J males to diet-induced obesity (Black et al. 1998; Mills et al. 1993; Rebuffe-Scrive et al. 1993; Surwit et al. 1988) and other features of metabolic syndrome, such as hypertension, insulin resistance, and dyslipidemia (Collins et al. 2004; Mills et al. 1993; Rebuffe-Scrive et al. 1993; Surwit et al. 1988, 1995; Farber et al. 2009). As in humans, inheritance of these traits is complex, involving multiple genes and gene-environment interactions. Therefore, these strains are an important model for studying the genetics and physiology of the common forms of diet-induced obesity and metabolic syndrome (Collins et al. 2004; Wuschke et al. 2007; Manolio et al. 2009).

The first comprehensive genetic analysis of diet-induced obesity in B6 and A/J male mice was performed using the B6–Chr$^{A/J}$/NaJ chromosome substitution strains (CSSs), a panel of 22 inbred strains in which a single chromosome from a donor strain (in this case, A/J) has been substituted onto a host strain background (in this case, B6) (Singer et al. 2004). When fed a high-fat diet, 17 CSSs were resistant to obesity relative to B6 (Singer et al. 2004). Thus, at least 17 obesity-resistance genes contribute to the differential susceptibility of B6 and A/J males to high-fat diet-induced obesity (Singer et al. 2004), a result that further emphasizes the genetic complexity of this trait in this pair of strains. Additional studies are needed to narrow these regions to facilitate gene discovery.

The goal of the present study was to further dissect the genetic control of resistance to diet-induced obesity in the A/J and B6 inbred strains as well as in CSSs and intercrosses derived from these progenitor strains. To this end, we assessed the impact of diet composition on the parental strains, surveyed the genetics of resistance to diet-induced obesity in replicate CSS surveys, compared results from the CSS surveys with results from intercrosses derived from each CSS and from intercrosses between the parental strains, and assessed dominance and temporal effects in the segregating crosses derived from CSSs.

Materials and methods

Abbreviations

We refer to our original CSS survey as O-SRV, which was performed at the CWRU Animal Resource Center (ARC) (Singer et al. 2004), the first CSS survey replicate as R-WOL, which was done in the CWRU Wolstein Animal Facility, and the second CSS survey replicate as R-ARC, which was performed at a later time in the ARC.

Mice

B6 and A/J males were obtained from the Jackson Laboratory (Bar Harbor, ME). For all studies except R-WOL, B6 and A/J males from Jackson Laboratory were used for body weight studies. For R-WOL, B6 and A/J colonies were established at CWRU using mice obtained from Jackson Laboratory, and males from these colonies were used for analysis. The C57BL/6J–Chr$^{A/J}$/NaJ CSSs were raised at CWRU.

Husbandry

All mice were weaned at 3-4 weeks of age and housed in micro-isolator cages with a 12 h:12 h light:dark cycle. Unless otherwise noted, mice were maintained on LabDiet 5010 autoclavable rodent diet (27.6 kcal% protein, 13.5 kcal% fat, 59.0 kcal% carbohydrate) ad libitum (Labdiet, Richmond, IN). All studies and procedures were approved by the CWRU Institutional Animal Care and Use Committee.

Intercrosses

Crosses between each CSS and B6 (except CSS-Y and CSS-Mito) were made in one direction—CSS females × B6 males—to control for possible parental and grandparental effects among intercross progeny. F1 offspring were intercrossed to generate F2 male progeny (75-93 per CSS cross). Similarly, B6 and A/J mice were crossed to generate F1 mice (B6 × A/J) that were then intercrossed to generate 94 F2 progeny.

Genotyping

Both microsatellite markers and single nucleotide polymorphisms (SNPs) (average intermarker interval = 11.2 Mb; greatest intermarker interval = 40.5 Mb) were used as genetic markers for the CSS intercrosses. For microsatellite markers, DNA was isolated from tail samples with standard methods (proteinase K digestion, Invitrogen, Carlsbad, CA), and genotyping was performed using PCR and polyacrylamide gel electrophoresis. For SNPs used in CSS intercrosses and in the parental strain intercross, DNA was isolated from tail samples with the Qiagen DNeasy Kit (Qiagen, Valencia, CA), and genotyping was performed with the Sequenom MassArray 7 K system and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom, San Diego, CA). For each marker scored with mass spectrometry, genotypes were scored independently with both the Sequenom software (version 3.3) and an alternative method developed by Dr. Joel Hirschhorn (personal communication). Markers in which 90% of the typed individuals had concordant positive (not "no call") genotypes using both methods were used for the mapping analyses. Among the concordant calls for usable markers, we analyzed the distribution of genotypes in the CSS intercrosses to test whether they were consistent with the expected Mendelian distribution of genotypes in an F2 cross, which was evaluated with a χ^2 test at $\alpha = 0.000052$, which corresponds to the Lander-Kruglyak threshold of $\alpha = 0.05$ for intercross analyses (free model) (Lander and Kruglyak 1995). Marker segregation was consistent with Mendelian expectations in every case. Markers used in the CSS intercross analysis and the corresponding p values derived from χ^2 analysis are provided in Supplementary Table 1A. Markers (SNPs) used in the B6 × A/J intercross were similarly genotyped and are presented in Supplementary Table 1B.

Phenotyping

Male mice (A/J and B6 parental strains, CSSs, and intercross progeny) were introduced to one of the four test diets at 5 weeks of age. All test diets were obtained from Research Diets (New Brunswick, NJ). The following four test diets were used: HFSC, high-fat, simple carbohydrate (Research Diets D12331, 58.0 kcal% fat, 25.5 kcal% carbohydrate—sucrose and maltodextrin, 16.4 kcal% protein); HFCC, high-fat, complex carbohydrate (Research Diets D12330, 58.0 kcal% fat, 25.5 kcal% carbohydrate—cornstarch and maltodextrin, 16.4 kcal% protein); LFSC, low-fat, simple carbohydrate (Research Diets D12329, 10.5 kcal% fat, 73.1 kcal% carbohydrate—sucrose and maltodextrin, 16.4 kcal% protein); and LFCC, low-fat, complex carbohydrate diets (Research Diets D12328, 10.5 kcal% fat, 73.1 kcal% carbohydrate—cornstarch and maltodextrin, 16.4 kcal% protein). For the parental strains, R-WOL, O-SRV, the LFCC survey, and the intercrosses, mice were weighed every 2 weeks for approximately 100 days after introduction of the diet. In contrast, for R-ARC, weights were collected only at select time points (IW and FW). For R-ARC, the final time point was 120 days (∼4 months) rather than 100 days.

The following traits (or a subset as described in the text) were analyzed (Fig. 1): IW (weight in grams at ∼35 days of age), MW (weight in grams at ∼90 days of age), FW (weight in grams at ∼135 days of age), BMI [FW in grams/(final nasoanal length in centimeters)^2], EWG (mean weight gain per day in grams/day for first ∼55 days), FWG (mean weight gain per day in grams per day for second ∼45 days), and WG (mean weight gain per day in grams/day). For the parental strains, five traits were analyzed (MW, FW, WG, EWG, FWG); for the original HFSC and LFCC surveys, six traits (IW, MW, FW, WG, EWG, FWG) were analyzed; for the replicate CSS surveys, only IW, FW, and BMI were analyzed; for the CSS intercross analysis, all seven traits (IW, MW, FW, BMI, WG, EWG, FWG) were analyzed; and for the parental strain intercross analysis, four traits (IW, FW, BMI, WG) were analyzed.

Statistical analyses and QTL mapping

All statistical analyses were performed with the R statistical software (Ihaka and Gentleman 1996) unless otherwise noted. QTL analysis was performed with R/qtl, an add-on package for R (Broman et al. 2003).

Standard three-way ANOVA was used to test the effects of strain and dietary content of fat and carbohydrate

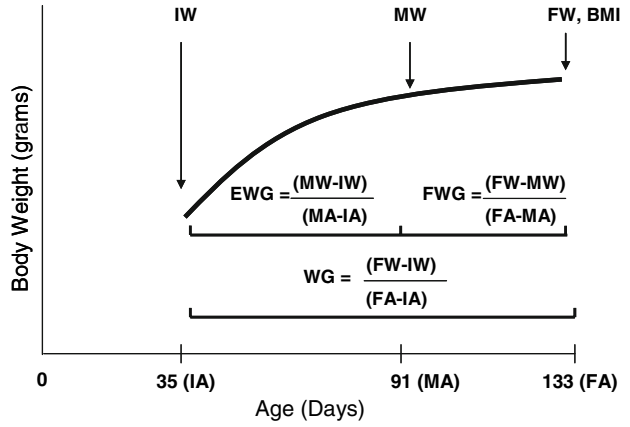

Fig. 1 Time course, traits, and metrics for body weight studies. IW = initial weight, MW = midpoint weight, FW = final weight, BMI = body mass index, EWG = mean weight gain per day during the first half of the study, FWG = mean weight gain per day during the second half of the study, WG = mean weight gain per day during the entire study, IA = initial age (days), MA = midpoint age (days), FA = final age (days). This study design was used for all studies except R-ARC (see Methods)

composition on MW, FW, EWG, FWG, and WG in B6 and A/J males. Unpaired *t* tests were used to compare IW, MW, FW, EWG, FWG, and WG from each CSS with those from B6 for the HFSC and LFCC surveys. To test whether a CSS and B6 differed in response to the high-fat diet, the difference in the mean trait value for a CSS on the HFSC versus LFCC diets was compared to the corresponding difference for B6. *P* values, adjusted for the search across the 22 chromosomes and across the six traits (IW, MW, FW, WG, EWG, FWG), were determined using a permutation test, with 100,000 permutations of individuals with respect to their strain assignments. The same *t* statistics used with the observed data were calculated with the permuted data. The maximum *t* statistic across strains and phenotypes was recorded for each replicate. The adjusted *p* value for a particular strain and phenotype was the proportion of permutation replicates at which the maximal *t* statistic (maximized across strains and phenotypes) was greater than or equal to the observed *t* statistic.

We used two methods to test reproducibility in the replicate CSS surveys. First, the difference between mean trait values (IW, FW, BMI) for each CSS in R-WOL and R-ARC was plotted versus the mean trait value for each CSS in R-WOL and R-ARC, and then the mean difference among all CSSs and the 95% confidence intervals (±2 standard deviations) were then plotted to identify outliers. Next, unpaired *t* tests were used to compare the trait values for each CSS in the replicate surveys to those of B6 using similar methods as described above. *P* values, after adjusting for the search across the 22 chromosomes and the three phenotypes, were again calculated with 100,000 permutations. This method identified strains that were reproducibly resistant to obesity relative to B6.

For the intercross analyses, F2 males that lost more than 10% of their body weight in a 2-week interval (suggestive of illness) were removed from the analysis of IW, MW, FW, BMI, WG, EWG, and FWG. The FW of each of the 20 intercrosses was compared to that of B6 using an unpaired *t* test followed with 20,000 permutations of the data set to determine significance thresholds that adjust for the search across chromosomes. QTL analysis was performed using standard interval mapping (Lander and Botstein 1989). Age was used as a covariate for all body weight and BMI analyses to account for small variations in ages of the mice at specific time points (IW: 34 ± 4 days, MW: 89 ± 7 days, FW: 33 ± 7 days). Confidence intervals for the location of the QTL were estimated by calculating 1.5 LOD support intervals. To establish statistical significance of the QTL mapping results, we took account of the search across the 20 chromosomes and across the seven (IW, MW, FW, BMI, WG, EWG, FWG) traits; we sought to control the family-wise error rate, namely, the chance of identifying a QTL for at least one chromosome or trait, if in fact there are no QTLs for any of the traits. Because the intercrosses for individual chromosomes varied in size and marker density, chromosome-specific significance thresholds are required. However, the seven traits have similar marginal distributions, so a common threshold can be used. We used a permutation test, with 100,000 permutation replicates. Let M_{ijk} denote the maximum LOD score on chromosome i for phenotype j in permutation replicate k, and let M_{ik} denote the maximum, across trait j, of the M_{ijk}. For a single trait, the $1 - \alpha$ quantile of the M_{ik} (across k) is a significance threshold adjusted for the scan across the seven phenotypes but not for the scan across the genome. We derived chromosome-specific significance threshold using a previously published approach (Broman et al. 2003). Let L_i denote the genetic length of chromosome i, and let L denote the sum of these lengths. We use the $1 - (1 - \alpha)^{Li/L}$ quantile of the M_{ik} (across permutation replicates k) as the significance threshold for chromosome i, adjusting for the scan across phenotypes and across the genome. We used the accepted thresholds for suggestive ($\alpha = 0.63$) and significant ($\alpha = 0.05$) QTLs (Lander and Kruglyak 1995).

To investigate the possibility of linked or interacting QTLs, we performed two-dimensional, two-QTL scans. For each pair of sites on a chromosome, we fit an additive model (two QTLs acting additively) and a full model (two QTLs allowed to interact). From these results we calculated four LOD scores: L_f, comparing the full, two-QTL model to the null model (of no QTLs); L_i, comparing the full model to the additive two-QTL model (concerning the interaction term); L_{ci}, comparing the full model to the best

single-QTL model for that chromosome; and L_{ca}, comparing the additive model to the best single-QTL model. L_{ca} and L_{ci} indicate evidence for a second QTL on that chromosome; L_i indicates evidence for an interaction between the QTLs. Two QTLs on a chromosome are indicated when L_f is large and either L_{ca} or L_{ci} are large. The QTLs are indicated to interact when L_i is also large. The analysis was performed using the R/qtl software (Broman et al. 2003).

To evaluate inheritance patterns associated with QTLs detected in the intercrosses, progeny in each cross were sorted by genotype at the marker nearest the peak. Then, a one-way ANOVA with Tukey's post-test was used to compare the trait values among the genotypes (GraphPad Prism, version 3.03, GraphPad Software, Inc., La Jolla, CA). A Bonferroni correction accounting for the number of chromosomes on which QTLs were detected and number of traits tested was also applied to correct for multiple testing.

The traditional intercross (using the parental strains) was analyzed much like the CSS intercrosses, though the derivation of significance thresholds to adjust for the genome scan did not require the special adjustments, described above, to obtain chromosome-specific thresholds.

Results

Effects of diet content

To assess the relative contribution of dietary fat and carbohydrate to weight gain in B6 and A/J mice, males from each strain were raised on one of four diets that differed in fat content (58 vs. 11 kcal%) and carbohydrate source (simple versus complex carbohydrate) (Fig. 2). We used body weight as a surrogate for obesity in this study because previous work showed a strong correlation between body weight and fat pat weights in this model (Buchner et al. 2008). Mice were evaluated for five traits: EWG, FWG, MW, FW, and WG (Fig. 1, Supplementary Table 2A). IW was omitted from this analysis because our objective was to assess diet effects rather than differences in body size. For FW, three-way ANOVA showed a strong strain effect ($p < 2.0 \times 10^{-16}$), a strong fat effect ($p < 2.0 \times 10^{-16}$), but not a carbohydrate effect; a strong interaction was detected between strain and fat ($p < 2.0 \times 10^{-16}$; Supplementary Table 2B). Similar results were found for EWG, MW, FWG, and WG as well as in previous studies (Surwit et al. 1995). Thus, although B6 males were heavier than A/J males on all four diets, the differences were exaggerated on the high-fat versus low-fat diets, indicating that B6 males were genetically predisposed to high-fat, diet-induced differences in body weight.

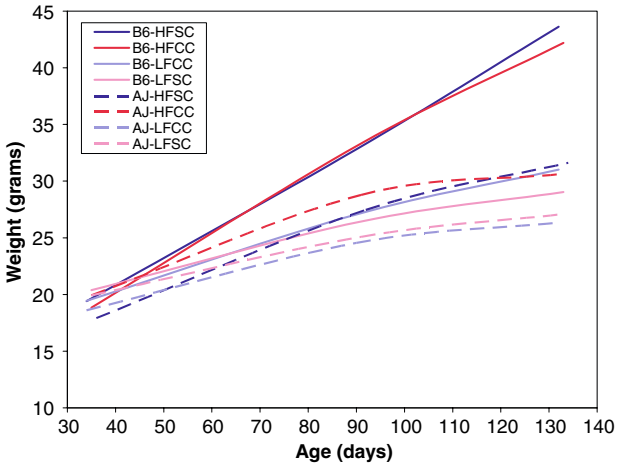

Fig. 2 Genetic and diet effects on patterns of weight gain. Male mice (n = 24-30 per strain) were raised on one of four diets and weighed at 2-week intervals. The mean body weight for each strain-diet combination at each time point is plotted. Diet abbreviations: HFSC = high-fat, simple carbohydrate; HFCC = high-fat, complex carbohydrate; LFSC = low-fat, simple carbohydrate; LFCC = low-fat, complex carbohydrate

Diet versus body size effects

Our previous CSS survey (O-SRV, see below and Supplementary Table 3A) revealed 17 A/J-derived chromosomes that confer resistance to HFSC diet-induced obesity (Singer et al. 2004). However, that survey did not evaluate the relative contributions of diet-induced obesity versus body weight independent of diet. Thus, we evaluated measures of weight gain in CSSs and B6 male mice fed a low-fat (LFCC) diet using a similar study design as O-SRV. The LFCC diet was selected because we and others demonstrated that this diet does not induce obesity in B6 males (Surwit et al. 1995). To this end, we compared the six body weight traits (IW, MW, FW, WG, EWG, FWG) for the CSSs and B6 males fed the LFCC diet (Supplementary Table 3B), and we also tested whether we could detect a "diet effect" for each CSS on the LFCC diet. For example, if differences in trait measures between CSSs and B6 on the alternative diets varied significantly (diet effect detected), we could conclude that the reduced trait measures in the CSSs fed the HFSC diet resulted from resistance to diet-induced obesity. Alternatively, if differences between a CSS and B6 were similar regardless of diet, we could conclude that trait differences were independent of diet (no diet effect) and instead reflect diet-independent differences in body size.

Among the 17 CSSs that were resistant to diet-induced obesity (Singer et al. 2004), we found a significant diet effect in 10 CSSs (CSS-3, -4, -6, -7, -8, -12, -15, -17, -Y, and –M; Supplementary Table 3C), suggesting that they were indeed resistant to HFSC-diet-induced obesity. By

contrast, the effect of the diet was not significant for the other CSSs that were resistant in the original survey (O-SRV; CSS-5, -10, -11, -13, -14, 16-, and -18, Supplementary Table 3C), suggesting that their reduced FW was due to a body size effect that was independent of diet. Hence, variation in FW appears to result from both differences in body size and resistance to diet-induced obesity, depending on the CSS.

Replicate surveys of obesity resistance

Vagaries in environmental factors such as season, diet lot, and mouse facility can lead to difficulty reproducing results among independent studies. Furthermore, studies have demonstrated that epigenetics may play a role in body weight in C57BL/6J mice (Koza et al. 2006) and, thus, could also contribute to variability in genetically identical mice. To test reproducibility of responses to the HFSC diet, R-WOL and R-ARC replicate CSS surveys were performed in different mouse facilities at CWRU, in addition to the original survey (O-SRV) (Singer et al. 2004). In contrast to the O-SRV and LFCC-diet surveys, we focused on IW as a baseline trait prior to diet introduction and FW as a measure of body weight at the end of the study. We also added a new trait, BMI, as a measure of adiposity because we have previously demonstrated that BMI correlates with gonadal fat pad size in the parental strains (Buchner et al. 2008). We tested reproducibility among the replicates for these three traits.

We began by plotting the difference in CSS trait values in the R-WOL and R-ARC replicate surveys versus the average trait value for each CSS in the replicate surveys. We also calculated the mean difference among all CSSs and 95% confidence interval (±2 SD). For IW, the mean difference between the two surveys was −2.26 g with 95% confidence intervals (± 2 SD) ranging from −5.81 to 1.30 g. The difference in IW of a single strain (A/J) was outside the confidence intervals (Fig. 3a). For FW, the mean difference was −2.08 g with 95% confidence intervals (±2 SD) ranging from −6.33 to 2.16 g. Only CSS-X had a FW difference outside of these confidence intervals (CSS-X) (Fig. 3b). Finally, for BMI, the mean difference was −0.01 g/cm^2 with 95% confidence intervals (±2 SD) ranging from −0.04 to 0.02 g/cm^2, and only the value for CSS-X was outside of these confidence intervals (Fig. 3c). For all three traits, the mean difference was negative because, on average, the CSSs in R-ARC had higher trait values than the CSSs in R-WOL (Table 1). Thus, results of the CSS surveys were remarkably consistent, with only three discrepant results among the 69 tests for three traits in the R-WOL and R-ARC surveys.

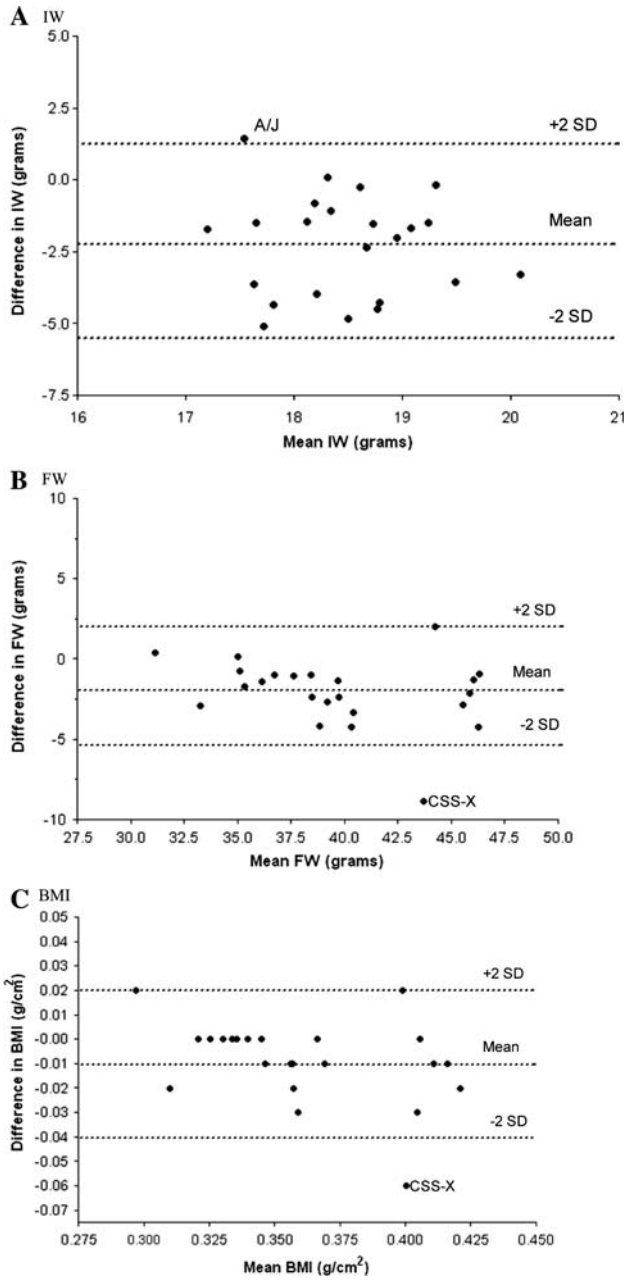

Fig. 3 Mean-difference plots for replicate CSS surveys. The difference between the mean trait values (IW, FW, BMI) for each CSS in R-WOL and R-ARC was calculated and plotted versus the mean trait value for each CSS in R-WOL and R-ARC. The mean difference among all CSSs and the 95% confidence intervals (±2 standard deviations) for the mean difference were calculated and plotted. Parental strains and CSSs that are outside the 95% confidence intervals are identified

CSS intercrosses

We next tested for QTLs that affect MW, FW, EWG, WG, and FWG among progeny of intercrosses between each CSS (except CSS-Y and CSS-Mito) and the B6 host strain (Table 2, Fig. 4; see also Supplementary Table 5). We also

Table 1 Final body weight results for the three replicate CSS surveys

Strain	O-SRV	R-WOL	R-ARC
C57BL/6J	42.71 (5.97; 20)	45.37 (5.25; 20)	46.68 (3.98; 29)
A/J	31.62 (3.82; 24)	31.32 (3.15; 6)	30.94 (2.91; 30)
CSS-1	41.59 (5.21; 17)	45.83 (4.86; 7)	46.78 (3.90; 26)
CSS-2	43.55 (5.19; 12)	44.11 (5.71; 11)	46.98 (6.27; 22)
CSS-3	31.56 (4.19; 16)	35.07 (3.09; 11)	34.92 (5.08; 15)
CSS-4	31.58 (3.11; 20)	36.23 (4.18; 23)	37.22 (3.48; 25)
CSS-5	37.40 (4.28; 20)	38.99 (5.49; 25)	40.35 (3.68; 21)
CSS-6	32.93 (4.25; 12)	35.44 (4.64; 23)	36.84 (3.41; 24)
CSS-7	30.47 (2.69; 18)	31.78 (3.00; 23)	34.67 (4.25; 22)
CSS-8	33.17 (3.55; 21)	37.12 (4.19; 22)	38.15 (4.30; 17)
CSS-9	40.60 (5.50; 17)	44.15 (5.88; 22)	48.37 (4.87; 19)
CSS-10	35.16 (7.02; 13)	37.92 (6.42); 25	38.91 (5.96; 25)
CSS-11	35.40 (5.50; 16)	38.77 (4.92; 20)	42.07 (5.86; 19)
CSS-12	33.77 (3.43; 12)	38.55 (3.70; 23)	40.89 (4.84; 26)
CSS-13	36.04 (4.56; 20)	34.48 (4.31; 6)	36.16 (5.25; 20)
CSS-14	35.56 (3.95; 17)	37.83 (2.43; 20)	40.52 (4.21; 23)
CSS-15	34.35 (1.86; 17)	38.20 (5.78; 11)	42.41 (6.50; 22)
CSS-16	36.73 (4.42; 20)	37.30 (3.52; 12)	39.68 (3.05; 21)
CSS-17	33.49 (2.57; 17)	34.73 (3.88; 24)	35.48 (4.44; 30)
CSS-18	35.36 (4.25; 10)	44.78 (6.60; 23)	46.91 (4.89; 18)
CSS-19	43.79 (6.79; 16)	45.28 (8.30; 19)	43.24 (5.82; 20)
CSS-X	47.52 (4.65; 14)	39.24 (5.29; 16)	48.12 (3.06; 14)
CSS-Y	34.82 (4.38; 14)	36.73 (4.23; 20)	40.90 (4.15; 18)
CSS-Mito	36.21 (3.47; 23)	41.69 (6.10; 31)	nd

Final body weight (FW, g) is provided for the original (O-SRV) (Singer et al. 2004) and the replicate (R-WOL and R-ARC) CSS surveys with standard deviation and sample size in parentheses

tested IW in the intercrosses as a baseline trait. The intercross analysis revealed significant QTLs on chromosomes 1 (BMI, WG), 6 (BMI), 10 (MW, EWG), 11 (WG), and 13 (MW). In addition, suggestive QTLs were found on 1 (FW, MW), 6 (FW, MW, WG, FWG), 10 (FW, IW, WG), 11 (FW, BMI, FWG), 13 (FW, BMI, WG, FWG), and 17 (MW). Interestingly, on chromosome 6, two suggestive QTLs with nonoverlapping 1.5 LOD support intervals were detected for FW, WG, and FWG.

To test whether pairs of QTLs with additive or interactive effects might explain why we were unable to detect QTLs on chromosomes that reproducibly conferred resistance in CSS surveys, we performed a two-dimensional, two-QTL scan for each chromosome and trait among the 20 intercrosses. For each trait we compared the best two-QTL model (additive or interacting) with the best one-QTL model and provided a LOD score for each comparison. Obviously, the small number of recombinant males in each intercross limited the sensitivity of this study. Regardless, the two-dimensional, two-QTL scan provided suggestive evidence (additive model) of two QTLs for FWG on chromosome 6 ($p = 0.26$), two QTLs for FW on chromosome 13 ($p = 0.43$, additive model), and two QTLs for FWG on chromosome X ($p = 0.61$) (Supplementary Figs. 1 and 2). Thus, despite the limited sensitivity of this analysis, suggestive evidence was found for at least two QTLs on three chromosomes.

Based on the results of the CSS replicate surveys, we expected to detect FW QTLs on at least 13 chromosomes in the intercrosses. Because we detected fewer than the expected number of QTLs, we hypothesized that segregating QTLs may be present but not detected using conventional intercross analysis. To test this hypothesis, we assessed the mean FW in intercross progeny versus B6 (Fig. 5). Intercross progeny with mean FW that differed significantly from B6 suggested that QTLs affecting FW were segregating in the cross, regardless of whether QTLs were detected with conventional analyses. If QTLs were not segregating, the mean weight of intercross progeny and B6 should be similar. This test is conservative because, depending on dominance, a substantial portion of the intercross population was expected to be genetically and phenotypically similar to B6. Remarkably, the mean FW for 13 intercrosses (CSS-3, -4, -5, -6, -7, -8, -10, 12, -13, -15, 17, -18, and X) was significantly less than that of B6, suggesting that at least one QTL with strong effects was segregating in intercross progeny producing resistance in the population relative to B6. These results provide independent evidence demonstrating that many QTLs were present but not detected with conventional CSS intercross analysis (Fig. 6).

QTL inheritance patterns and allelic effects

We expected to detect many A/J-derived resistance QTLs because of the large number of A/J chromosomes that conferred resistance to diet-induced obesity in the surveys. Hence, we tested whether A/J-derived alleles conferred resistance to diet-induced obesity in the intercrosses. To this end, intercross mice were sorted by their genotype at the marker nearest the significant or suggestive LOD score peaks, and the mean FW was calculated for mice with each genotype at that marker. Then, the FWs for each genotype were compared using a one-way ANOVA with Tukey's post-test and a Bonferroni correction was applied to account for the large number of tests performed (across chromosomes and traits). Various modes of inheritance and allelic effects were observed, including over-dominance on chromosomes 6 (*Obrq1* and *Obrq3*) and 17 (*Obrq8*), A/J-derived alleles on chromosomes 1 (*Obrq4*) and 11 (*Obrq6*) that promote obesity in a dominant manner, and A/J-derived alleles on chromosomes 10 (*Obrq5*) and 13 (*Obrq7*) that tend to promote resistance to diet-induced

Table 2 Significant and suggestive QTLs detected in CSS intercross analysis

Chromosome (trait)	QTL name	Location (cM)	1.5 LOD support interval (cM)
Significant			
1 (BMI)	*Obrq4*	67	57–69
1 (WG)	*Obrq4*	60	47–69
6 (BMI)	*Obrq1*	56	51–62
10 (MW)	*Obrq5*	65	57–90
10 (EWG)	*Obrq5*	95	79–103
11 (WG)	*Obrq6*	49	38–55
13 (MW)	*Obrq7*	24	12–31
Suggestive			
1 (FW)	*Obrq4*	62	47– 69
1 (MW)	*Obrq4*	65	37–69
6 (FW)	*Obrq3*	28	20–33
6 (FW)	*Obrq1*	57	37–76
6 (MW)	*Obrq1*	56	39–64
6 (WG)	*Obrq3*	28	19–33
6 (WG)	*Obrq1*	43	36–73
6 (FWG)	*Obrq3*	28	18–33
6 (FWG)	*Obrq1*	72	51–83
10 (FW)	*Obrq5*	62	25–103
10 (IW)	*Obrq5*	65	56–80
10 (WG)	*Obrq5*	90	68–103
11 (FW)	*Obrq6*	49	31–57
11 (BMI)	*Obrq6*	47	32–57
11 (FWG)	*Obrq6*	49	40–58
13 (FW)	*Obrq7*	38	8–53
13 (BMI)	*Obrq7*	15	0–51
13 (WG)	*Obrq7*	23	0–47
13 (FWG)	*Obrq7*	35	6–51
17 (MW)	*Obrq8*	0	0–11

IW Initial weight, *MW* midpoint weight, *FW* final weight, *BMI* body mass index, *EWG* mean weight gain per day during the first half of the study, *FWG* mean weight gain per day during the second half of the study; *WG* mean weight gain per day during the entire study

The location of the QTL peak in cM and 1.5 LOD support interval are listed for each significant and suggestive QTL

obesity in a dominant, recessive, or additive manner (Table 3). Therefore, we discovered A/J-derived resistance QTLs but also new A/J-derived obesity-promoting QTLs that were not detected in the CSS surveys.

Duration of diet exposure reveals distinct timing of QTL action

To test whether QTLs act after different times during diet exposure, we evaluated the intercross mapping results for traits at different time points. For example, *Obrq6* affected weight gain in the second half of the study because significant peaks were detected for BMI, FW, WG, and FWG, but not for EWG or MW. In contrast, *Obrq5* affected weight gain in the first half of the study because QTLs for both EWG and MW were detected. Chromosome 6 was also complex with at least two QTLs: *Obrq3* near 28 cM affecting FWG, WG, and FW, and *Obrq1* near 56 cM affecting MW, FW, BMI, WG, and FWG. *Obrq1* may influence body weight earlier than *Obrq3* because *Obrq1* showed an effect for MW whereas *Obrq3* did not. Thus, individual QTLs appear to dynamically control weight gain (Table 2).

Parental strain intercrosses

To test whether QTLs detected in the CSS intercrosses are comparable to those detected using a traditional intercross between the parental strains, we performed a QTL analysis using 94 B6 × A/J intercross progeny and compared the results with those obtained using the CSS intercrosses. We used only 94 intercross progeny in the parental strain analysis because this number is comparable to the number of mice used in the CSS intercross analysis (i.e., each chromosome segregates only ~100 times in both studies). No significant QTLs were detected in the

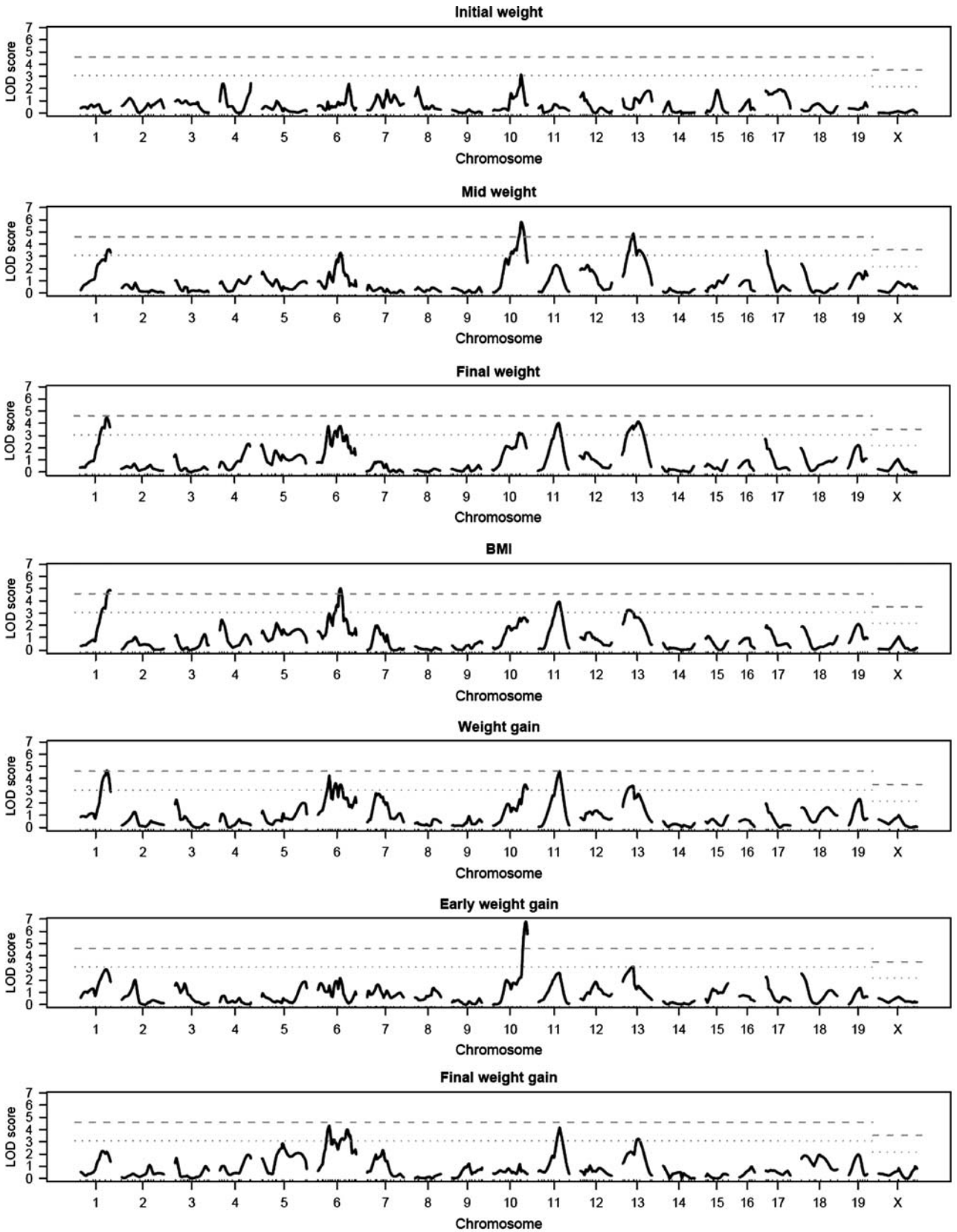

Fig. 4 Genome surveys of intercross progeny derived from the 20 CSS intercrosses. The LOD scores for each cross are plotted. 0.05 (—) and 0.63 (…) thresholds are indicated. These thresholds were calculated after correction for testing 7 traits and 20 crosses

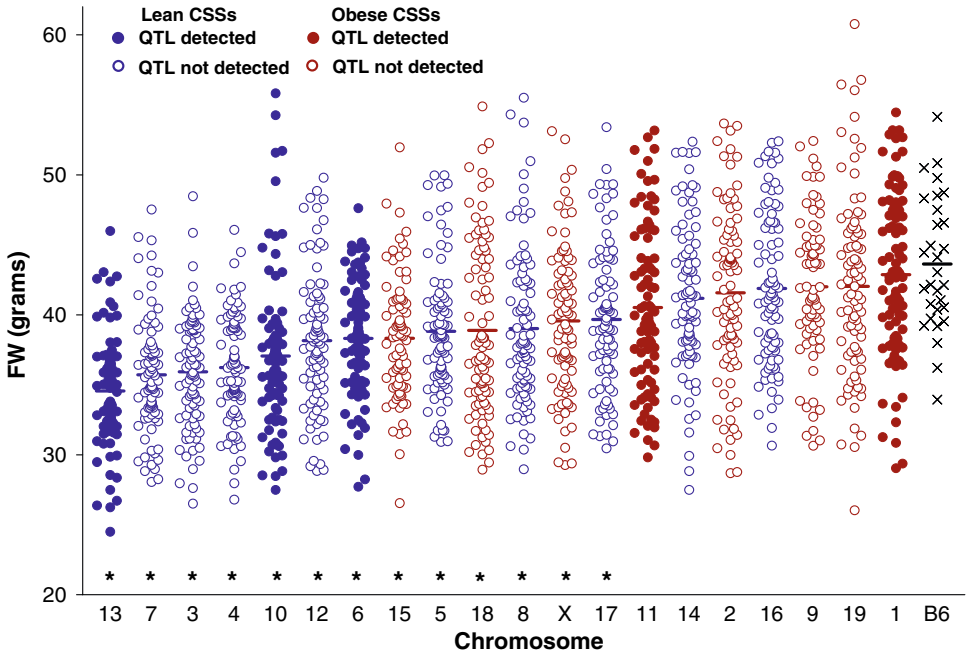

Fig. 5 FW for CSS intercross progeny versus B6. The CSS from which each intercross population was derived is indicated (*x* axis), the mean FW from each intercross is indicated by a horizontal line, and * along the *x* axis indicates that the FW for the intercross was significantly different from B6 ($p < 0.05$) after multiple-testing correction (Supplementary Table 6). Individuals are marked with a *filled circle* if a significant or suggestive QTL was detected in the intercross analysis, and with a *open circle* if a QTL was not detected. X indicates B6. Obesity-resistant and obesity-susceptible strains were categorized according to results of the replicate HFSC surveys (consistently resistant strains) and are highlighted in blue and red, respectively

Study	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y
O-SRV																					
LFCC SRV																					
R-ARC																					
R-WOL																					
Intercross QTLs																					NR
Mean FW of F2																					NR

Fig. 6 Summary of diet-induced obesity in CSSs. The results of the CSS surveys, the CSS intercrosses (FW QTLs), and the comparisons of the mean FW for intercross progeny versus B6 are presented for each chromosome (listed at the top of each column). Studies were conducted on the high-fat, simple carbohydrate (HFSC) diet, unless otherwise indicated (low-fat, complex carbohydrate, LFCC). A gray box indicates that a QTL for FW was detected. For the CSS intercrosses, both significant and suggestive QTLs detected are presented. NR = not relevant

parental strain intercross. For traits related to diet exposure, suggestive QTLs were discovered on only chromosomes 6 (FW, $p = 0.407$; WG, $p = 0.583$) and 10 (FW, $p = 0.347$; BMI, $p = 0.184$). As in the CSS analysis, at least two suggestive QTLs were discovered on chromosome 6 (FW at 24.4 cM and WG at 60.6 cM). In addition, QTLs for weight at the onset of the study (prior to diet exposure) were discovered on chromosomes 13 ($p = 0.494$) and 17 ($p = 0.526$). Interestingly, unlike the suggestive QTLs on chromosomes 6 and 10, the IW QTLs on chromosomes 13 and 17 were not detected in the CSS intercross analysis. LOD curves are provided in Supplementary Fig. 3 and suggestive QTLs are listed in Supplementary Table 7.

We also performed a two-dimensional genome scan for additive and nonadditive effects using the parental strain intercross progeny. Significant evidence for additive effects involving QTLs on chromosomes 6 and 10 for FW [$p(L_{ca}) = 0.0008$, comparison of additive model vs. single QTL model] was discovered even though the individual QTLs on chromosomes 6 and 10 were not significant in the parental strain (B6 × A/J) intercross progeny. Similarly, suggestive evidence was discovered for interactions between these same loci on chromosomes 6 and 10 for BMI and WG. Finally, suggestive evidence was discovered for additive effects for IW (chromosomes 3 and 17, 13 and 17), interactive effects for BMI (chromosomes 1 and 6), and additive effects for WG (chromosomes 6 and 11, 10 and 11).

Table 3 QTL inheritance and allelic effects on growth and obesity-related traits

Chr	Marker	Trait	B	H	A	*p* value			Conclusion
						B vs. A	B vs. H	A vs. H	
1	m7	FW	39.27	44.50	43.39	ns	ns	ns	Inconclusive
1	m8	MW	31.94	35.28	35.73	ns	<0.027	ns	Dominant A/J obesity allele?
1	m8	BMI	0.34	0.39	0.39	<0.027	<0.027	ns	Dominant A/J obesity allele
1	m7	WG	0.20	0.26	0.24	ns	<0.027	ns	Dominant A/J obesity allele?
6	m9	FW	37.04	39.77	35.92	ns	ns	<0.027	Over-dominance?
6	m18	FW	37.55	39.69	35.56	ns	ns	<0.027	Over-dominance?
6	m18	MW	30.97	32.74	30.71	ns	ns	ns	Inconclusive
6	m18	BMI	0.35	0.37	0.33	ns	ns	<0.027	Over-dominance?
6	m9	WG	0.19	0.21	0.17	ns	ns	<0.027	Over-dominance?
6	m15	WG	0.18	0.21	0.17	ns	ns	<0.027	Over-dominance?
6	m9	FWG	0.14	0.17	0.12	ns	ns	<0.027	Over-dominance?
6	m22	FWG	0.16	0.17	0.12	ns	ns	<0.027	Recessive A/J
10	m9	FW	40.77	37.11	34.52	<0.027	ns	ns	Resistance allele? Additive: A/J resistance allele?
10	m10	MW	33.45	31.29	28.92	<0.027	ns	ns	Additive: A/J resistance allele?
10	m10	IW	20.46	20.38	18.50	ns	ns	ns	Inconclusive
10	m11	WG	0.20	0.16	0.16	ns	ns	ns	Inconclusive
10	m11	EWG	0.23	0.18	0.19	ns	<0.027	ns	Dominant AJ resistance allele?
11	m7	FW	36.58	40.78	44.03	<0.027	ns	ns	Dominant A/J obesity allele?
11	m7	BMI	0.32	0.36	0.38	<0.027	ns	ns	Dominant A/J obesity allele?
11	m7	WG	0.17	0.21	0.24	<0.027	ns	ns	Dominant A/J obesity allele?
11	m7	FWG	0.13	0.18	0.23	<0.027	ns	ns	Dominant A/J obesity allele?
13	m9	FW	38.05	34.65	31.82	<0.027	ns	ns	Additive?
13	m6	MW	30.64	30.59	27.37	<0.027	ns	<0.027	Recessive A/J resistance allele
13	m5	BMI	0.33	0.33	0.30	<0.027	ns	ns	Recessive A/J resistance allele?
13	m5	WG	0.18	0.17	0.13	<0.027	ns	ns	Recessive A/J resistance allele?
13	m9	FWG	0.15	0.11	0.09	<0.025	ns	ns	Dominant A/J resistance allele?
17	m1	MW	31.31	33.52	30.50	ns	ns	<0.025	Over-dominance?

ns A statistically non-significant result, *A* homozygosity for the A/J-derived allele, *B* homozygosity for the B6-derived allele; *H* heterozygosity

The marker nearest the peak LOD score of each significant and suggestive QTL was identified. Mice were sorted according to their genotype at this marker. Mean body weight was then calculated for each genotype and compared using a one-way ANOVA with Tukey's post-test. *p* values were corrected for multiple testing ($n = 27$). We categorized inheritance patterns according to the following criteria: *additive* indicates a significant difference between the "A" and "B" genotypes, with the "H" genotype not significantly different from either parental genotype; *dominant A/J obesity allele* indicates obesity in mice that were homozygous or heterozygous for the A/J-derived allele, with homozygotes for the B allele weighing significantly less than A homozygotes and H heterozygotes; *dominant A/J obesity allele?* indicates that two of three tests were consistent with dominant obesity in mice with the A/J-derived allele; *dominant A/J resistance allele?* indicates that two of three tests were consistent with dominant resistance to diet-induced obesity in mice with the A/J-derived allele; *recessive A/J resistance allele* indicates obesity in mice that were homozygous for the A/J-derived allele with A homozygotes weighing significantly less than the B homozygotes and the H heterozygotes; *recessive A/J resistance allele?* indicates that two of three tests were consistent with recessive resistance in mice with the A/J-derived allele; *over-dominance?* indicates that the trait in heterozygotes was significantly more extreme than one of the parental strains and in the same direction, although not significantly, with the other parental strain; and three ns results indicate an inconclusive result. See Supplementary Table 1 for details about the identity and location of the *m* markers. IW, MW, and FW are presented in g. EWG, FWG, and WG are presented in g/day. BMI is presented in g/cm^2

Discussion

CSS surveys

Many metabolic and physiologic studies have investigated the genetic control of susceptibility to body weight-related traits and diet-induced obesity in various combinations of inbred strains (Wuschke et al. 2007). These studies are based on segregating populations, heterogeneous genetic backgrounds such as recombinant inbred strains, and heterogeneous stocks. The present study is the first comprehensive analysis of diet-induced changes in body weight in a panel of CSSs. CSS surveys are based on testing inbred strains with single, defined, and nonoverlapping genetic

differences on a uniform and inbred background (Malek et al. 2006; Nadeau et al. 2000; Shao et al. 2008; Singer et al. 2004). This paradigm enables rigorous and statistically powerful assessments of the number of QTLs that affect complex traits, their phenotypic effect sizes, and the nature of their interactions (Belknap 2003; Nadeau et al. 2000; Shao et al. 2008; Singer et al. 2004; Stylianou et al. 2006). In this report we focused on the genetic control of resistance to diet-induced obesity, with an emphasis on characterizing diet effects, testing reproducibility of CSS surveys, mapping obesity-resistance QTLs in CSS intercrosses, and assessing dominance and temporal QTL effects.

Reproducibility of CSS surveys

In humans, replication of linkage and association studies is important but challenging (Altschuler et al. 2008; Morgan et al. 2007). Despite statistical fluctuations, the sometimes significant vagaries of animal husbandry, and evidence indicating a role of epigenetic control of body weight in mice (Koza et al. 2006), remarkably little effort has been made to assess reproducibility for most obesity genetics studies in mice. The unique nature of CSSs as genetically defined inbred strains provides opportunities, and perhaps an obligation, to test reproducibility. Trends for IW, FW, and BMI among replicate CSS surveys, which were made at different times and in two animal facilities, were remarkably consistent, with only 3 of 69 tests showing inconsistent results (Fig. 3). CSS surveys therefore yielded highly reproducible results.

Obesity-resistant CSSs

Using the CSSs, we found obesity-resistance QTLs on at least 13 chromosomes. Twelve of these chromosomes had been previously associated with obesity in mice but in different strain combinations. These CSS surveys provide the first evidence for an obesity-related genetic variant on the mouse Y chromosome. In all three replicates, CSS-Y males weighed less than B6 males (Table 1). Interestingly, several reports of human patients with structural abnormalities involving the Y chromosome include obesity as one of several clinical features, although no causative gene has been identified (Calzolari et al. 1993; Castro et al. 2004; De Rosa et al. 1997; Velissariou et al. 2007).

QTLs on substituted chromosomes

CSS intercrosses in which only a single chromosome segregated were used to determine the number and location of QTLs on individual A/J-derived chromosomes. Surprisingly, these intercrosses revealed evidence for QTLs on only six chromosomes (1, 6, 10, 11, 13, and 17) of the 20 chromosomes that were tested. Of note, using a B6 × A/J intercross with a comparable number of intercross progeny, QTLs were detected on only four of these chromosomes (6, 10, 13, 17), with the QTLs on chromosomes 13 and 17 being "new" IW QTLs not detected in the CSS intercrosses. Interestingly, of the 13 CSSs that showed evidence for an obesity-resistance QTL in the CSS surveys, A/J-derived resistance QTLs were detected on only three of these chromosomes: 6, 10, and 13. In addition, one of these CSS intercrosses, involving CSS-6, showed convincing evidence for two QTLs with significant or suggestive effects: *Obrq3* (proximal) for FW, FWG, and WG, and *Obrq1* (distal) for BMI, FW, MW, FWG, and WG (Table 2). Furthermore, the two-dimensional, two-QTL scan provided suggestive evidence for two QTLs with additive effects on chromosomes 6 and 13 and two interacting QTLs on chromosome X. Thus, most of the CSSs that were reliably detected in CSS surveys were not found in the intercrosses.

As an alternative method to study the genetics of obesity resistance in intercrosses, we analyzed the mean FW for each intercross relative to that of B6. This test was based on the premise that segregating QTLs should lead to a significant difference in FW among intercross progeny versus the B6 host strain. Dominance effects of segregating QTLs make this test relatively conservative. Interestingly, the mean FWs in intercrosses derived from seven of the nine chromosomes on which QTLs were expected but not detected based on CSS survey results were significantly lower than that of B6 (Fig. 5). Presumably at least two QTLs with contrasting effects on FW are located on the nine chromosomes on which QTLs were expected but not detected in the single- or two-QTL intercross analysis. Thus, two independent lines of evidence, namely, the replicated CSS surveys and this analysis of mean FWs in intercrosses, demonstrate evidence for resistance QTLs on these chromosomes.

Issues related to sample size and the corresponding implications for the number of informative recombinant chromosomes may have led to the limited power to detect QTLs in the CSS intercrosses versus CSS surveys. In general, CSS surveys have considerably greater statistical power than crosses and as a result the same QTL can be detected in many fewer mice in CSS surveys than in intercrosses (Belknap 2003). We therefore analyzed approximately 90 males per CSS intercross, expecting that statistical power would be adequate to detect and then localize QTLs in CSS intercrosses. However, the proportion of intercross progeny that inherited recombinant chromosomes was relatively small and apparently sufficient for detecting many QTLs but insufficient for resolving their location precisely. This issue may explain why the

CSS surveys and the mean FW of intercross progeny detected more QTLs than the CSS intercrosses.

B6 and A/J were not intentionally selected to have particular phenotypes such as susceptibility to weight gain in response to a high-fat diet. As a result, these inbred strains are expected to have a combination of diet-induced obesity-promoting and diet-induced obesity-resistance QTLs, but only obesity-resistance QTLs were detected in the CSS surveys. In contrast, the CSS intercrosses, but not the traditional intercross analysis, provided evidence for these heterogeneous effects. The basis for these contrasting effects in CSSs versus crosses is unclear.

Two interesting examples of A/J-derived obesity-promoting QTLs were those for FW on chromosomes 1 and 11. First, although FW in CSS-1 was not significantly different from B6, an A/J-derived (*Obrq4*) QTL was detected in the CSS-1 intercross (cf. Vogel et al. 2009). *Obrq4* was similar in location to an obesity-promoting QTL previously reported with crosses derived from B6 and A/J fed a regular diet (Zhang and Gershenfeld 2003). Similarly, the dominant FW QTL on chromosome 11 (*Obrq6*) was also an A/J-derived obesity-promoting QTL. An A/J-derived resistance allele was expected because CSS-11 was reproducibly resistant in the CSS surveys. This finding suggests that additional A/J-derived resistance QTLs must conceal these obesity-promoting QTLs to produce resistance when an intact chromosome 1 or 11 was present in these CSSs.

Body weight and BMI were selected for these studies as surrogate traits to measure obesity because previous work by our lab demonstrated that fat pad weights correlate with both body weight and BMI in the parental strains (Buchner et al. 2008). A caveat is that the correlation between fat pad weight and these two traits may vary across strains, and body weight and BMI may not be predictors of obesity in all strains. Thus, in some strains, body weight may reflect muscle mass or organ weight rather than obesity.

Timing of QTL action

Mapping studies for traits at various points during the time course of the study demonstrated that some QTLs became apparent after short durations of diet exposure or at relatively young ages (e.g., MW or EWG), whereas others required longer diet exposures or increased age (e.g., FW or FWG) before showing an effect. For example, *Obrq6* appeared later and *Obrq5* appeared earlier in the study. Time-dependent or stage-specific QTLs affecting body weight, obesity, or size-related traits in the mouse have been previously described (Brockmann and Bevova 2002; Cheverud et al. 1996; Morris et al. 1999; Rocha et al. 2004). Perhaps QTLs act independently at different time points. Alternatively, QTLs may act sequentially such that the action of one QTL requires the previous action of another. QTLs that act early in the disease progression may provide targets to prevent weight gain prior to the development of obesity. Congenic strains derived from these CSSs could be used to isolate the effects of particular QTLs and thereby determine whether later-acting QTLs depend on the prior action of other QTLs. In this way the sequence and functional dependencies of gene action during pathogenesis can be studied.

Crosses versus congenic strains

Both segregating crosses and panels of congenic strains are appropriate ways to follow-up CSS surveys, and both have been used previously with strikingly different results. A backcross analysis with the 129-Chr19^{MOLF} CSS (Matin et al. 1999) provided evidence for a single-susceptibility QTL for spontaneous testicular germ cell tumors (TGCTs) on the central segment of chromosome 19. By contrast, a panel of 13 single- and double-congenic strains derived from the same CSS suggested at least five QTLs with enhancer or suppressive effects and that act additively or epistatically, depending on genetic background (Youngren et al. 2003). Similarly, we made and characterized panels of congenic strains for three CSSs and in every case found multiple QTLs for most traits (Buchner et al. 2008; Shao et al. 2008). Although larger intercross populations may increase the power to detect QTLs in CSS intercrosses, the congenic strain results suggest that they are more powerful than modest intercross populations ($n \cong 100$) for QTL detection. A further advantage of congenic strains is their immediate utility as resources for further genetic and functional studies.

Comparison of B6 × A/J intercross versus CSS intercrosses

Comparison of results using traditional F2 intercross progeny versus CSS intercross progeny showed that the two approaches provide complementary results. Although the two analyses produced similar results for chromosomes 6 and 10 (with significant p values obtained only with the CSS intercrosses), the B6 × A/J intercross detected "new" IW QTLs on chromosomes 13 and 17. Furthermore, perhaps the most interesting result of the traditional analysis was the detection of an additive effect between QTLs on chromosomes 6 and 10. A common concern of the CSS methodology is that CSSs do not detect interacting or additive QTLs, but detection of additive effects involving QTLs discovered using the CSS intercrosses demonstrates that the CSS analyses can detect QTLs on individual chromosomes that are involved in additive or interactive effects. Furthermore, the large effects conferred by

individual QTLs detected in the CSSs provide evidence that CSSs can be used to detect epistatic QTLs (Shao et al. 2008). These results contribute to the increasing focus on epistasis (Ankra-Badu et al. 2009) and on gene-diet interactions (Gordon et al. 2008).

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References

Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC et al (1996) The heritability of body mass index among an international sample of monozygotic twins reared apart. Int J Obes Relat Metab Disord 20:501–506

Altshuler D, Daly MJ, Lander ES (2008) Genetic mapping in human disease. Science 322:881–888

Ankra-Badu GA, Pomp D, Shriner D, Allison DB, Yi N (2009) Genetic influences on growth and body composition in mice: multilocus interactions. Int J Obes 33:89–95

Belknap JK (2003) Chromosome substitution strains: some quantitative considerations for genome scans and fine mapping. Mamm Genome 14:723–732

Black BL, Croom J, Eisen EJ, Petro AE, Edwards CL et al (1998) Differential effects of fat and sucrose on body composition in A/J and C57BL/6 mice. Metabolism 47:1354–1359

Brockmann GA, Bevova MR (2002) Using mouse models to dissect the genetics of obesity. Trends Genet 18:367–376

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Buchner DA, Burrage LC, Hill AE, Yazbek SN, O'Brien WE et al (2008) Resistance to diet-induced obesity in mice with a single substituted chromosome. Physiol Genomics 35:116–122

Calzolari E, Patracchini P, Palazzi P, Aiello V, Ferlini A et al (1993) Characterization of a deleted Y chromosome in a male with Turner stigmata. Clin Genet 43:16–22

Castro A, Zambrano N, Kaune H, Madariaga M, Lopez P et al (2004) YqTER deletion causes arrest of spermatogenesis in early puberty. J Pediatr Endocrinol Metab 17:1675–1678

Cheverud JM, Routman EJ, Duarte FA, van Swinderen B, Cothran K et al (1996) Quantitative trait loci for murine growth. Genetics 142:1305–1319

Collins S, Martin TL, Surwit RS, Robidoux J (2004) Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol Behav 81:243–248

De Rosa M, De Brasi D, Zarrilli S, Paesano L, Pivonello R et al (1997) Short stature and azoospermia in a patient with Y chromosome long arm deletion. J Endocrinol Invest 20:623–628

Farber CR, Aten JE, Farber EA, de Vera V, Gularte R et al (2009) Genetic dissection of a major mouse obesity QTL (*Carfhg2*): integration of gene expression and causality modeling. Physiol Genomics 37:294–302

Gordon RR, Hunter KW, Sorensen P, Pomp D (2008) Genotype × diet interactions in mice predisposed to mammary cancer. I. Body weight and fat. Mamm Genome 19:163–178

Hinney A, Nguyen TT, Scherag A, Friedel S, Brönner G et al (2007) Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. PLoS One 2:e1361

Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q et al (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131–141

Ihaka R, Gentleman R (1996) A language for data analysis and graphics. J Comput Graph Stat 5:299–314

Kelly T, Yang W, Chen CS, Reynolds K, He J (2008) Global burden of obesity in 2005 and projections to 2030. Int J Obes 32:1431–1437

Koza RA, Nikonova L, Hogan J, Rim JS, Mendoza T et al (2006) Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. PLoS Genet 2:e81

Krude H, Biebermann H, Luck W, Horn R, Brabant G et al (1998) Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat Genet 19:155–157

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Lindgren CM, Heid IM, Randall JC, Lamina G, Steinthorsdottir V et al (2009) Genome-wide association scan meta-analysis identifies three loci influencing adiposity and fat distribution. PLoS Genet 5:e10000508

Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH et al (2008) Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet 40:768–775

Maes HH, Neale MC, Eaves LJ (1997) Genetic and environmental factors in relative body weight and human adiposity. Behav Genet 27:325–351

Malek RL, Wang HY, Kwitek AE, Greene AS, Bhagabati N (2006) Physiogenomic resources for rat models of heart, lung and blood disorders. Nat Genet 38:234–239

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA et al (2009) Finding the missing heritability of complex diseases. Nature 461:747–753

Matin A, Collin GB, Asada Y, Varnum D, Nadeau JH (1999) Susceptibility to testicular germ-cell tumours in a 129.MOLF-Chr 19 chromosome substitution strain. Nat Genet 23(2):237–240

Mills E, Kuhn CM, Feinglos MN, Surwit R (1993) Hypertension in C57BL/6J mouse model of non-insulin-dependent diabetes mellitus. Am J Physiol 264(1 Pt 2):R73–R78

Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H et al (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903–908

Morgan TM, Krumholz HM, Lifton RP, Spertus JA (2007) Nonvalidation of reported genetic risk factors for acute coronary syndrome in a large-scale replication study. JAMA 297:1551–1561

Morris KH, Ishikawa A, Keightley PD (1999) Quantitative trait loci for growth traits in C57BL/6J × DBA/2J mice. Mamm Genome 10:225–228

Mutch DM, Clement K (2006) Unraveling the genetics of human obesity. PLoS Genet 2:e188

Nadeau JH, Singer JB, Matin A, Lander ES (2000) Analysing complex genetic traits with chromosome substitution strains. Nat Genet 24:221–225

National Institutes of Health (1998) Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: the evidence report. National Institutes of Health. Obesity Res 6(2):51S-209S

Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ et al (2006) Prevalence of overweight and obesity in the United States, 1999–2004. JAMA 295:1549–1555

Rebuffe-Scrive M, Surwit R, Feinglos M, Kuhn C, Rodin J (1993) Regional fat distribution and metabolism in a new mouse model (C57BL/6J) of non-insulin-dependent diabetes mellitus. Metabolism 42:1405–1409

Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M (2008) Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 371:569–578

Rocha JL, Eisen EJ, Van Vleck LD, Pomp D (2004) A large-sample QTL study in mice: I. Growth. Mamm Genome 15:83–99

Schelbert B (2009) Comorbidities of obesity. Prim Care 36:271–285

Shao H, Burrage LC, Sinasac DS, Hill AE, Ernest SR et al (2008) Genetic architecture of complex traits: large phenotypic effects and pervasive epistasis. Proc Natl Acad Sci USA 105:19910–19914

Singer JB, Hill AE, Burrage LC, Olszens KR, Song J et al (2004) Genetic dissection of complex traits with chromosome substitution strains of mice. Science 304:445–448

Stylianou IM, Tsaih SW, DiPetrillo K, Ishimori N, Li R et al (2006) Complex genetic architecture revealed by analysis of high-density lipoprotein cholesterol in chromosome substitution strains and F2 crosses. Genetics 174:999–1007

Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN (1988) Diet-induced type II diabetes in C57BL/6J mice. Diabetes 37:1163–1167

Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE et al (1995) Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism 44:645–651

Vaisse C, Clement K, Guy-Grand B, Froguel P (1998) A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat Genet 20:113–114

Velissariou V, Sismani C, Christopoulou S, Kaminopetros P, Hatzaki A et al (2007) Loss of the Y chromosome PAR2 region and additional rearrangements in two familial cases of satellited Y chromosomes: cytogenetic and molecular analysis. Eur J Med Genet 50:291–300

Vogel H, Nestler M, Ruschendorf F, Block M-D, Tischer S et al (2009) Characterization of Nob3, a major quantitative locus for obesity and hyperglycemia on mouse chromosome 1. Physiol Genomics 38:226–232

Walley AJ, Asher JE, Froguel P (2009) The genetic contribution to non-syndromic human obesity. Nat Rev Genet 10:431–441

Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK (2008) Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. Obesity 16:2323–2330

Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM et al (2009) Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41:25–34

Wuschke S, Dahm S, Schmidt C, Joost H-G, Al-Hasani H (2007) A meta-analysis of quantitative trait loci associated with body weight and adiposity in mice. Int J Obesity 31:829–841

Yan LL, Daviglus ML, Liu K, Stamler J, Wang R et al (2006) Midlife body mass index and hospitalization and mortality in older age. JAMA 295:190–198

Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG et al (1998) A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat Genet 20:111–112

Youngren KK, Nadeau JH, Matin A (2003) Testicular cancer susceptibility in the 129.MOLF-Chr19 mouse strain: additive effects, gene interactions and epigenetic modifications. Hum Mol Genet 12:389–398

Zhang S, Gershenfeld HK (2003) Genetic contributions to body weight in mice: relationship of exploratory behavior to weight. Obes Res 11:828–838

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L et al (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432