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Genetic loci affecting body weight and fatness in a C57BL/6J × PWK/PhJ mouse intercross

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Abstract

To determine the genetic variation that contributes to body composition in the mouse, we interbred a wild-derived strain (PWK/PhJ; PWK) with a common laboratory strain (C57BL/6J; B6). The parental, F_1, and F_2 mice were phenotyped at 18 weeks old for body weight and composition using dual-energy X-ray absorptiometry (DEXA). A total of 479 (244 male and 235 female) F_2 mice were genotyped for 117 polymorphic markers spanning the autosomes. Twenty-eight suggestive or significant linkages for four traits (body weight, adjusted lean and fat weight, and percent fat) were detected. Of these, three QTLs were novel: one on the proximal portion of Chr 5 for body weight (*Bwq8*; LOD = 4.7), one on Chr 3 for lean weight (*Bwtq13*; LOD = 3.6), and one on Chr 11 for percent fat (*Adip19*; LOD = 5.8). The remaining QTLs overlapped previously identified linkages, e.g., *Adip5* on Chr 9. One QTL was sex-specific (present in males only) and seven were sex-biased (more prominent in one sex than the other). Most alleles that increased body weight were contributed by the B6 strain, and most alleles that increased percent fat were contributed by the PWK strain. Eight pairs of interacting loci were identified, none of which exactly overlapped the main-effect QTLs. Many of the QTLs found in the B6 × PWK cross map to the location of previously reported linkages, suggesting that some QTLs are common to many strains (consensus QTLs), but three new QTLs appear to be particular to the PWK strain. The location and type of QTLs detected in this new cross will assist in future efforts to identify the genetic variation that determines the ratio of lean to fat weight as well as body size in mice.

Introduction

During domestication, laboratory mice were selected for early, rapid growth and high fertility (Silver 1995) and this has biased and reduced their gene pool (Guenet and Bonhomme 2003). However, this bias can be side-stepped and new genetic diversity infused by pairing traditional strains with recently “wild-caught” inbred strains (Ishikawa et al. 2000). Some wild-caught strains have limited usefulness because there are few genetic resources available to characterize them, but a balance between benefits and drawbacks is offered by the PWK/PhJ (PWK) strain: It is a relatively new, wild-derived strain, but it is also well-described genetically (Bogue and Grubb 2004; Churchill et al. 2004; Gregorova and Forejt 2000; Jansa et al. 2005; Petkov et al. 2004).

Although early work on obesity in mice tended to concentrate on extreme phenotypes (Danforth 1927) and single-gene mutations (Bultman et al. 1992; Naggert et al. 1995; Noben-Trauth et al. 1996; Tartaglia et al. 1995; Zhang et al. 1994), more recent work has concentrated on what might be considered normal variation in fatness and its associated genetic variation (Reed et al. 2007; Svenson et al. 2007; Wuschke et al. 2006). The measurement of normal variation in body fat is important because the outcome of linkage studies can change depending on the

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exact method used to express the trait (Lang et al. 2005). In addition to the types of obesity measures used, the strength of sex-by-genotype (Farber and Medrano 2007) and gene-gene interactions (Brockmann et al. 2000) influence the trait. These types of interactions may be as important as additive genetic effects in their contribution to the heritability of body composition. As investigators move toward gene identification for obesity in mice, these issues have taken on greater practical meaning because it is difficult to isolate and identify genes with small additive effects if these other influences are not well understood. Therefore, in this study we paired PWK mice with the B6 strain to exploit the genetic diversity and resources of this combination, and we also investigated linkage outcomes in male versus female mice and the influence of epistatic interactions. We used two methods of assessing fatness: fat weight divided by body weight (percent fat) and fat weight adjusted for body weight by regression analysis. The value of this work is twofold: we can compare the identified QTLs with other strain pairings to assist in positional identification strategies like multiple cross and haplotype mapping (Hitzemann et al. 2002; Wade et al. 2002), and we can pursue QTLs that may be unique to the PWK strain.

Methods

Mice

Ten male and ten female mice from the C57BL/6J strain (B6; stock No. 000664) and PWK/PhJ strain (PWK; stock No. 003715) were purchased from The Jackson Laboratory (Bar Harbor, ME) and the F_1 and F_2 hybrids were bred at the Monell Chemical Senses Center. F_2 pups were weaned at 21–23 days of age and reared in same-sex groups. A total of 479 F_2 mice (235 ♀ and 244 ♂) were bred from two types of reciprocal crosses: 236 and 243 F_2 mice were obtained from (B6 ♀ × PWK♂) F_1 ♀ × (B6 ♀ × PWK ♂) F_1 ♂ and (PWK ♀ × B6 ♂) F_1 ♀ × (B6♀ × PWK ♂) F_1 ♂, respectively. The reciprocal cross design was unbalanced because male F_1 hybrids with an X chromosome from their PWK mothers are sterile (Storchova et al. 2004). The mice were housed in a temperature-controlled vivarium at 23°C on a 12:12-h light:dark cycle and had free access to water and pelleted AIN-76A diet (12% of energy as fat; Dyets Inc., Bethlehem, PA; catalogue No. 100000). Prior to the measurement of body weight and adiposity, mice were tested to determine their preference for noncaloric taste solutions, and the results of these experiments will be reported elsewhere. All mice (B6, PWK, F_1, and F_2) were treated similarly before they were measured for body composition. Protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Monell Chemical Senses Center.

Body composition measurements

All mice were euthanized by CO_2 asphyxiation when they were 18 weeks old and weighed to nearest 0.1 g on an electronic balance. Measurements of body fatness were made by dual-energy X-ray absorptiometry (DEXA) using a Lunar PixiMus II densitometer (GE, software version 2.00; Lunar Corp., Madison, WI). This was calibrated daily according to the manufacturer's instructions using a quality control phantom (Phantom values: percentage fat = 10.0%). Mice were placed on a positioning tray ventral side down with the legs extended away from the body. Because large mice were longer than the image (80 mm × 65 mm), the head of each mouse was excluded as a region of interest. Using these procedures, the precision of measurement of fat weight has a coefficient of variation of less than 2%. Prior work in our laboratory has validated the DEXA method using a wide range of mouse strains (including B6 and PWK) and suggests that differences between this measure and other measures of body composition are minor. Previous investigators have used this method successfully to conduct genetic analyses of body composition in mice (Jerez-Timaure et al. 2005; Masinde et al. 2002c; Srivastava et al. 2006; Vitarius et al. 2006).

Phenotype analysis

The four phenotypes used in the linkage analysis were body weight, lean weight, fat weight, and percent fat (fat weight/body weight × 100). Strain and reciprocal cross differences were evaluated using a two-way ANOVA with group and sex as factors. When significant main effects of strain or sex were found, group means were evaluated by LSD post hoc tests. Differences in the homogeneity of variance between males and females were assessed with Levene's test. Correlations among phenotypes in the F_2 offspring were computed and tested to determine whether the correlation coefficient significantly differed (a) from zero and (b) between males and females. We used a criterion of $p < 0.01$ to control for the effects of multiple testing for the correlation analysis. These statistical analyses were conducted with Statistica 6.1 (StatSoft, Tulsa, OK).

DNA extraction and genotyping

Genomic DNA was extracted and purified from mouse tails by a sodium hydroxide method (Truett et al. 2000). A genome scan was conducted in two steps with the end result being that the average distance between markers was approximately 10 cM, with no gap greater than 30 cM (Table 1). First, simple sequence repeat markers known to be polymorphic between the parental strains were selected to evenly cover all 19 autosomes (Witmer et al. 2003). These fluorescently labeled microsatellite primers were amplified by PCR and the PCR products were scanned by an ABI 3100 capillary sequencer (Applied Biosystems, Forest City, CA). This genotyping was conducted by CIDR (Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD). DNA purchased from The Jackson Laboratory as well as parental DNA provided by our laboratory was included as control samples in the genotyping analysis. As a second quality control, blind duplicate DNA samples were genotyped. After the typing was completed, the code was broken and duplicate samples were matched and the data compared, with no disagreement among duplicates. Second, when gaps between the simple sequence length markers were greater than 30 cM, we genotyped single nucleotide polymorphisms, using fluorescently labeled primers and probes designed to discriminate between alleles, with an ABI Prism 7000 Real Time PCR system (ABI Assay-by-Design, Applied Biosystems, Foster City, CA). Genotypes were checked by determining whether they were compatible with the pre-existing haplotype; suspicious genotypes such as those that created double recombinants were reassayed.

Linkage analysis

Genome-wide scans of the F_2 mice involving the four traits were conducted using markers from all 19 autosomes. The genetic map, genotyping errors, and linkage between individual traits and marker genotypes were assessed with algorithms implemented by the R/qtl 1.04 - 53 package of R (Broman et al. 2003). All traits were analyzed with sex as an additive and interacting covariate; for fat and lean weight, body weight was also used as a covariate. For main effects, thresholds for suggestive and significant linkage followed established guidelines (Lander and Kruglyak 1995) and were based on values obtained from 1000 permutations of the observed data. Characterization of the most likely mode of inheritance of each QTL according to free, dominant, recessive, and additive genetic models was determined using MapMaker/QTL 1.1 (Lander et al. 1987). Each QTL's confidence interval was considered to be the genetic distance between 1.5-LOD drops from its peak score. To identify sex-specific loci, we used a method that allows the male and female genotype effects to be in opposite directions, e.g., one genotype may increase the trait value in males but reduce the trait value in females (Solberg et al. 2004). The analysis method was similar to that described earlier (Farber et al. 2006), with some modification to the thresholds for suggestive (sex-biased) and significant (sex-specific) linkage. Linkage results were defined as sex-biased when the LOD scores differed by 0.9 unit and sex-specific when the LOD scores differed by 3.1 units. We

chose the suggestive criterion because it was very similar to the one used in other studies, and we chose the second and more stringent criterion because permutation testing suggested it corresponded to $p < 0.001$. Two-way interactions (epistasis) were estimated with a two-QTL scan. Statistical significance for these gene-gene interaction tests were based on $p < 0.05$, using 1000 permutations of the observed data. Significant interactions found in the two-QTL scan were confirmed with two-way ANOVAs.

Exploratory candidate gene assessment

The implication of linkage peaks is that one or more genes that fall underneath the peak are allelic and cause the individual differences in phenotype. We assessed the most likely genes that would account for peaks using software tailored for this purpose. We focused on two regions, one on chromosome 2 and one on chromosome 11, both of which were linked to percent fat. We chose the QTL on chromosome 2 because it is the best studied of all obesity QTLs, and we chose the QTL on chromosome 11 because it was novel. Therefore, these two QTLs are at the extremes in terms of how well scrutinized the genes that fall under the peak are likely to be. We used a 1-LOD drop to capture the confidence interval and mapped the endpoints to Build 36 of the mouse genome to get physical position (Mb). We used a shorter confidence interval to search for candidate genes than to report the linkage. This was to reduce the number of genes captured and considered without substantially increasing the possibility that the gene would be missed. For the automated search, we used the algorithms implemented in Positional Medline Database, which filters candidate genes by location, function, gene expression, and function networks (see Web References section). We used the words “adiposity” and “percent fat” as key search terms.

Results

Obesity-related traits

B6 mice were heavier than PWK mice [Table 2; $F(3,584) = 24.6$, $p < 0.001$], and males were heavier than females [$F(1,584) = 54.4$, $p < 0.001$], with the sex difference being larger in the B6 than PWK mice [$F(3,584) = 5.0$, $p = 0.002$]. A similar pattern was observed for lean weight and fat weight (all main effects and interactions, $p < 0.001$). Percent fat differed significantly by strain [$F(3,584) = 24.7$, $p < 0.001$] and sex [$F(1,584) = 5.6$, $p < 0.05$], but the strain × sex interaction was not significant [$F(1,584) = 1.9$, $p = 0.14$] (Table 2). Tests of homogeneity of variance showed that relative to male mice, female mice were significantly more variable in body weight [$F(1,481) = 4.0$, $p < 0.01$], lean weight [$F(1,481) = 9.4$, $p < 0.01$)], and fat weight [$F(1,481) = 7.2$, $p < 0.01$], but equally variable in percent fat [$F(1, 481) = 1.03$, $p = 0.79$]. There were no differences in the mean trait values of the F_2 offspring by reciprocal cross type ($p > 0.05$).

Correlations among traits

Measures of body composition were moderately or strongly correlated in the F_2 population (all p values < 0.01) and the strength of each correlation was influenced by sex (Table 3). The most striking result was that for female mice, body weight was only moderately correlated with percent fat, whereas in male mice, body weight was almost perfectly correlated with percent fat. Lean weight and fat mass were correlated in both sexes, but to a lesser degree in female mice.

Linkage

In total, 28 main-effect QTLs for four traits were identified on chromosomes 1, 2, 3, 4, 6, 7, 9, 10, 11, 13, and 16 (Fig. 1 and Table 4). For body weight and lean weight, 50% of the linkages were statistically significant. For body weight, lean weight, and fat weight, the “plus” or “trait-

increasing" alleles were typically contributed by the B6 strain (i.e., congruent QTLs, recapitulating the differences between the parental strains). However, for the two significant QTLs for percent fat, the PWK strain contributed the trait-increasing allele (i.e., antagonistic QTLs).

We named the new QTLs using rules developed by the International Committee on Standardized Genetic Nomenclature for Mice (see Web References section). They were designated as *Bwq8*, a body weight QTL in the proximal region of Chr 5; *Bwtq13*, a lean weight QTL identified on Chr 3; and *Adip19*, a linkage for percent fat on Chr 11. *Bwq* denotes a QTL that affects body weight, including lean and fat weight. *Bwtq* was adopted from another study (Stylianou et al. 2006) and is used to indicate a QTL for lean weight. *Adip* has usually been assigned to QTLs that affect fat weight when adjustments for body size are made (Cheverud et al. 2001). In the current study, body weight was used as a covariate in the analysis of fat weight, and therefore the new QTL on Chr 11 was called *Adip19.* The remaining QTLs were detected in previous studies and, therefore, those symbols were adopted here (Table 4). In cases where multiple symbols were previously used that were equally appropriate, one was adopted arbitrarily.

Sex-specific loci

Twenty-two body weight QTLs and 19 adiposity QTLs were sex-biased. They were clustered on Chrs 2, 3, 4, 5, and 11 (Table 4). There were more male- than female-dependent and female-biased QTLs. *Adip19* was sex-dependent: It was detected only in male mice, with the PWK strain contributing the trait-increasing, additive allele (Fig. 2).

Epistasis

A pairwise scan was conducted to identify all marker-marker interactions (117 markers; 6786 possible interactions), applying the same covariates used as those for screening the single-effect QTLs. A 5% threshold level was set by permutation testing and confirmed by two-way ANOVA (associated nominal $p < 0.0000074$). Eight significant epistatic interactions were identified based on the pairwise scan (Fig. 3). A comparison of chromosome position of these epistatic loci with those of main-effect QTLs revealed that no epistatic QTLs overlapped the main-effect QTLs. Potential hubs were also identified: the QTL near *D17Mit143.2* interacted with a locus on Chr 11 (63 cM) influencing percent fat, and it also interacted with another locus on Chr 13 (9 cM) affecting lean weight (Fig. 3). Likewise, a QTL on Chr 6 (74 cM) interacted with two other QTLs, one on Chr 3 (79 cM) and the other on Chr 14 (48 cM).

Candidate genes

We conducted exploratory candidate gene assessment for the QTLs on chromosomes 2 and 11. For the chromosome 2 QTL, we searched between 142 and 175 Mb and identified 107 candidate genes. For the chromosome 11 QTL, we searched between 80 and 99 Mb and identified 58 genes. Genes were ranked in terms of likelihood based on the amount of evidence extracted during database searches; for the chromosome 2 QTL, the three highest-ranking genes were adenosine deaminase, cystatin C, and lipo-polysaccharide binding protein, and for the chromosome 11 QTL, the three most likely candidates were transcription factor 2, acetyl-coenzyme A carboxylase alpha, and gastric inhibitory polypeptide. These genes, like the others on the list, have well-known roles in obesity and body weight regulation and could be responsible for the QTL effects described. Details relevant to these genes and the efforts on the part of other investigators to map genes in this region of chromosome 2 are discussed below.

Discussion

Using a classical F_2 intercross design, we interbred mice from the PWK and B6 strains, conducted a genome scan using microsatellite and SNP markers, and assessed the presence of primary QTLs, sex-by-genotype interactions, and epistatic interactions. Body weights of the PWK strain were considerably less than those of the B6 strain but the two strains had similar percentages of carcass fat; if anything, the PWK strain tended be fatter. Sex differences in body composition were not remarkable. Like most mice strains (Reed et al. 2007), the males were fatter than the females. The F_1 B6 × PWK mice were intermediate between the parental strains in body weight, and the F_2 on average were heavier than the B6 strain. This pattern is typical for a complex trait like body weight, which reflects the action of multiple genes and hybrid vigor.

Several QTLs detected here probably replicate those described earlier. However, three new linkages were identified and named (*Bwq8*, *Bwtq13*, and *Adip19*). Sex-by-genotype interactions were of two types: There was one linkage that was detected in only one sex and many linkages detected in both sexes, but with a stronger effect in one than the other (mostly male-biased). Epistatic interactions between loci accounted for up to a third of the trait variance, which is consistent with other reports (Brockmann et al. 2000; Yi et al. 2006).

QTL comparison with earlier studies

A total of 28 primary QTLs were detected in this study, of which nine were significant and the remainder met the criterion for suggestive linkage. One way to determine the statistical reliability of QTLs is to check whether they replicate in other studies, so we compared these findings with earlier experiments. There are no published QTL studies (to the best of our knowledge) that paired the PWK with the B6 strain, but two studies have paired the PWK strain with different inbred partners, either KK (Komatsu et al. 2002) or NOD (Melanitou et al. 1998). The only phenotype in common with the current study was body weight (and then only for the study with the PWK × KK intercross). Although the inbred partner strain differed (KK vs. B6), two of the QTLs were replicated: one QTL on Chr 2 near the *agouti* locus, and the other on Chr 4, with the same marker (*D4Mit17*) associated with the highest LOD score in the current and earlier study. The QTL on Chr 2 is the most replicated body weight QTL (Cheverud et al. 1996; Chiu et al. 2007; Ishikawa et al. 2005; Vitarius et al. 2006). We use the symbol *Bwfq2* to label it here, but it has many aliases. The presence of the same QTL on Chr 2 detected in crosses involving many divergent strains could have several explanations: (1) It is ancient and was present in mouse strains prior to their dispersal to different continents. (2) It arose several times spontaneously due to a highly mutable gene. (3) It contains polymorphisms that improve survival. (4) It contains a tight cluster of genes that are allelic and influence body weight, i.e., a supergene. For the QTL on Chr 4 for body weight, the results suggest that the PWK strain has a body weight allele that differs from the alleles carried by both the B6 and KK strains. Because the linkage peak locations were nearly identical between the studies, this result suggests that these QTLs are due to the same underlying genotype.

Novel QTLs

In contrast to QTLs that are frequently identified in mouse genome scans, several of the QTLs were apparently novel. Our criteria for novelty were that no previously reported QTLs with the same or very similar phenotype had an overlapping confidence interval, and the behaviors of the QTLs (effect direction and mode of action) were the same or very similar. Using these criteria, we considered *Bwq8*, on the proximal portion of Chr 5, to be novel because although there are many linkages to body weight on this chromosome, none map to this confidence interval (Bennett et al. 2005; Brockmann et al. 1998, 2000; Ewart-Toland et al. 1999; Le Roy et al. 1999; Rance et al. 2005; Rocha et al. 2004b). Likewise, two other QTLs were novel

(*Bwtq13* and *Adip19*), although in the case of *Adip19*, there were a few points to consider before we concluded that this was a novel QTL. There were three previous reports of QTLs near *Adip19* but all previous QTLs had features that made it unlikely that they captured the same underlying genetic variation (Carlborg et al. 2005; Collin et al. 2005; Farber and Medrano 2007). Determining whether a QTL replicates is subject to some uncertainty regarding its location, confidence interval, and its effects on a particular genetic background, and thus the term “novel QTL” is applied here only with these considerations in mind.

Pleiotropy

When QTLs for two or more traits map near the same location, the same genetic variation may contribute to both traits, and this is much more likely to occur when the trait values are highly correlated. This issue is salient for obesity QTL mapping because measures of body size and body composition are generally correlated. In the simple case, QTLs that increase body weight do so because they add to either lean weight, fat weight, or both, and therefore body weight QTLs should overlap with one or both of these traits (Li et al. 2006). However, this result was rarely observed here (or in most QTL studies of body composition). For instance, for the six QTLs for lean weight, only one mapped to the same marker location as a body weight QTL (*D4Mit17*). For fatness, the lack of agreement in QTL locations for body weight and fat weight QTL phenotypes was even more extreme. The most common finding was that body weight QTLs were found on the same chromosome as lean and fat weight QTLs but they mapped to different locations, i.e., Chrs 2, 3, 4, 5, and 11. Therefore, these QTLs are likely to arise from independent and non-overlapping genetic signals. The most parsimonious explanation for our findings is that body weight QTLs reflect an equal increase in fat and lean weight, whereas the fat and lean QTLs underlie variation in body composition and not body size. However, it is also possible that some effects of body size on body composition were obscured by using body size as a covariate in analyses.

Sex-specific effects

There are several ways to identify sex-specific QTLs. The most common methods are to analyze data separately by sex and to estimate sex effects using linear models. Analyzing data separately by sex typically halves the sample size of each analysis, which is undesirable. Using sex as an additive covariate eliminates this problem but has the limitation that sex-antagonistic effects go undetected (Solberg et al. 2004). One advance has been the use of sex as an additive and interactive covariate. Using this approach, we detected several sex-biased QTLs and *Adip19*, a sex-dependent QTL that increased percent fat in male mice but had no effect in female mice. We detected no sex-antagonistic QTLs (alleles have opposing effects for each sex). One puzzling observation was that there were three times as many male- than female-biased QTLs, a result seen in several other mouse crosses (Cheverud et al. 2001; Farber and Medrano 2007; Reed et al. 2006). The mechanisms underlying sexually dimorphic QTLs remain unclear, but there are many potential explanations such as sex-specific gene expression (Yang et al. 2006) and epistatic effects of the Y chromosome (Ishikawa et al. 2005). Linkage analysis of gene expression has demonstrated that thousands of genes show sex-biased expression (Wang et al. 2006). Regardless of the mechanism by which it happens, sex-by-genotype interactions play a major role in the determination of mouse body composition.

Epistatic interactions

Previous investigators have tested for epistatic effects on body weight and composition using several approaches. One way is to examine the interaction of primary QTLs for a single trait; a variation of this method is to examine the interaction of primary QTLs among related traits. Another method is to examine all marker-marker interactions regardless of whether primary QTLs are present, which is the approach we used here. Eight pairs of interacting loci for body

composition were identified: three for fat weight, three for lean weight, one for percent fat, and one for body weight. Previous studies of epistasis suggest that interactions among primary QTLs is the rule rather than the exception (Brockmann et al. 2000; Yi et al. 2004, 2006). However, at least in our case, the strongest epistatic interactions were not near any of the primary QTLs. This suggests that important determinants of body composition are missed when only the effects of interactions among primary QTL are measured.

Setting the statistical criteria to declare that a given epistatic interaction is significant is not clear, and several approaches have been suggested, e.g., nominal *p* values in regression or ANOVA analysis and permutation testing. Another method to test the reliability of epistatic interactions is whether they replicate in other studies. With this point in mind, we compared the current pairwise result to those of other studies but found no instances of an identical or similar interaction present for the same or related phenotype (Brockmann et al. 2000, 2004; Carlborg et al. 2005; Collin et al. 2005; Ishikawa and Namikawa 2004; Ishikawa et al. 2005; Ishimori et al. 2004; Li et al. 2006; Masinde et al. 2002a, b; Reifsnyder et al. 2000; Rocha et al. 2004a, b; Stylianou et al. 2006; Yi et al. 2004, 2005, 2006). The results of this comparison suggest that although certain locations are likely to be network "hubs," i.e., locations that interact with many other genes to determine adiposity (Stylianou et al. 2006), the details are highly dependent on the specific mouse cross studied, the statistical model employed, and threshold for detection selected. The degree to which gene-gene interactions are replicable is probably best tested in two separate studies intercrossing the same strains and measuring the same phenotype.

Candidate genes

We surveyed candidate genes in the region of QTLs for percent fat on Chrs 2 and 11, which were chosen to represent a consensus QTL and a novel QTL. For the novel QTL on Chr 11 (*Adip19*), we identified three likely gene candidates: transcription factor 2 (*Tcf2*), acetyl-coenzyme A carboxylase alpha (*Acaca*), and gastric inhibitory polypeptide (*Gip*). If we assume that *Adip19* is detected only when the PWK and B6 strains are paired, because B6 is often paired with strains but this region has never before been implicated, the search for alleles unique to the PWK strain might be especially useful to triage the variation in this region. The other region in which we searched for candidate genes was on mouse Chr 2, near *D2Mit285*, which maps between the midpoint of the chromosome and its telomere. Many studies have identified this distal region of Chr 2 as important in body composition phenotypes, and it is the focus of several positional cloning efforts (Chiu et al. 2007; Diament et al. 2004; Farber et al. 2006; Jerez-Timaure et al. 2005). According to our candidate gene analysis, the three genes most likely to underlie the QTL on Chr 2 were adenosine deaminase, cystatin C, and lipo-polysaccharide binding protein. Other investigators, using congenic analysis, have identified other genes such as *Pcsk2* as potentially causal. These genes, like the others lower on the list, have well-known roles in obesity and body weight regulation and could be responsible for the QTL effects described. Comparisons of microcongenic or genetically engineered mouse strains will be needed to unequivocally identify the gene(s) (and alleles) in this region that account for this QTL.

We did not conduct a formal candidate gene analysis for all QTLs identified, but one point about the QTL on Chr 13 is worth mentioning. From the work of other investigators, we have learned that the B6 strain has a missing gene (*Nnt*), which is involved in mitochondrial ATP production. Its absence may reduce the ability of mitochondria (in brown adipocytes and other cell types) to create heat and thus partially account for dietary obesity (Freeman et al. 2006). *Nnt* is inside one QTL confidence interval reported here (for fat and lean weight), and so this linkage signal may be due to the missing gene in the segregating F_2 population. This point raises a general issue for the cross of recently wild-caught and laboratory mice: Genetically

distinct strains are often originally from different parts of the world (i.e., Europe, PWK and North America, B6) with different histories of inbreeding. Given that the PWK and B6 mouse strains are so different that they have started the process of speciation (some F_1 males are sterile), large genome variations like missing or relocated genes, large duplications, or rearrangements probably deserve as much consideration as do single nucleotide polymorphisms as the underlying causes of QTLs.

Future directions

A goal of this study and QTL analysis in general is to find the specific genetic variation that creates the linkage peaks and there are several ways to achieve this goal (Abiola et al. 2003). The B6 × PWK cross and the loci identified here have several features that will help support future attempts to identify specific loci. One feature is the existence of a series of chromosome substitution mice based on the B6 and PWD strains (the PWD strain is nearly identical to the PWK strain). These strains can provide a useful starting point for the development of interval-specific congenics. Another feature is the existence of BAC libraries for both strains, which will be useful in the creation of genetically engineered mice, for instance, for allele-swap studies to test candidate genes. With these tools in hand, newly identified QTLs such as *Adip19* may lead to the eventual identification of the causal genetic variation.

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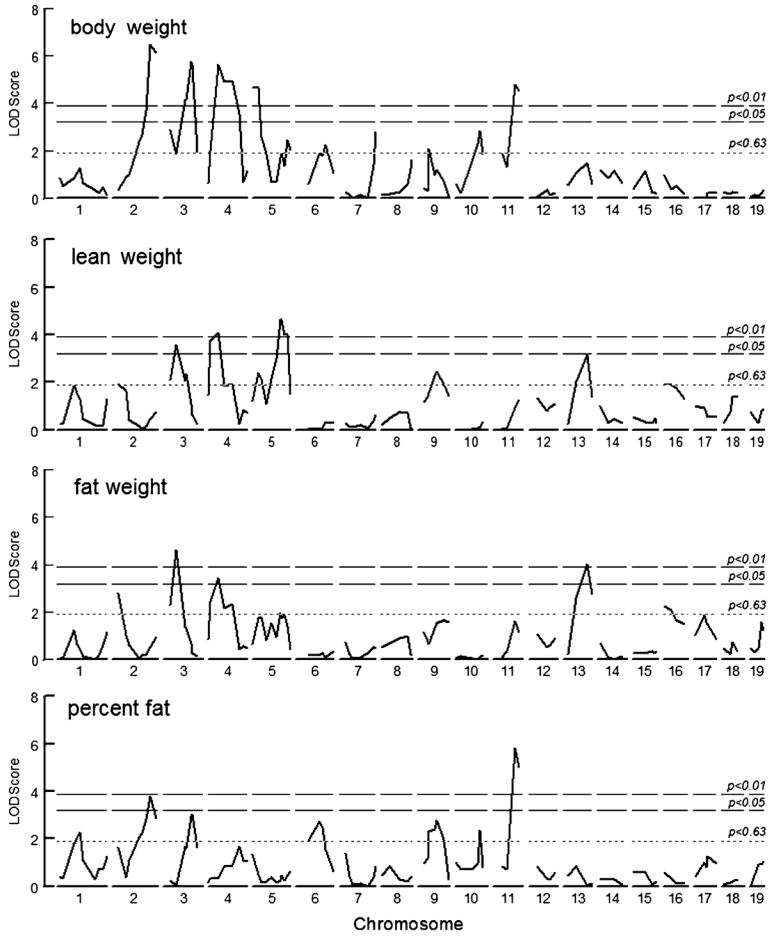

Fig. 1.

Genome scan results of an F_2 intercross between B6 and PWK inbred mouse strains. The interval maps show body weight, lean weight, fat weight, and percent fat (sex is an additive covariate for these analyses, and body weight was a covariate for lean and fat weight). The three horizontal lines represent LOD thresholds at genome-wide significant level ($p < 0.01$ and $p < 0.05$), and suggestive level ($p < 0.63$), respectively, as determined by 1000 permutation tests

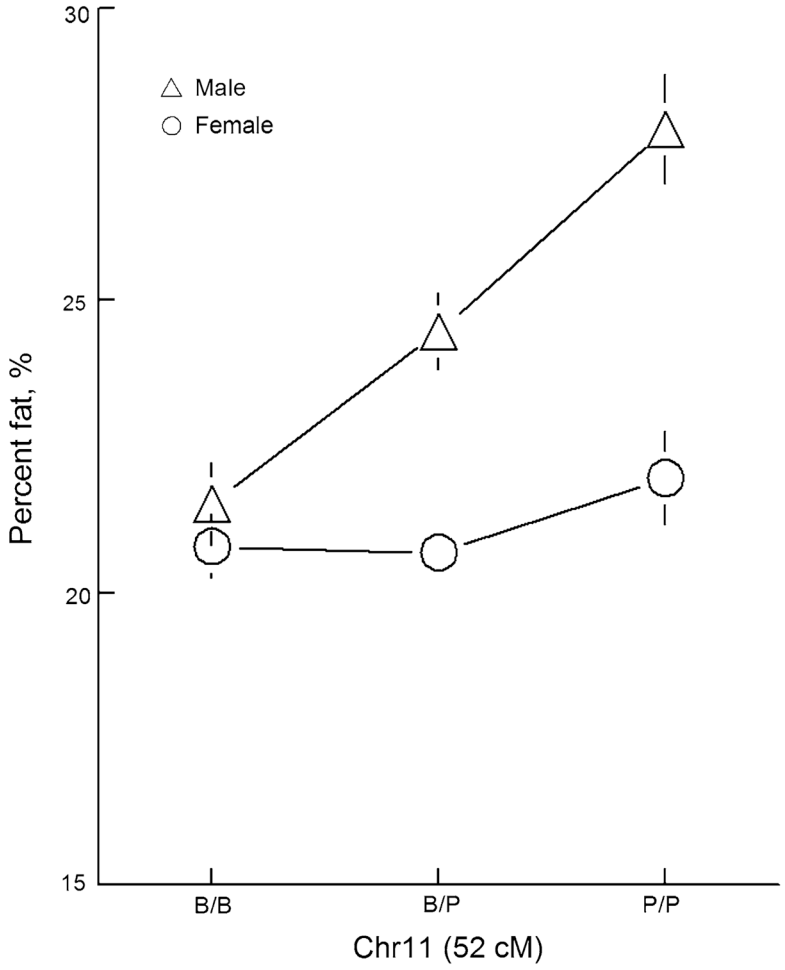

Fig. 2.

Effect plot for the interaction of a sex-specific QTL on chromosome 11. Genotypes B/B, B/P, and P/P represent B6 homozygous, B6/PWK heterozygous, and PWK homozygous, respectively

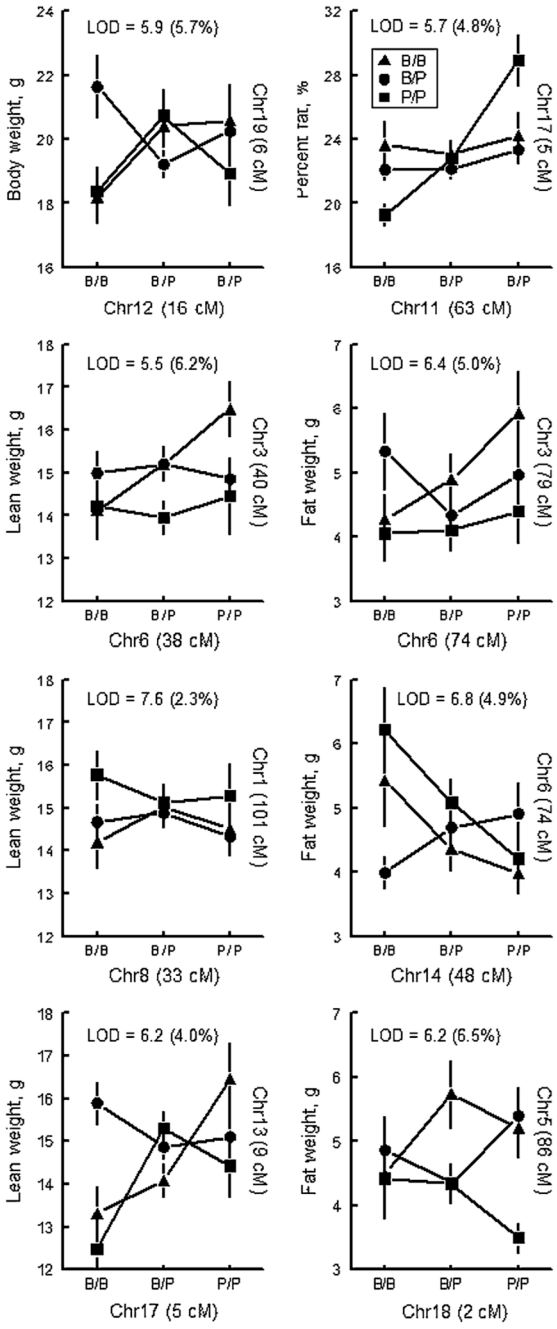

Fig. 3.

Plots of pairwise interactions. Chromosome number and peak position in cM are indicated for each QTL. Freestanding text = interaction LOD score and percentage of trait variance accounted for

Table 1

Markers used in autosomal genome scan

Markers	Position cM (Mb)	Markers	Position cM (Mb)	Markers	Position cM (Mb)	Markers	Position cM (Mb)
D1MIT64.1	5(12.83)	*D4MIT209.1*	79 (153.26)	*D8MIT120.1*	61 (121.23)	*D14MIT98.1*	3 (15.32)
D1MIT169.1	15 (24.02)	*rs13478074*	80 (154.70)			*D14MIT60.1*	15 (46.02)
D1MIT1000.1	33 (65.24)			*D9MIT126.1*	6 (22.91)	*D14MIT39.1*	30 (67.49)
D1MIT132.1	43 (77.03)	*D5MIT123.1*	3 (6.56)	*D9MIT2.1*	17 (37.14)	*D14MIT106.1*	48 (99.06)
D1MIT440.1	54 (90.62)	*D5MIT348.1*	8 (24.43)	*D9MIT129.1*	26 (43.64)		
D1MIT1001.1	67 (103.59)	*D5MIT352.1*	20 (35.93)	*D9MIT336.1*	35 (65.38)	*D15MIT13.1*	7 (3.41)
D1MIT102.1	73 (149.01)	*rs3713492*	NA (55.96)	*D9MIT123.1*	42 (73.33)	*D15MIT143.1*	21 (51.98)
D1MIT507.1	88 (166.88)	*D5MIT201.1*	42 (75.44)	*D9MIT347.1*	56 (103.12)	*D15MIT70.1*	48 (81.03)
D1MIT426.1	101 (182.25)	*rs13478400*	NA (96.08)	*D9MIT151.1*	72 (121.33)	*D15MIT242.1*	56 (90.22)
D1MIT155.1	112 (196.13)	*D5MIT314.1*	59 (109.92)			*D15MIT161.1*	69 (96.84)
		rs3719351	NA (114.25)	*D10MIT49.1*	2 (4.14)		
D2MIT293.1	11 (25.24)	*D5MIT95.1*	68 (125.12)	*D10MIT86.1*	17 (24.29)	*D16MIT107.1*	3 (5.62)
D2MIT242.1	29 (57.17)	*D5MIT98.1*	78 (138.45)	*D10MIT20.1*	35 (66.41)	*D16MIT60.1*	23 (32.62)
D2MIT327.1	40 (69.27)	*D5MIT143.1*	86 (151.27)	*D10MIT115.1*	38 (69.67)	*D16MIT169.1*	37 (49.75)
D2MIT100.1	48 (106.34)			*D10MIT95.1*	50 (91.93)	*D16MIT189.1*	55 (82.42)
D2MIT395.1	67 (119.22)	*D6MIT274.1*	21 (48.66)	*D10MIT96.1*	56 (98.99)		
D2MIT208.1	77 (134.97)	*D6MIT284.1*	38 (92.57)	*D10MIT233.1*	62 (113.79)	*D17MIT143.2*	5 (8.28)
D2MIT285.1	86 (152.55)	*D6MIT36.1*	46 (104.47)			*D17MIT51.1*	23 (42.97)
D2MIT113.1	103 (172.99)	*D6MIT198.1*	67 (116.44)	*D11MIT186.1*	17 (35.08)	*D17MIT180.1*	29 (50.89)
		D6MIT373.1	74 (147.01)	*D11MIT143.1*	32 (57.73)	*D17MIT20.1*	34 (56.91)
D3MIT178.1	14 (30.58)			*D11MIT285.1*	52 (89.74)	*D17MIt123*	56 (93.49)
D3MIT5.1	25 (50.93)	*D7MIT294.1*	8 (26.99)	*D11MIT126.1*	63 (103.76)		
D3MIT98.1	40 (86.27)	*D7MIT228.1*	18 (39.80)			*D18MIT64.1*	2 (6.11)
D3MIT311.1	45 (93.1)	*D7MIT83.1*	27 (51.67)	*D12MIT60.1*	16 (35.38)	*D18MIT12.1*	17 (36.01)
D3MIT57.1	55 (115.82)	*D7MIT350.1*	41 (83.46)	*D12MIT91.1*	29 (72.66)	*D18MIT194.1*	22 (43.79)
D3MIT256.1	66 (136.29)	*D7MIT98.1*	53 (114.71)	*D12MIT158.1*	38 (83.54)	*D18MIT208.1*	38 (61.05)
D3MIT147.1	79 (148.68)	*D7MIT109.1*	66 (136.35)	*D12MIT7.1*	50 (103.39)	*D18MIT48.1*	50 (77.01)
		D7MIT223.1	72 (143.88)				
D4MIT227.1	3 (9.93)			*D13MIT57.1*	9 (16.78)	*D19MIT68.1*	6 (3.65)
D4MIT196.1	12 (39.64)	*D8MIT155.1*	1 (4.98)	*D13MIT16.1*	10 (20.30)	*D19MIT96.1*	15 (21.91)
D4MIT17.1	32 (62.85)	*D8MIT190.1*	21 (37.46)	*D13MIT247.1*	32 (51.49)	*D19MIT88.1*	34 (37.32)
D4MIT9.1	45 (94.56)	*D8MIT178.1*	33 (73.97)	*D13MIT144.1*	48 (97.19)	*D19MIT17.1*	43 (45.60)
D4MIT308.1	57 (123.66)	*D8MIT211.1*	49 (105.61)	*D13MIT151.1*	71 (116.67)	*D19MIT103.1*	52 (53.82)
D4MIT170.1	67 (137.89)	*D8MIT88.1*	58 (117.72)				

Position = genetic position in centimorgans (cM) and physical position in megabases (Mb). cM locations are from the Mouse Genome Database, accessed February 2006. Physical locations are from NCBI Build 36.1 [February 2006, strain C57BL/6J, Ensembl (Hubbard et al. 2007); see Web References]

Table 2

Means ± standard errors of body composition of parental, F_1, and F_2 mice

	C57BL/6J	PWK/PhJ	F_1	F_2
Female				
Number of mice	14	16	32	235
Age (months)	4.7 ± 0.6	4.7 ± 0.3	4.9 ± 0.2	4.8 ± 0.4
Body weight (g)	19.0 ± 1.0^{c}	12.9 ± 1.0^{a}	16.3 ± 0.7^{b}	16.6 ± 0.3^{b}
Lean weight (g)	15.2 ± 0.6^{c}	10.3 ± 0.5^{a}	13.1 ± 0.4^{b}	12.6 ± 0.1^{b}
Fat weight (g)	3.4 ± 0.6^{a,b}	2.5 ± 0.6^{a}	2.6 ± 0.4^{a}	3.5 ± 0.2^{b}
Percent fat (%)	18.0 ± 1.5^{a,b}	19.6 ± 1.4^{b,c}	16.7 ± 1.0^{a}	21.0 ± 0.4^{c}
Male				
Number of mice	15	13	30	244
Age (months)	4.7 ± 0.9	4.7 ± 0.5	4.9 ± 0.7	4.8 ± 0.8
Body weight (g)	23.9 ± 1.0^{c}	15.3 ± 1.1^{a}	19.7 ± 0.7^{b}	23.1 ± 0.3^{c}
Lean weight (g)	19.2 ± 0.5^{d}	11.8 ± 0.6^{a}	15.8 ± 0.4^{b}	16.9 ± 0.1^{c}
Fat weight (g)	4.8 ± 0.6^{a,b}	3.3 ± 0.7^{a}	3.3 ± 0.4^{a}	6.0 ± 0.2^{b}
Percent fat (%)	19.8 ± 1.4^{a,b}	21.5 ± 1.5^{b,c}	17.1 ± 1.0^{a}	24.7 ± 0.4^{c}

$^{a–c}$Strains that do not share a common superscript differ by LSD post hoc tests

Table 3

Correlations in F_2 mice for body weight and body fatness by sex

Phenotype	Body weight	Lean weight	Fat weight	Percent fat
Body weight		*0.63*	*0.58*	*0.45*
Lean weight	**0.93**		*0.97*	*0.76*
Fat weight	**0.92**	**0.76**		*0.86*
Percent fat	**0.84**	**0.61**	**0.96**	

Male values are in the lower diagonal, in boldface; *Female* values are in the upper diagonal, in italics. All correlations differ significantly from zero, $p < 0.01$, and each female correlation differs from the corresponding male correlation, $p < 0.01$

Table 4

Summary of genome scan results and comparison with other QTLs

Chr	Phenotype	Marker[a]	Peak (CI) (cM)	LOD score[b]	Mode[c]	Plus allele	ΔLOD[d]	Sex effect[e]	QTL symbol and previous references[f]
1	Percent fat	*D1MIT440.1*	54 (43–66)	2.3*	recessive	B6	0.03		*Fatq1* (Moody et al. 1999)
2	Body weight	*D2MIT285.1*	86 (78–92)	6.5***	free	B6	1.91	M	*Pbwg11* (Ishikawa et al. 2005); *D2Mit22* (Cheverud et al. 1996); *Bw20* (Vitarius et al. 2006)
2	Fat weight	*D2MIT293.1*	11 (0–15)	2.8*	dominant#	B6	0.12		*Adip10* (Stylianou et al. 2006)
2	Percent fat	*D2MIT285.1*	86 (72–98)	3.8**	dominant	B6	0.39		*Gnf3* (Jerez-Timaure et al. 2005)
3	Body weight	*D3MIT57.1*	55 (45–67)	5.8***	free	B6	1.52	M	*Wt10Q2* (Moody et al. 1999)
3	Lean weight	*D3MIT5*	25 (17–36)	3.6**	dominant	B6	0.36		*Bwtq13* this report
3	Fat weight	*D3MIT5*	25 (14–32)	4.6***	dominant	B6	0.48		*Afw1* (Brockmann et al. 2000)
3	Percent fat	*D3MIT256.1*	66 (57–72)	3.0*	dominant	B6	2.62	M	*Afp(1)* (Brockmann et al. 1998)
4	Body weight	*D4MIT17.1*	32 (26–43)	5.6***	free	B6	1.65	M	*Bw6b* (Keightley et al. 1996)
4	Lean weight	*D4MIT17.1*	32 (13–39)	4.1***	additive	B6	0.79		*Epb4.1* (Moody et al. 1999) (body weight)
4	Fat weight	*D4MIT17.1*	32 (17–46)	3.5**	dominant	B6	0.31		*Adip11* (Stylianou et al. 2006)
5	Body weight	*D5MIT123.1*	3 (0–10)	4.7***	free	B6	0.70		*Bwq8* this report#
5	Lean weight	*rs3719351*	49 (43–55)	4.7***	free	B6	0.14		*Bw6d* (Keightley et al. 1996); *Bwob* (Ewart-Toland et al. 1999) (body weight)
5	Fat weight	*D5MIT314.1*	59 (42–71)	2.0*	additive	B6	0.77		*Afw(3)* (Brockmann et al. 1998)
6	Body weight	*D6MIT198.1*	67 (52–79)	2.2*	dominant	PWK	0.41		*Body weight QTL 3* (Anunciado et al. 2001); *Bwtq10* (Stylianou et al. 2006)
6	Percent fat	*D6MIT284.1*	38 (26–47)	2.8*	additive	PWK	0.32		*Pfat2* (Keightley et al. 1998)
7	Body weight	*D7MIT223.1*	72 (58–79)	2.8*	dominant	B6	1.46	M	*Pbwg3* (Ishikawa et al. 2005)
9	Body weight	*D9MIT129.1*	26 (15–41)	2.1*	free	PWK	0.96		*Bodw4* (Carlborg et al. 2005); *Bwnd4wk5* (Brockmann et al. 2004)
9	Lean weight	*D9MIT123.1*	42 (26–55)	2.5*	dominant	B6	1.24	F	*D9Mit164* (Brockmann et al. 2000) (body weight)
9	Percent fat	*D9MIT123.1*	42 (33–49)	2.8*	free	PWK	0.43		*Adip5* (McDaniel et al. 2006)
10	Body weight	*D10MIT96.1*	56 (32–65)	2.9*	recessive	B6	0.92		*D10Mit10* (Cheverud et al. 1996)
10	Percent fat	*D10MIT96.1*	56 (49–61)	2.3*	recessive	B6	0.61		*Adip15* (Stylianou et al. 2006)

Chr	Phenotype	Marker[a]	Peak (CI) (cM)	LOD score[b]	Mode[c]	Plus allele	ΔLOD[d]	Sex effect[e]	QTL symbol and previous references[f]
11	Body weight	*D11MIT285.1*	52 (44–67)	4.8***	free	PWK	2.35	M	*Bw4* (Brockmann et al. 2004)
11	Percent fat	*D11MIT285.1*	52 (47–57)	5.8***	free	PWK	3.16	M	*Adip19* this report[§]
13	Fat weight	*D13MIT144.1*	48 (37–59)	4.0**	dominant	B6	0.75		*Fatpad3* (Yi et al. 2006)
13	Lean weight	*D13MIT144.1*	48 (39–56)	3.2*	dominant	B6	0.58		*Bw10* (Brockmann et al. 2000) (body weight)
16	Lean weight	*D16MIT107.1*	3 (0–16)	1.9*	additive	PWK	0.28		*Fatq1* (Moody et al. 1999)
16	Fat weight	*D16MIT107.1*	3 (0–11)	2.2*	dominant[#]	B6	0.18		*Adip17* (Stylianou et al. 2006)

Chr = chromosome; CI = confidence interval

[a] Marker positions in Mb were obtained using the University of California at San Diego Genome Bioinformatics browser (Kent et al. 2002) accessing Build 36 of the mouse genome, see Web References

[b] LOD score for corresponding mode of inheritance. Genome-wide significance determined by permutation tests:

*** $p<0.01$;

** $p< 0.05$;

* $p < 0.63$

[c] Mode = estimate by MAPMAKER/QTL for additive, dominant/recessive and unconstrained (free) models. # indicates that the dominant allele reduced the trait value

[d] ΔLOD indicates the difference between a model with sex as additive or additive and interactive covariate

[e] Sex effect indicates a QTL that is either sex-biased (ΔLOD>0.9) or sex-specific/sex antagonistic (ΔLOD>3.1); M = male-biased QTL, F = female-biased QTL

[f] List of QTLs: When the previous QTL was for a slightly different trait, the original trait is given in parentheses, e.g., (body weight). The list of overlapping QTLs is representative and does not include every QTL known to map to the region.

[#] After *Bwq8* was assigned to this locus but before publication, an overlapping QTL (*Bw21*) was reported (Vitarius et al. 2006).

[§] An unnamed QTL for an adiposity index maps near here (Collin et al. 2005) but it has features that suggest it is not equivalent to *Adip19*