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Fine-mapping of Obesity-related Quantitative Trait Loci in an F_{9/10} Advanced Intercross Line

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Abstract

Obesity develops in response to a combination of environmental effects and multiple genes of small effect. Although there has been significant progress in characterizing genes in many pathways contributing to metabolic disease, knowledge about the relationships of these genes to each other and their joint effects upon obesity lags behind. The LG, SM advanced intercross line (AIL) model of obesity has been used to characterize over 70 loci involved in fatpad weight, body weight, and organ weights. Each of these quantitative trait loci (QTLs) encompasses large regions of the genome and require fine-mapping to isolate causative sequence changes and possible mechanisms of action as indicated by the genetic architecture. In this study we fine-map QTLs first identified in the F₂ and F_{2/3} populations in the combined F_{9/10} advanced intercross generations. We observed significantly narrowed QTL confidence regions, identified many single QTL that resolve into multiple QTL peaks, and identified new QTLs that may have been previously masked due to opposite gene effects at closely linked loci. We also present further characterization of the pleiotropic and epistatic interactions underlying these obesity-related traits.

INTRODUCTION

As science enters the genomic era, there is new appreciation for the complex genetics underlying many common diseases. Obesity is one of the most prevalent diseases in developed countries (1). Complex diseases, such as obesity, are a result of some combination of genes and their interaction with the environment (2). Typically, the genetic contribution to complex diseases is due to multiple genes of small effect as opposed to few

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/oby>

DISCLOSURE

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genes of relatively large effect (3). Estimates of the heritable variation contributing to obesity range from 30–60% in family studies to 60–80% in twin studies (3).

A number of genes have been identified to date that contribute to the etiology of obesity in humans. Mutations of large effect in single genes rarely cause obesity. Examples include *ob(Lep)* (4), *LEPR* (5) and *MC4R* (6). Several important genes (*CAPN10* (7), *ENPP1* (8), *SLC6A14* (9), and *GAD2* (10)) have been identified using genome-wide linkage analysis and positional cloning. More recently genome-wide association studies have been used to identify genes that contribute to obesity in human populations, including *FTO* and the adjacent *FTM* gene (11). Although *FTO* has been identified as an obesogenic gene in multiple ethnic populations (12), it only explains ~1% of the heritable variation. Genes of smaller effect, such as *MTMR9* and *PFKP* (13,14), have also been identified using genome-wide association studies. Genes such as *INSIG2* (15) are controversial and may exhibit nonadditive, epistatic effects on fat deposition. Relevant heritable complexity in obesity due to epistasis and other genetic architectural effects have been underrepresented in the literature to date.

Although genome-wide association studies and family-based association studies have been successful in identifying human obesogenic genes, questions remain about the complexity of the underlying genetic architecture. Animal-based quantitative trait locus (QTL) studies have been useful in studying characteristics of genetic architecture such as pleiotropy, epistasis, and dominance, which are difficult to ascertain in human populations. Many obesity QTLs have previously been mapped (3,16,17) in F₂ animal model populations. Recent advances in computational power and modeling have facilitated the study of epistatic (18,19) and imprinting (20) contributions to heritable variation. Modeling efforts are ongoing to separate QTLs with direct effects on obesity from those whose effect is mediated by another phenotype, such as skeletal muscle mass (21).

The LG, SM advanced intercross line (AIL) is a well-studied model of population variation in the development of obesity and diet effects (18,22–25). Initial F₂ mapping studies have identified several QTLs affecting fatpad weight, as well as QTLs affecting associated traits such as organ weights, body weight, and growth (22,26). Fine-mapping efforts have been ongoing (24,27,28) to narrow QTL intervals and facilitate identification of causal genes. Evidence for a larger than expected impact of genetic architecture has been revealed in studies targeting the proportions of heritable variance explained by epistasis and imprinting (29). Few mapping studies have continued intercrossing through the F₁₀ generation, leaving uncertainty about the genetic and interaction structure underlying each large F₂ QTL. This study examines the architecture of QTLs contributing to obesity and organ weights in the LG, SM AIL combined generation F_{9/10} population with five times the resolution of the earlier F₂-based studies.

METHODS AND PROCEDURES

Animal husbandry and phenotyping

Animal husbandry and breeding details for the LG, SM AIL can be found in publications by Cheverud *et al.* (18,30,31). Briefly, animals were fed on PicoLab Rodent Chow (no. 5053). Animals were 12–40 weeks of age at necropsy (see age correction below). A 12 h light/ dark cycle was maintained, with a constant temperature of 21 °C. All phenotypes were collected upon animal necropsy. Weights to the nearest 0.01 g were obtained for the carcass (body), reproductive fatpad (fatpad), liver (liver), each kidney (kidney), heart (heart), and spleen (spleen). Tail length (tail) was measured using digital calipers to 0.01mm. Data were corrected for effects due to litter size, date of birth, age at necropsy, and sex. The

phenotypes were each regressed onto the collection of covariates and the regression residuals added to the means of each phenotype being studied.

Genotyping

DNA was extracted from liver using the DNeasy DNA extraction kit (Qiagen, Valencia, CA) or the QIAmp DNA extraction kit (Qiagen) from 150 F₉ and 1305 F₁₀ animals in the intercross population. Each animal was single-nucleotide polymorphism genotyped at 1,470 single-nucleotide polymorphisms using the Illumina Golden Gate platform at the Washington University Genome Center (St Louis, MO). The average intermarker interval was 4.94 F_{9/10} cM (~1.0 F₂ cM), correlating to an approximate fivefold expansion of the F₂ map. Map distances were generated via Haldane's algorithm in R/qtl (32), treating the F_{9/10} as an F₂ to reflect the accumulated recombination.

One QTL mapping

QTL analysis was performed using regression interval mapping (33) as described previously (18) with the following exceptions. We imputed genotype scores every 1 F_{9/10} cM throughout the genome, interspersing markers at their mapped locations. Trait values were regressed onto the genotypic scores using the program R (32,34). Logarithm probability ratios (LPRs), a logarithm of the odds equivalent, were derived using:

$$\text{LPR} = -\log_{10}(\text{Prob}). \quad (1)$$

Chromosome- and genome-wide thresholds that accounted for both family-substructure and multiple comparisons were generated via simulation in R. A detailed description of this methodology can be found in Fawcett *et al.* (18). The genome-wide multivariate 5% significance threshold per trait is as follows: fatpad (7.64), body (7.97), tail (10.24), heart (5.58), kidney (6.77), spleen (6.11), and liver (5.72). These thresholds are inflated relative to those used in a F₂ population because family members in this population are related to each other and not genetically independent. Confidence intervals (CIs) were defined by an empirical formula:

$$\text{CI} = \pm 1.96 \times \exp(-0.416 - 0.438 \times \ln[R^2/h^2]), \quad (2)$$

where CI is the 95% confidence interval containing the peak, R^2 is the multiple squared r that was calculated for the single-trait QTL at each locus, and h^2 is the narrow-sense heritability of the trait calculated using the sibship method (Table 1). R^2/h^2 represents among family variance at that locus. The CI equation was determined using the F_{9/10} population pedigree information. A more detailed description is provided in Norgard *et al.* (31).

All QTLs underwent composite interval mapping where effects ~25 F_{9/10} cM upstream and downstream of the QTL peak were controlled (35). The composite interval mapping model was:

$$Y_{ijk} = \mu + a \times X_{ai} + d \times X_{dj} + e_{ijk} | X_{al} X_{dl} X_{am} X_{dm}, \quad (3)$$

where Y_{ijk} is the vector of trait values for all individuals, μ is the constant, X_{ai} and X_{dj} , respectively represent the additive and dominance scores, a and d reflect the additive and dominance genotypic values, e_{ijk} represents the residual error, and X_{al} , X_{dl} , X_{am} , and X_{dm} represent the additive and dominance genotypic scores for markers forming the boundary of the interval. Each QTL on each chromosome was considered independently, with closely placed QTL peaks being individually controlled to verify their placement and presence.

Composite interval mapping is used to narrow QTL intervals and resolve multiple QTL signals.

Sex interaction QTLs were analyzed as in Fawcett *et al.* (18). Briefly, a one-qtL model with sex by additive and sex by dominance interactions as the independent variables was fit controlling for the direct effects of locus and sex. Locations of significant ($P < 0.05$) sex interaction were verified by mapping separately by sex to determine the type and degree of sexual dimorphism in gene effects. Pleiotropy was estimated by grouping traits with QTLs within 20 F_{9/10} cM together. Traditional methods of pleiotropic analysis were not sensitive enough to analyze the F_{9/10} population. Naming conventions are as in Fawcett *et al.* (18).

Epistatic mapping analysis

Epistatic mapping was performed on the peak locations of the direct effects QTLs identified in the one-QTL analysis above. Briefly, mapping was performed as in Cheverud *et al.* (22). Significance thresholds were calculated based upon the prior knowledge of main effects and accounting for family-substructure as detailed below. Computational limitations prevented full simulation of the population, so thresholds were estimated using three empirical equations:

$$\text{Intercept} = 1.4143587 + 4.3190134 \times h^2 \quad (4)$$

$$\text{Slope} = 0.4320926 + 1.5272199 \times h^2 \quad (5)$$

$$\text{Threshold} = \text{Intercept} + \text{Slope} \times \log(M_{\text{eff}}), \quad (6)$$

where M_{eff} is the effective number of loci on the chromosome and h^2 is the among sibling heritability. The M_{eff} (36,37), or effective number of markers, was calculated for each threshold scale. QTL-by-QTL tests had one effective comparison. Heritabilities were generated using the among-litter variance approach (25). QTL-by-QTL thresholds were: fatpad (3.18), body (3.34), tail (4.42), heart (2.21), kidney (2.77), spleen (2.46), and liver (2.27). Naming conventions were as in Fawcett *et al.* (18).

RESULTS

Single-trait QTLs

We identified 113 single-trait QTLs, affecting seven traits associated with overall body size and obesity. Ninety of the single-trait QTLs were significant at the genome-wide level. This group also contains QTLs with protected thresholds from identification in the F₂ and/or F₃ LG, SM populations. The trait distribution of single-trait QTLs is: fatpad (21), body (23), tail (25), heart (10), kidney (16), spleen (14), and liver (4) (Supplementary Table S1 online). Chromosomes 3, 12, 14, and 16 did not exhibit multiple QTLs for any trait. There were no significant QTLs on chromosome 12.

Sexual dimorphism

Significant sex-interaction effects, primarily affecting males, were observed on chromosomes 1–7, 9–11, and 17 (Supplementary Table S1 online). 13.3% of single-trait QTLs are sex-specific, with 80.0% of the sex-specific QTLs affecting males. All sex-specific QTLs displayed at least a twofold greater LPR score in the affected relative to the unaffected sex. The distribution of sex-specific single-trait QTLs (male, female) was: fatpad (0%, 0%), body (13.0%, 0%), tail (4.0%, 4.0%), heart (20.0%, 0%), kidney (37.5%, 0%),

spleen (0%, 14.3%), and liver (0%, 0%). Only fatpad and liver showed no sex-specificity. Kidney exhibited a very high degree of male-specific QTLs. Spleen was the only trait that exhibited sex-specific QTLs solely affecting females.

Gene effects

Single-trait QTL gene effects are typically additive in nature (Figure 1). Here 59.3% of loci exhibited solely additive effects. Some loci exhibited both significant additive and dominance gene effects (15.0%). Maximum gene effects were <0.5 s.d. units. Although the LG/J allele typically results in a larger measured phenotype, we observed the SM/J allele contributing to a larger phenotypic effect in 16.8% of loci, with the following loci (denoted by proximal single-nucleotide polymorphisms) exhibiting this effect: fatpad (*rs6316481*, *rs6299531*, *rs3699522*, *rs6296621*, *rs4152838*), body (*rs6316481*, *rs6212358*), tail (*UT-8-57.168981*, *rs6316481*), heart (*rs3663950*, *rs13480601*), kidney (*rs3722447*, *rs6173859*, *rs3663950*, *rs13478204*, *rs6316481*), spleen (*rs13459120*, *rs3692040*, *rs3683495*). None of the liver QTLs exhibited a larger phenotype correlated with the SM/J allele. Variance explained in males was greater in all traits but spleen, as is expected from the higher proportion of male-specific QTLs. Total variance explained by all single-trait QTLs was between ~2.8% and 35.6% by trait. Broken down by trait (male, female), the variance explained was: fatpad (18.3%, 18.3%), body (25.8%, 24.2%), tail (35.1%, 35.0%), heart (8.7%, 7.6%), kidney (16.2%, 10.7%), spleen (13.9%, 16.0%), and liver (2.8%, 2.8%).

Dominance effects were much less common, with only 30.1% of loci significant. Overdominance ($d > 1.5$ s.d. units) and underdominance ($d < -1.5$ s.d. units) were exhibited in few (13 and 10) QTLs. Codominance ($-0.5 < d < 0.5$ s.d. units) was seen in the majority of the loci (52 out of 107) (Figure 1). Dominance of the LG/J allele was ~1.5-fold more common than dominance of the SM/J allele.

Two genome-wide significant fatpad QTLs did not exhibit significant additive or dominance gene effects. These were on chromosome 18 (*Adip8a* and *Adip8b*), where the QTLs were closely linked. There were opposite gene effects between these loci as well as significant epistatic interactions, which might account for a dampening of overall observed effect given their close linkage. This issue should be resolved with further fine-mapping efforts.

Pleiotropic QTLs

Pleiotropy occurs when a single locus contributes to multiple phenotypes. We estimated that all single-locus QTLs residing within 20 F₁₀ cM of any other single-locus QTL were pleiotropic. Previous work (18,38) utilized a Pearson correlation test of closely linked QTLs for lack of pleiotropy. That test was not sensitive enough to detect nonpleiotropy in loci located within 20 F₁₀ cM in this study. We observed 29 pleiotropic QTLs, leaving 45 single-trait QTLs (Table 2). Most of the pleiotropic QTLs were significant at the genome-wide level for at least one of the traits included (53.3%), as were 54.5% of the remaining single-trait QTL. Eleven (37.9%) of the pleiotropic QTLs exhibit sex-specificity in at least one trait. CIs for pleiotropic loci were narrow (~4.1 F₂ cM), matching the single-trait QTL trend.

Recent work (18,38,39) hypothesizes that traits affected by pleiotropic loci exhibit modularity. In this study we did not observe a clear-cut demonstration of modularity, such as the clustering of organ weights or isolation of tail length as a measure of skeletal morphology. Body (63.3%) and fatpad (40.0%) were the two most likely traits exhibiting a pleiotropic relationship with other traits, most often with organ weights (79.2%).

Fine-mapping

Fine-mapping and significantly reduced QTL confidence regions result from accumulated recombination in a $F_{9/10}$ advanced intercross. We observed narrowing of QTL support intervals relative to the F_2 generation, and splitting of pleiotropic QTL into multiple peaks, each affecting either one or a few of the traits. Thirty-five percent of the QTLs that replicated from the previous $F_{2/3}$ study (18) split into two or more closely linked peaks. Each trait exhibited QTL splitting: fatpad (*Adip2*, *Adip3A*, *Adip5*, *Adip18*, *Adip8*), body (*Adip3A*, *Wtn11.1*, *Adip20*), tail (*Adip15*, *Adip18*, *Bod19.1*), heart (*Wtn11.1*), kidney (*Bod2.1*, *Adip11*), spleen (*Adip20*), and liver (*Adip18*). The new QTL peaks were verified by composite interval mapping. Gene effects and variance calculations were double-checked for significance for all QTL on each chromosome for each trait to verify effects of closely linked QTLs. All of the QTLs exhibited significant gene effects except for *Adip8a* and *Adip8b* on chromosome 18.

Epistatic QTLs

Epistasis occurs when the interaction of two or more genes causes a change in the phenotypic effects of alleles. Epistatic interactions were studied only between identified direct effects QTLs (QTL-by-QTL interactions). We identified 66 epistatic interactions between 73 epistatic loci affecting all studied traits. Supplementary Table S2 online displays the epistatic loci and their relationships. The number of epistatic loci and interactions are listed respectively by trait: fatpad (10, 12), body (9, 11), tail (21, 20), heart (5, 7), kidney (13, 12), spleen (6, 8), and liver (2, 3). Although each trait exhibited epistasis, we observed much more for tail than for any other trait ($\sim 2\times$), possibly related to the greater number of direct effects for this trait. None of the epistatic interactions affected more than one trait, which is consistent with the expectation that fine-mapping will separate apparently pleiotropic loci by trait. We anticipate that at least as many additional epistatic interactions would be identified in a full genome-by-genome epistatic study.

All epistatic interactions exhibited at least one significant form of epistasis. Each of the four types of epistatic interaction was represented with the s.d. unit effect size varying between 0.097 and 0.641 s.d.: $33 a \times a$ (0.097–0.254 s.d.), $30 a \times d$ (0.145–0.526 s.d.), $21 d \times a$ (0.155–0.288 s.d.), and $19 d \times d$ (0.206–0.641 s.d.). The magnitudes of genotypic values for the epistatic interactions are similar to those observed for the direct effects. Epistasis contributed to the genetic variance explained for each of the traits: fatpad (8.1%), body (12.3%), tail (26.0%), heart (4.0%), kidney (10.5%), spleen (4.1%), and liver (0.07%). The low contribution to liver is due to the relatively low number of direct effects QTLs contributing to this trait. Epistasis between QTLs without direct effects were not considered here but are likely to exist and contribute to trait variation (40).

LG, SM QTL replication

Many of the QTLs reported previously (18,22,27,41) replicated in this study. 54.5% of the $F_{2/3}$ QTLs (18) replicated. *Adip1*, *Adip4*, and *Adip6* (22) did not replicate at the current significance thresholds. At the peak locations, identified on the physical genome map, from the $F_{2/3}$ work, the LPRs for these loci in the $F_{9/10}$ were 2.07, 2.34, and 2.04, respectively. Two loci were immediately adjacent to the CI for *Adip1* from both the F_2 (22) and $F_{2/3}$ (18) studies, located at 5.18 Mb (LPR 3.22) and 71.58 Mb (LPR 3.59), but neither exceeded the unprotected significance threshold. Earlier work on the LG, SM F_2 (36,41) and the LG $\times$ SM recombinant inbred lines (27,28) replicated at lower rates, as shown in Table 2. There is no significant correlation (Pearson) between the magnitude of the LPR in the original study and the probability of replication in the current study.

Replication of LG, SM epistatic effects

Despite the high epistatic thresholds and different populations, we observed replication of several previously identified LG, SM epistatic interactions (18,22). Five of the interactions between *Adip* QTLs, which primarily affect fatpad weight, replicated from the F_2 (22) study (Figure 2). Despite moderate replication for epistatic effects between direct effects QTLs in the $F_{2/3}$ (18), we did not observe replication of $F_{2/3}$ epistasis between loci not significant for direct effects. 94.7% of the $F_{9/10}$ epistatic interactions identified herein appear novel.

DISCUSSION

This study focused upon the fine-mapping of previously identified *LG*, *SM* QTLs. Although increased resolution of known QTLs is of significant value in itself, biological complexity in the form of epistasis or fragmenting of previously linked effects can confound replication and fine-mapping efforts. Therefore we performed an unbiased full-genome scan, with lowered significance thresholds for locations containing previously identified QTLs. We identified many QTLs (>100) contributing to a number of obesity-related traits including fatpad and organ weights. Stringent Bonferonni- and family-substructure adjusted thresholds restricted the overall number of QTLs that were identified. Many (35.3%) of the QTLs identified in the $F_{2/3}$ study that replicated also resolved into at least two QTLs in the $F_{9/10}$ study. In addition to resolution of multiple QTL peaks in the $F_{9/10}$, each independent QTL CI was much narrower, as is expected when mapping in an advanced intercross. We observed a general pattern of $F_{9/10}$ QTLs resolving around the peak of the originally identified $F_{2/3}$ peak, but not under it, suggesting that the location of some QTL effects in earlier generations resulted from an average of multiple true, linked QTL positions.

Many $F_{9/10}$ QTLs were replicates of QTLs identified previously (18,22,27,38). Approximately 32.5% of the $F_{9/10}$ QTLs are novel. There are several possible causes for identification of novel QTLs in an advanced generation. First, earlier populations may have exhibited occluding epistatic effects. In this situation, the unique nature of the epistatic interactions present within the population and the contribution of epistasis to the additive genetic variance (29) may inhibit resolution of small effect genes or genes with a large epistatic component. Second, despite the large population sizes used in the $F_{2/3}$ study (18), there is a probability of failure to replicate QTLs of relatively low LPR (42). Third, opposing gene effects in closely linked QTLs may not have been resolved in early generations due to the lack of accumulated recombination. As a result, some QTLs that are now resolvable from neighboring QTLs would have been invisible. One such situation occurred on chromosome 2 for tail length. *SkI2.1* exhibits underdominance and no significant additive gene effects and *SkI2.2* exhibits LG/J dominance and a small but significant additive effect. Finally, the separation of effects due to fine-mapping may result in lowered individual LPR scores that are unable to exceed the stringent Bonferonni- and population substructure-adjusted thresholds. Studies are therefore strengthened by assuming an unbiased approach to QTL identification even in advanced generations used primarily for fine-mapping.

As expected from previous mapping experiments (18,22,31), the predominant contribution to genetic variance was from additive effects, with dominance and epistasis each contributing a smaller portion. Seventy-seven percent of $F_{9/10}$ QTLs exhibited significant additive gene effects. Dominance patterns were similar to those observed previously (18), with most effects being codominant, and the LG/J allele most often contributing to a larger phenotypic value.

Most of the QTLs in the current study affected single traits, suggesting a large false-positive rate for pleiotropy in early generations. The pleiotropy test utilized in the $F_{2/3}$ study was

unable to separate closely linked effects, and so $F_{9/10}$ pleiotropy was determined by comparing the composite interval mapping results with all QTLs residing within close proximity ($\sim 20 F_{10}$ cM). 72.4% of the QTLs that exhibited pleiotropy in the $F_{9/10}$ clustered very closely (within $10 F_{10}$ cM) to one another, as compared to the $F_{2/3}$ pleiotropic results where the QTL CIs were quite broad and accommodated widely separated single-trait QTLs (up to $30 F_2$ cM). This effect was acknowledged in earlier studies examining pleiotropy (38). Pleiotropy results done on early generations of an advanced intercross should be interpreted cautiously.

Epistasis contributed significantly to genetic variation in the $F_{9/10}$ population. The greatest epistatic contribution was to tail length, where 26.0% of genetic variation was due to epistasis. Although a large number of epistatic interactions were identified that contributed to each of the examined traits, only a small proportion (5.3%) of the epistatic QTLs represented replication from previous work (18,22). We expected low replication rates due to the nature of these interactions which are unique to each population. Furthermore, apparent epistasis between linked regions in the F_2 may be caused by each locus bearing different effects.

AILs allow fine-mapping progress genome-wide, which is of particular importance in studying complex traits. Fine-mapping efforts are often conducted using congenic or sub-congenic lines. AI lines permit verification of QTL effects, fine-mapping progress and tools to study epistasis with any QTL of interest, rather than being restricted to single genomic regions as in the use of congenic lines for fine-mapping. Fine-mapping in the LG, SM AIL $F_{9/10}$ population allowed narrowing of 30 QTLs that had been previously identified in the Cheverud lab (18,22,27,41). Additionally, we were able to replicate 15 epistatic interactions (Figure 2) affecting fatpad and body weight from earlier work (22).

Complex trait studies identify QTLs that may be more or less likely to be identified in any given population, and QTLs that can replicate between populations are of high interest. Fine-mapping QTLs of particular interest to the study of obesity have resulted in the narrowing of QTLs on chromosome 2, as well as identification of epistatic effects between the murine chromosome 2 location, orthologous to the human HSA20 obesity locus (43), and other genomic loci (43). We replicated several QTLs affecting kidney weight, body weight, and tail length from a study of the congenic MMU2 and MMU11 lines (43). Additionally, we replicated three fatpad or body weight QTLs from work using an F_2 C57BL/6J $\times$ 129S1/SvImJ, with CIs narrowed via bioinformatics tools (44). As might be expected, recent work in an F_2 of SM/J $\times$ NZB/BINJ was replicated more thoroughly than other studies that shared no common inbred lines with our study. Three of the eleven QTLs replicated from the work by Stylianou *et al.* (45) were located just distal to our QTLs, with estimated CIs overlapping. Table 3 lists the various replicated QTLs by study from fine-mapping efforts. Work in the (M16i $\times$ CAST/Ei) $F_1 \times$ M16i backcross population has identified a number of direct effects loci and epistatic interactions contributing to obesity-related traits (46–48). None of these loci appeared in our study, most likely due to different populations being used. The population-specific nature of epistatic interactions combined with the ability of epistasis to prevent detection of direct effects likely contributed to the dearth of replication of epistatic results from non-LG, SM populations.

We analyzed QTLs for candidate genes based upon selection for replication from the F_2 , the number of traits affected, and resolution into multiple peaks. Genes were considered to be candidate genes if they have been shown to directly impact obesity, carbohydrate metabolism, type 2 diabetes mellitus, hyperglycemia, insulin resistance or body size. Additional candidate genes were identified if they exhibited direct physical interaction with a gene known to affect body size or obesity. Table 4 displays the selected QTLs with their

locations, numbers of genes within the region, and candidate genes. QTLs containing candidate genes known to have large effects upon the development of obesity exhibited genome-wide significant results. Examples include *Adip25 (IGF1R)*, *Adip3B (UCP2)*, and *Adip8d (PPARGC1B)*. Future fine-mapping and congenic studies of these reported direct effect and epistatic QTLs will result in enhanced clarity of molecular networks affecting obesity.

We have identified a large number of QTLs affecting fat pad weight, body weight, tail length, and organ weights in the LG, SM AIL. Compared to earlier studies of these strains, we show that many previously mapped QTLs represented the effects of multiple gene loci resolved as separate effects in the F_{9/10} population. Furthermore, these loci are now mapped to much reduced genomic regions with compelling positional candidate genes. In order to identify causal genes underlying these well-characterized QTLs, we are pursuing a number of complimentary approaches. Sequencing of the LG/J and SM/J genomes has been an ongoing effort to facilitate identification of potentially functional polymorphisms within both coding and noncoding regions. Fine-mapping efforts are ongoing for more advanced generations (F₁₆) of the LG, SM AIL with the added feature of variation in diet. Finally, we are performing differential complementation studies to identify relevant genes within *Adip1 (CAPN10, (49))* and *Adip8d (PPARGC1B)*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

$F_{9/10}$ gene effects. Typical gene effects were small ($a/s.d. < 0.3$) and codominant ($-0.5 < d < 0.5$). **(a)** Additive gene effects were normalized to using the s.d. for the appropriate trait ($a/s.d.$). Absolute values are displayed to demonstrate effect sizes. **(b)** Dominance effects were normalized to additivity (d/a). Any d/a value where the additive gene effect was extremely low and nonsignificant was excluded from the analysis due to dramatic inflation of dominance ratio.

Figure 2.

Fatpad and necropsy weight epistasis networks. **(a)** Four loci from the original F₂ study (22) replicated epistatic effects. Only the loci interacting were replicative, not the type of epistasis. **(b)** Nonreplicative epistatic loci and interactions affecting fatpad weight. **(c)** Nonreplicative epistatic loci and interactions affecting necropsy weight.

Table 1Heritabilities for F₁₀ necropsy traits

	Mean	s.d.	h^2	V_p
Fatpad (g)	0.77	0.45	0.47	0.204
Necropsy weight (g)	31.8	4.28	0.51	18.283
Tail (mm)	89.75	5.41	0.80	29.322
Heart (g)	0.16	0.04	0.21	0.001
Kidney (g)	0.29	0.05	0.36	0.002
Spleen (g)	0.11	0.03	0.27	0.001
Liver (g)	1.97	0.4	0.22	0.156

Trait units, mean values, and s.d. are listed. Heritabilities were determined using the sibship method (Materials and Methods).

Table 2

Table of pleiotropic and nonpleiotropic $F_{9/10}$ QTLs

QTL name	Traits	Chr	Prox SNP	LPR	Loc (cM)	CI (cM)	Loc (Mb)	CI (Mb)	Previous name
Sk11.1	T	1	rs6293581	10.42	158.51	152.84–164.18	64.68	63.61–67.69	
Bod1.1a	K _M	1	rs13475922	4.33	180.48	176–195	73.67	72.42–79.83	Bod1.1 ^a
Bod1.1b	T, K	1	rs13475945	28.18	210.64	192.37–214.10	88.25	79.31–90.47	Bod1.1 ^a
Sk11.2	T	1	rs13475973	26.78	219.00	215.42–222.58	92.05	90.75–93.22	
Sk11.4	T	1	rs6382880	11.55	286.00	280.93–291.07	145.05	140.64–152.18	Adip1 ^a
Sk11.6	T	1	rs3685643	10.41	317.16	312.19–322.12	164.09	160.24–165.12	Adip1 ^a
Org2.1	L	2	rs3710094	2.40	230.00	224.44–235.56	75.35	73.58–77.90	BOD2.1(m) ^{a,b}
Bod2.1a	K	2	rs6220079	3.46	254.96	247.96–261.96	97.29	84.57–102.36	370–409 ^c , BOD2.1(m) ^{a,b}
Bod2.1b	W, H, K	2	rs3715478	4.53	272.98	264.03–280.33	109.58	102.81–114.12	370–409 ^c , BOD2.1(m) ^{a,b}
Sk12.1	T	2	rs13476787	12.30	317.00	312.30–321.70	134.96	133.14–136.07	
Sk12.2	T	2	rs6249968	17.50	328.88	324.64–333.11	137.48	136.83–139.44	
Bod2.2	W, T	2	rs6195594	17.18	345.00	337.50–349.34	145.96	141.13–147.46	
Bod2.3	W, H _M	2	rs6209403	7.70	419.45	402–427	158.43	154.64–159.84	
Adip21	F, W _M	3	rs3689046	10.52	18.00	12.37–23.63	19.18	15.99–21.01	
Org3.1	K	3	rs3722447	5.95	371.00	364.46–377.54	149.25	147.26–150.67	
Wtn4.1	W	4	rs13477659	7.52	81.00	75.58–86.42	40.02	36.70–43.67	
Adip23	F, S _F	4	gnf04.062.327	12.70	154.26	129–163.55	70.31	56.86–81.88	
Adip24	F, W	4	rs13477816	12.68	171.00	167.0–180.51	85.60	84.00–89.97	
Adip11a	F, W, S	4	rs6255772	5.38	202.00	195.89–213.98	99.10	96.68–105.53	BOD4.1 ^b , Adip11 ^a , 148–150 ^c
Adip11b	K	4	rs6173859	5.03	241.00	232.91–249.09	119.29	116.58–123.71	BOD4.1 ^b , Adip11 ^a
Adip11c	H, K _M	4	rs3663950	4.32	310.00	272–323.92	138.00	133.73–140.05	BOD4.1 ^b , Adip11 ^a
Org5.1	K	5	rs3718492	3.55	79.00	71.88–86.12	33.38	30.16–39.43	Adip12 ^a
Org5.2	K	5	rs13478204	4.28	89.54	82.06–97.01	40.96	36.88–44.10	Adip12 ^a
Org5.3	S _F , L	5	CEL-5-120064766	5.14	278.27	274.00–305.07	122.81	121.64–130.96	
Org6.3	S	6	rs13478730	4.96	72.84	68.34–77.34	44.96	41.80–46.35	
Adip2a	W, K _M	6	CEL-6-83434907	10.24	161.14	156.20–165.80	84.86	81.45–88.91	Wt2(f) ^d , Adip2(f) ^{a,d} , BOD6.1 ^b

QTL name	Traits	Chr	Prox SNP	LPR	Loc (cM)	CI (cM)	Loc (Mb)	CI (Mb)	Previous name
Adip2b	F, T	6	mCV23042866	5.65	256.00	248.60–263.40	117.23	113.16–119.97	236–186 ^c , 209–14 ^c , 198–201 ^c , Adip2 ^a
Adip13	W	6	rs6152631	8.29	320.33	314.33–326.33	144.01	143.35–144.70	Adip13 ^a
Adip3Aa	W	7	rs13479163	4.07	37.00	30.07–43.93	27.37	26.48–29.19	Adip3 ^d , BOD7.1 ^b , Adip3A ^a
Adip3Ab	F	7	rs3694031	4.01	73.00	66.29–79.71	35.86	34.90–38.15	Adip3A ^a
Adip3Ac	F	7	rs3689409	7.57	126	121.32–130.68	55466687	53.58–58.78	Adip3A ^a
Adip3Ad	F, W, H	7	rs3679779	12.09	136.81	130.92–141.78	66.16	58.56–68.52	Adip3 ^d , BOD7.1 ^b , Adip3A ^a , ORG7.1 ^b
Adip25	F, W, K	7	mCV25303361	9.39	166.00	161.02–191.12	74.30	72.93–91.56	
Adip3B	F, W, H _M , K _M	7	rs3656074	11.59	260.00	223–264.43	123.10	106.01–124.10	ORG7.1 ^b , Adip3B ^{a,d}
Org8.1	H	8	rs13479628	3.81	52.00	47.48–56.52	23.56	21.37–25.74	
Sk18.1	T	8	CEL-8-25677705	12.62	120.00	114.97–125.03	52.37	49.89–54.86	LBN8.1 ^b , Adip4 ^a
Sk18.2	T	8	UT-8-57.168981	9.50	143.77	138.28–149.26	60.35	59.54–64.63	LBN8.1 ^b , Adip4 ^a
Sk18.3	T	8	rs13479811	16.89	155.90	151.51–160.29	69.98	64.88–72.17	LBN8.1 ^b , Adip4 ^a
Bod8.1	T, K	8	rs13479814	17.05	171.00	158.51–175.49	76.78	70.74–79.08	58–80 ^c , LBN8.1 ^b , BOD8.1 ^b , Adip4 ^{a,d}
Adip5a	F, W, K _M	9	rs6316481	8.20	309.94	302.83–317.38	118.82	117.77–121.18	Adip5 ^{a,d}
Adip5b	F, T	9	rs13480454	8.06	315.00	308.41–324.76	120.12	118.56–123.21	Adip5 ^{a,d}
Adip5c	F	9	rs6299531	7.74	326.00	321.28–327.34	123.35	122.14–123.64	Adip5 ^{a,d}
Adip14	W, H, S	10	rs6212358	7.70	102.38	89.16–108.06	45.81	40.29–53.65	Adip14 ^a
Bod10.3	T _F , K	10	rs13480630	5.35	144.00	135.94–162.47	68.03	63.23–76.74	
Org10.2	S	10	rs13480703	7.62	206.00	202.13–209.87	91.59	91.10–92.21	
Org10.3	S	10	rs3710293	6.82	229.62	225.55–233.69	99.54	96.76–100.99	
Bod10.1a	W, S	10	rs6174062	6.75	248.00	241.93–254.90	109.53	105.82–111.89	295–305 ^c , ORG10.1 ^{a,d} , BOD10.1 ^b , Adip15 ^a
Adip15a	W, T	10	rs6363315	8.23	271.10	258.14–277.23	116.26	112.97–118.88	295–305 ^c , ORG10.1 ^{a,d} , Adip15 ^a
Adip15b	F	10	rs13480792	6.68	282.85	275.66–290.04	119.81	118.07–121.71	295–205 ^c , Adip15 ^a
Sk110.1	T	10	rs6290842	10.60	313.69	308.07–313.69	128.52	126.73–128.52	
Wtm11.2	W	11	rs13481161	7.43	260.55	255.07–266.02	92.28	91.31–94.91	
Wtm11.1a	W _M , H	11	rs3686162	7.48	275.00	269.71–285.51	98.26	96.57–101.06	349–41 ^c , WTN11.1 ^{a,d}
Sk111.1	T	11	rs6180460	9.39	300.00	294.11–305.89	103.69	103.14–104.25	Sk15(m) ^d , Wtm11.1 ^a
Wtm11.1b	W, H	11	gnf11.121.400	4.78	353.08	346.40–359.77	112.46	112.01–114.70	349–41 ^c , WTN11.1 ^{a,d}

QTL name	Traits	Chr	Prox SNP	LPR	Loc (cM)	CI (cM)	Loc (Mb)	CI (Mb)	Previous name
Adip18a	F	13	rs3699522	4.50	111.00	104.18–117.82	53.97	52.06–55.09	(Adip7) ^d , 147–35 ^c , Adip18 ^a
Sk113.1	T	13	rs3688781	12.15	150.93	145.85–156.02	73.32	71.70–75.56	
Adip18b	F, T	13	rs6296621	16.45	182.00	177.56–193.24	89.89	88.14–94.43	147–35 ^c , Adip18 ^a
Sk16	T	13	rs13481958	15.56	201.78	197.42–206.14	97.62	95.92–99.71	Sk16(f) ^d , Adip18 ^a
Adip18c	T, K, L	13	rs13481990	10.08	237.00	231.67–254.45	107.33	106.16–111.96	Adip18 ^a
Adip18d	W, L	13	rs13482028	4.19	275.04	268.70–276.55	117.37	115.08–118.46	Adip18 ^a
Bod14.2	H	14	rs3658866	3.93	32.00	27.51–36.49	28.72	26.98–30.46	S1–266 ^c , Bod14.2 ^a
Org15.1	S	15	rs3692040	6.22	97.00	92.78–101.22	51.28	49.47–54.73	
Org15.2	S	15	rs3683495	5.08	124.62	120.00–129.25	61.03	58.67–63.93	
Adip19	F	16	rs4152838	4.09	30.00	23.50–36.50	16.55	13.84–19.25	28–152 ^c , BOD16.1 ^a , Adip19 ^a
Adip20a	W _M , T _M , S	17	rs13482875	13.71	51.00	28.24–56.72	25.49	9.53–27.86	ORG17.1 ^b , Adip20 ^a
Org17.1	S	17	rs3705058	8.88	77.00	73.41–80.59	33.39	31.36–41.03	ORG17.1 ^b , Adip20 ^a
Adip20b	W, S	17	rs3090988	9.42	87.54	84.04–100.93	46.22	42.26–49.30	ORG17.1 ^b , Adip20 ^a
Adip8a	F	18	rs6230993	3.93	64	59.32–68.68	40.07	37.12–42.35	Adip8 ^{a,d}
Adip8b	F	18	rs3684561	9.73	90	85.32–94.68	49.61	47.29–51.09	Adip8 ^{a,d}
Adip8c	F	18	rs13483382	9.73	120.22	115.55–124.89	58.52	57.79–59.61	Adip8 ^{a,d}
Adip8d	F	18	rs13483394	10.09	130.03	125.55–134.51	62.08	59.61–63.73	Adip8 ^{a,d}
Org18.1	S	18	rs13483398	5.69	139.00	133.10–144.90	64.25	63.47–65.19	Adip8
Bod19.1a	T	19	rs6316813	4.94	14.19	6.67–21.72	11.39	9.66–14.13	111–137 ^c , Bod19.1 ^a
Bod19.1b	T	19	UT-19–18.800709	6.258	54.00	46.59–61.41	21.04	19.71–22.01	111–137 ^c , Bod19.1 ^a

All pleiotropic and nonpleiotropic QTLs are listed in physical order by chromosome. Traits are indicated as: fatpad weight (F), necropsy weight (W), tail length (T), heart weight (H), kidney weight (K), spleen weight (S), and liver weight (L). Sex effects are designated for the appropriate trait with a subscripted M (male) or F (female). Locations and confidence intervals are shown in both centimorgans (cM) and megabases (Mb) for comparison purposes. LPR values in boldface were significant at the genome-wide level. QTLs that are replicates of previously identified QTLs have the previous QTL name listed in the right-most column, and the references indicated as follows:

^a ref. 18

^b ref. 41

^c ref. 27

^d ref. 22.

Table 3

QTL replicates to non-LG, SM populations

QTL name	Chr	(Mb) peak	(Mb) CI	Trait	Replicated QTL	Reference
Bod2.1b	2	109.58	102.81–114.12	KID	Kwq7	43
SK12.1	2	134.96	133.14–136.07	TL	Tailq7	43
Bod2.2	2	145.96	141.13–147.46	WTN/FP	Fatq1, FAT (145.7 Mb)	43, 50
Bod2.3	2	158.43	154.64–159.84	WTN/FP	Fatq2, FATP (159.1 Mb)	43, 50
Adip24	4	85.60	84.00–89.97	FP	Obq26, Adip11	48, 45
Adip2	6	84.86	81.45–88.91	FP	Obwq3	45
Adip5a	9	118.82	117.77–121.18	FP	Adip14	45
Adip5b	9	120.12	118.56–123.21	FP	Adip14	45
Adip5c	9	123.35	122.14–123.64	FP	Adip14	45
Wtn11.1b	11	103.69	103.14–104.25	TL	Tailq8	43
Wtn11.1c	11	112.46	112.01–114.70	WTN	Obq33	48
Adip19	16	16.55	13.84–19.25	FP	Obq35	48
Adip20a	17	25.49	9.53–27.86	WTN	Adip18	45
Adip20b	17	46.22	42.26–49.30	WTN	Obwq4	45
Adip8a	18	40.07	37.12–42.35	FP	Obq21	45
Adip8b	18	49.61	47.29–51.09	FP	Obq21	45
Adip8c	18	58.52	57.79–59.61	FP	Obq21	45
Adip8d	18	62.08	59.61–63.73	FP	Obq21	45

The F9/10 QTL name is listed, followed by chromosomal location, F9/10 peak location (Mb), F9/10 confidence interval (Mb), affected trait, replicated QTL name, and referenced study.

Table 4

Candidate genes for selected $F_{9/10}$ QTLs

QTL	Chr	CI (cM)	CI (Mb)	# genes	$F_{2/3}$ genes	Candidate genes
Adip2	6	156.2–165.8	81.5–88.9	127	2293	Gfp11 (ENSMUSG000000029992)
						Rpn1 (ENSMUSG000000030062)
						Hk2 (ENSMUSG000000000628)
Adip3Aa	7	30.1–43.9	26.5–29.2	82	2143	Tgfa (ENSMUSG00000029999)
						Tgfb1 (ENSMUSG00000002603)
						Akt2 (ENSMUSG00000004056)
Adip3Ab	7	66.3–79.7	34.9–38.2	35		Cebpa (ENSMUSG000000034957)
						Gpi1 (ENSMUSG000000036427)
						Ldha (ENSMUSG000000063229)
Adip3Ac	7	121.3–130.7	53.6–58.8	59		Ldhe (ENSMUSG000000030851)
						Tph1 (ENSMUSG000000040046)
						Gabra5 (ENSMUSG000000055078)
Adip3Ad	7	130.9–141.8	58.6–68.5	36		Gabrb3 (ENSMUSG000000033676)
						Gabrg3 (ENSMUSG000000055026)
						Dgat2 (ENSMUSG000000030747)
Adip3B	7	223–264.4	106.0–124.1	436	1115	Iik (ENSMUSG000000030890)
						P2ry2 (ENSMUSG000000032860)
						P2ry6 (ENSMUSG000000048779)
Adip8a	18	59.3–68.7	37.1–42.3	63	362	Pde3b (ENSMUSG000000030671)
						Ucp2 (ENSMUSG000000033685)
						Nr3c1 (ENSMUSG000000024431)
Adip8b	18	85.3–94.7	47.3–51.1	9		Hsd17b4 (ENSMUSG000000024507)
						Slc27a6 (ENSMUSG000000024600)
						Htr4 (ENSMUSG000000026322)
Adip8c	18	115.6–124.9	57.8–59.6	8		Adrb2 (ENSMUSG000000045730)
						Slc6a7 (ENSMUSG000000052026)
						Ppargc1b (ENSMUSG000000033871)
Adip8d	18	125.5–134.5	59.6–63.7	48		Csflr (ENSMUSG000000024621)

QTL	Chr	CI (cM)	CI (Mb)	# genes	F _{2/3} genes	Candidate genes
Adip11a	4	195.9–214.0	96.7–105.5	93	387	Lepr (ENSMUSG0000000057722)
						Prkaa2 (ENSMUSG0000000028518)
						Jak1 (ENSMUSG0000000028530)
Adip18a	13	104.2–117.8	52.1–55.1	27	571	Drd1a (ENSMUSG0000000021478)
						Hrh2 (ENSMUSG0000000034987)
						Auh (ENSMUSG0000000021460)
Adip18b	13	177.6–193.2	88.1–94.4	37		Vcan (ENSMUSG0000000021614)
Adip25	7	161.0–191.1	72.9–91.6	148	n/a	Igf1r (ENSMUSG000000005533)
Bod2.1b	2	264.0–280.3	102.8–114.1	179	2432	Plin (ENSMUSG0000000030546)
						Bdnf (ENSMUSG0000000048482)

QTLs examined for candidate genes were typically pleiotropic, replicated from the F₂ or F_{2/3} studies (18,22), and of large effect. Genes were chosen on the basis of their effects upon obesity, body size, or known involvement with obesity-related phenotypes such as hyperglycemia, type 2 diabetes mellitus, or insulin resistance. Confidence intervals of each QTL are listed in both centimorgans (cM) and megabases (Mb). The number of genes contained within the original F_{2/3} confidence intervals are listed to provide comparison to the number of genes contained within the much narrower F_{9/10} confidence intervals.