Genetic Architecture of Body Fat Composition in Mice

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Abstract

Body fat composition is a quantitative measure of obesity, a major health concern in humans. Laboratory mice are considered an excellent model for dissecting the genetic basis of obesity due to the genetic variation present in inbred strains for body size and fat composition, as well as their historical use as a model organism for human disease studies. To identify the loci controlling fat pad weights and body weight in mice, we performed a quantitative trait loci (QTL) analysis of 513 (SM/J x NZB/BINJ) F2 individuals fed a high-fat diet for 14 weeks. Our analysis separated fat composition genetic effects from those affecting overall body sizes in mouse. Composite interval mapping (CIM) results showed that body weight was conditioned by three major additive QTLs, explaining 3 to 30% of the phenotypic variation. One significant QTL on chromosome 19 conditioned all fat pads with the exception of the inguinal fat weight, which was controlled by a different QTL also on chromosome 19. Significant QTLs associated with fat compositions were detected on chromosomes 17 and 19 and differed from those of body weight. The fat pad QTLs also showed mainly additive gene effects and they explained 2 to 7% of variation in fat composition. Joint analysis of correlated traits detected five additional large effect QTLs on five different linkage groups. These findings have indicated that fat composition and body weight in mouse are conditioned by one to three major additive genes and can therefore be potentially manipulated in controlling obesity.

Keywords: body weight, fat pads, gene action, mouse, obesity, QTL mapping

1. Introduction

The common mouse (Mus musculus) (2n=20), is an important model organism often used to study human-related diseases. A number of these studies have focused on cardiovascular diseases which may often be associated with dyslipidemia, obesity, hypertension, and diabetes (Isomaa et al., 2001). Traits characterizing these disorders are complex and are controlled by many genes with large environmental influences (Stylianou et al., 2006). Modern medical research relies on the ability to study human diseases in closely-related species in which controlled studies and experiments can be completed.

Obesity is one of the greatest health challenges in the developed world. The Centers for Disease Control (CDC) estimate that over a third of the adult population in the United States is considered obese (Ogden et al., 2012). As such, it is a crucial area of research for both scientists and medical practitioners, who seek to lower this rate and increase general health of the global population. Regarding obesity, much is known about the nature and effect of environmental factors relating to the disease such as diet and exercise, yet relatively little is known about its underlying genetic control (Ogden et al., 2012). The genetic components of such a condition are complex, intricately associated with other traits, and thus difficult to parse out. An increased knowledge of the primary genetic determinants of obesity will enhance our understanding of the pathophysiological background and may provide novel molecular targets for intervention.

Mouse crosses have been utilized to localize and identify genes underlying complex traits (Allayee et al., 2003, Ishimori et al., 2004, Stylianou et al., 2006). Mice have a large amount of genetic diversity, yet maintain enough similarity to humans in both genetics and physiology to allow some generalizations to be made to human health and medicine. Paigen et al. (2003) showed that mice of different inbred strains exhibited great variation in plasma

lipoproteins and obesity when exposed to a high-fat diet. This genetic diversity has been exploited to map more than 200 obesity QTLs in mouse (Snyder et al., 2004). Past studies have shown variation in defining the underlying genetic factors of obesity, and as such it is unclear whether this complex trait is governed by additive, dominance and/or epistatic interactions (Stylianou et al., 2006).

In this study we used an F2 population obtained from the cross of SM/J x NZB/BINJ inbred mouse lines to elucidate the types of genes conditioning obesity and to map the chromosomal regions associated with this quantitative trait. The ultimate goal of this research is to increase knowledge of the genetics underlying fat composition in mice. Such information may allow physicians and medical researchers to better target and identify genetic risk factors for obesity in humans, alleviating the pressure of one of today's most significant health concerns.

2. Method

The data for this study was provided by Stylianou et al. (2006) and consists of 513 F2 individuals derived from the intercross of SM/J x NZB/BINJ inbred mouse lines. The lines are known to differ widely in their body fat compositions, where NZB genotypes are generally lean while SM genotypes tend to have high adiposity. Of the 513 individuals, there were 260 females and 253 males.

Traits measured were primarily related to the fat pads on the left side of the mouse, which were doubled to account for both sides of the individual. These fat pads include the inguinal, gonadal, retroperitoneal, and mesenteric regions of the body. Individual fat pad weights were summed up to obtain total fat pad weight. Body weight at 4 weeks was also considered. Sex was initially included among the phenotypes and was scored as 0=female and 1=male. Body weight and sex were treated as covariates by Stylianou et al. (2006). All weights were measured in grams.

A total 153 SNP markers were used to genotype the individuals and were dispersed across all 20 chromosomes. For this project, the sex chromosome was excluded since no sufficient information regarding the 'paternal grandmother' (pgm) was provided with the data set. The sex chromosome was analyzed using only three markers and was therefore not as densely covered as the remaining autosomes. The final analysis utilized the remaining 150 markers on 19 autosomes.

The markers were initially tested for segregation distortion in "R/qtl package" (Broman et al, 2003), with an adjustment for multiple testing using a Bonferroni correction at alpha=0.05. The data was examined for missing phenotypic and genotypic scores, and the proportion of missing data comprised less than 10% of our overall dataset. This low level of missing data is generally considered an acceptable amount for QTL analyses.

The genetic map was constructed by "OneMap" statistical package in R (Margarido et al. 2007). Pairwise recombination fractions were estimated based on a LOD score of 4 and a maximum recombination fraction of 0.35. The Kosambi map function was employed, and the markers were ordered using the **Rapid Chain Delineation (**RCD) method (Doerge, 1996) ; LOD score of 4, maximum recombination fraction of 0.2, and rippled with "ws" = 3 and LOD=4. All markers aligned to the expected 19 linkage groups. Marker order and distance inferred by "OneMap" were used to find the QTL using composite interval mapping (CIM) implemented by "R/qtl package". CIM was performed using the expectation maximization (EM) algorithm method (Lander and Botstein, 1989), with 3 marker covariates and the mapping function set to Kosambi. A total of 1000 permutations were performed on all traits to establish genome-wide LOD significance threshold at a 0.05 probability (Churchill and Doerge, 1994). QTLs were considered to exist only at positions where a LOD score exceeded the corresponding significance threshold.

3. Results

3.1. Segregation of markers and map construction

Most of the markers used segregated in an expected 1 :2: 1 ratio in the F2 population. One marker (D3Mit258) produced a significantly distorted segregation at an alpha of 0.05. This particular marker, located on chromosome 3, had 42% missing data points, which could be the reason for inflation of segregation distortion, in addition to possible genotyping errors. However, at a threshold of $P < 1E-10$, the marker could not be dropped. A total of 150 markers were used to construct a linkage map covering 2398.9 cM (Figure 1). The average distances between markers were 16 cM. There were gaps of > 25 cM, especially in linkage groups 9, 10, and 13. The marker order constructed in this study was in good agreement with the published standard mouse genetic map (Cox et al, 2009).

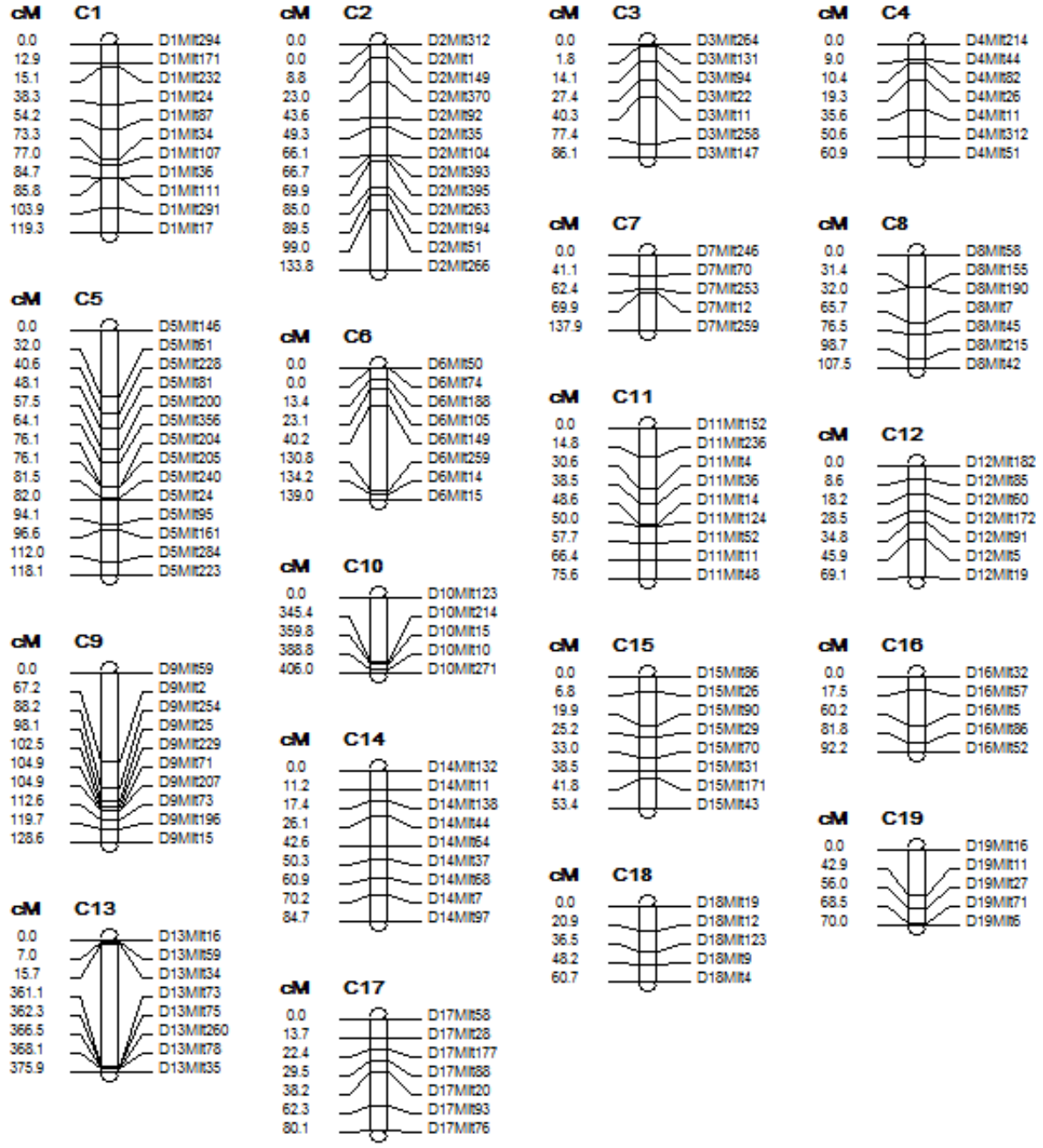

Figure 1. Genetic map of mouse constructed using 150 markers, genotyped on 513 F2 individuals from the cross of SM/J x NZB/BINJ inbred mouse lines. Map distances and order estimated using "OneMap" package in R with **Rapid Chain Delineation (**RCD), a LOD score of 4, and a maximum recombination fraction of 0.2 (Margarido et al 2007). Graphics generated using WinQTL Cartographer version 2.5 (Wang and Zeng 2007b). The order of the markers was consistent with the reference genome for mice as published by Cox et al (2009).

3.2. Phenotypic features

Distributions of measured quantitative traits are provided in Figure 2. These distributions indicate that there are possible QTLs associated with the measured traits due to the observed departure from normality (Lynch and Walsh, 1998). Specifically, the histograms illustrating the various fat pad traits are skewed, which is indicative of a few major QTL at play.

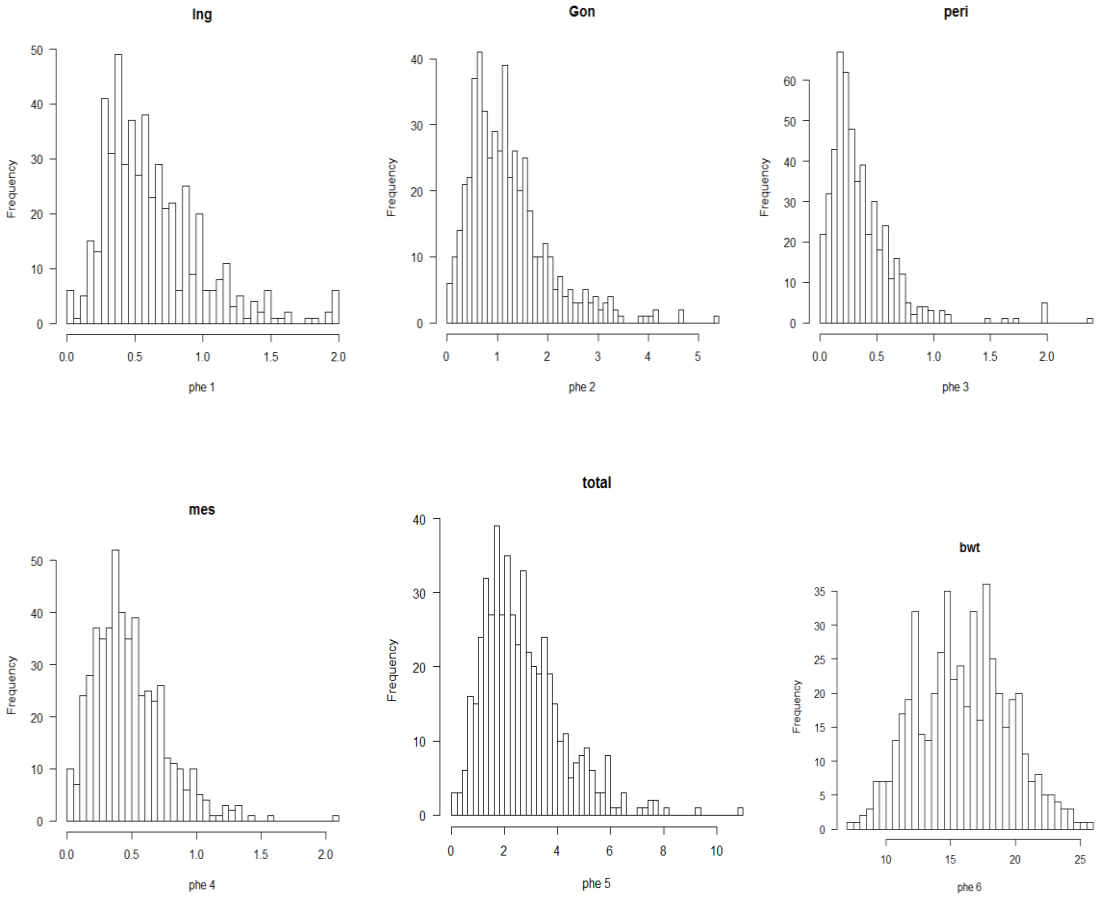

Figure 2. Distribution of measured quantitative traits. In this figure, Ing = inguinal fat pad, Gon = gonadal fat pad, Peri=retroperitoneal fat pad, Mes=mesenteric fat pat, total = total fat pad, and bwt=body weight at 4 weeks. The traits show skewed distributions, suggesting presence of major QTLs (Lynch and Walsh 1998)

Correlation analysis among the traits revealed that body weight had a weak correlation with the remaining fat pads and total fat (Figure 3). It was also found that sex was weakly correlated with all traits (Table 1), and was therefore not treated as a covariate in this analysis. However, the five fat pads were highly correlated with each other (Table 1). As such, a multiple trait analysis (MTA) was performed during QTL mapping on the five pads traits to counteract the covariance effects.

Table 1. Correlation values showing relationships among the quantitative traits used in the study. Sex is weakly correlated with all traits, all five fat pads are highly correlated with each other, and body weight is weakly correlated with fat pads weights.

	Gonadal	Peritoneal	Mesenteric	Total fat	Body weight	sex
Inguinal	0.71	0.77	0.76	0.88	0.24	0.28
Gonadal		0.58	0.72	0.94	0.26	-0.03
Peritoneal			0.68	0.78	0.19	0.34
Mesenteric				0.86	0.27	0.29
Total fat					0.28	0.17
Body weight						0.17
Sex						

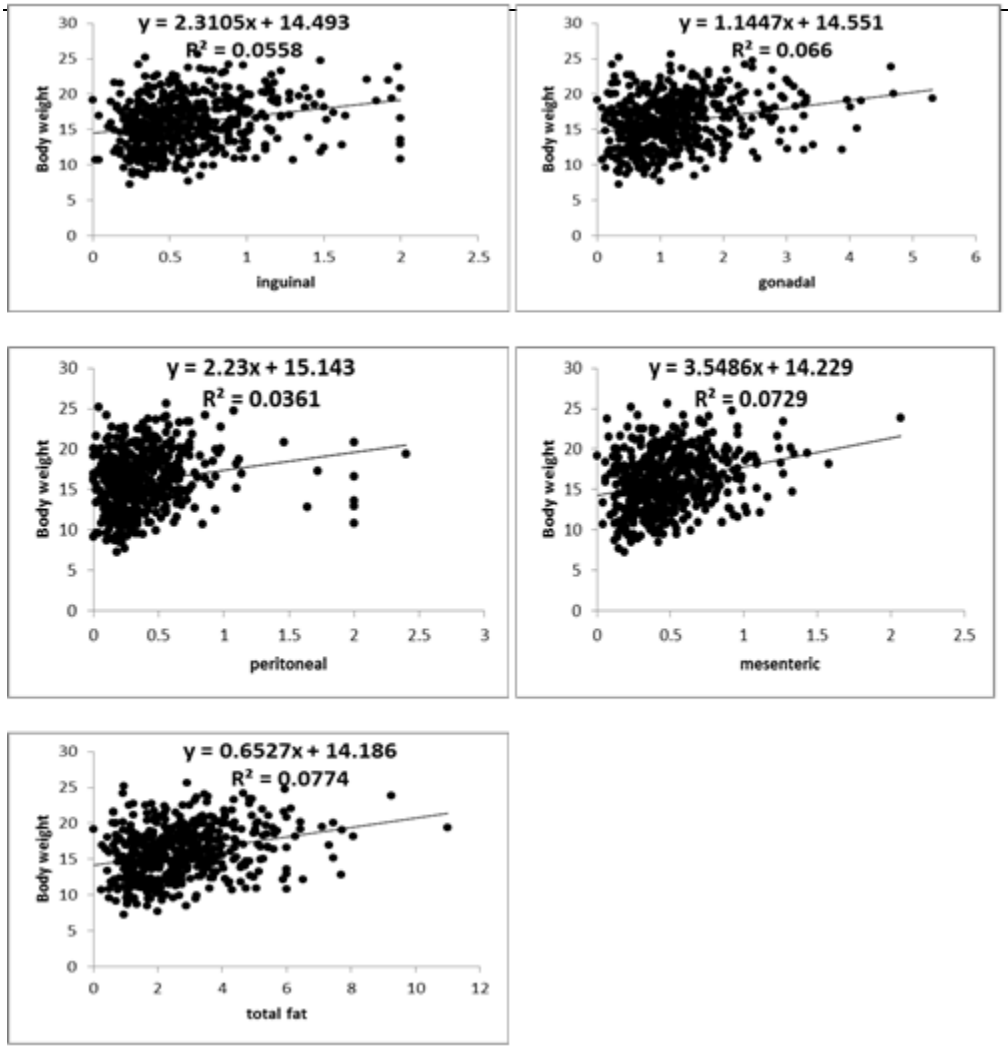

Figure 3. Scatter plots of distributions of each of the five fat pat pad weights relative to body weights.

The low R2 values in these plots show that fat pads explained less proportion of variation in body weight, an indication of weak relationships. As such, the multiple trait analysis did not look at the joint effects of body weight with the various fat pad weights.

QTL analyses of fat pads and body weight traits

A quick scan of the genome was conducted using single marker analysis (SMA) in order to detect major QTLs and localize regions of interest for further analyses. Results from the SMA indicated 24 markers as having significant regression coefficients and the proportion of variation (R2 values) ranged from 0.09 to 0.06 (Table 2). This result provided a preliminary visualization of markers that were possibly linked to QTLs.

Upon further examination of each chromosome, at least 2 QTLs were detected for each trait measured. It was also evident that the QTLs for fat compositions were clustered on chromosomes 17 and 19, but the body weight QTLs were localized on chromosomes 2, 5 and 6. Only gonadal fat pad and body weight shared a QTL detected on chromosome 5. The summary of the traits and the corresponding number of QTL detected is shown in Table 3.

Table 2. Single marker analysis (SMA) showed 24 markers possibly linked to QTLs of interest; methodology was

based on simple linear regression (significance level of 0.05) showing chromosome (Chrom.) and markers with significant regression coefficients bo and b1

Level of significance is denoted by asterisks, where * =0.05, ** =0.01, *** = 0.001.

Chrom.	Marker	b_0	b_1	-2ln(L0/L1)	F(1,n-2)	R^2
4	3	0.636	0.048	4.411	4.413*	0.0086
4	4	0.636	0.059	6.634	6.651*	0.0128
4	5	0.633	0.067	8.757	8.797**	0.0166
4	6	0.632	0.052	5.386	5.393*	0.0104
5	3	0.634	-0.056	5.791	5.802*	0.0112
5	4	0.634	-0.054	5.307	5.314*	0.0103
5	5	0.634	-0.05	4.493	4.495*	0.0087
5	6	0.634	-0.054	5.262	5.269*	0.0102
5	7	0.634	-0.052	5.016	5.021*	0.0083
5	8	0.634	-0.052	5.016	5.021*	0.0097
8	1	0.636	-0.086	7.629	7.656**	0.0057
8	2	0.636	-0.078	11.405	11.488***	0.0197
12	2	0.634	0.05	4.711	4.714*	0.0092
14	4	0.634	0.045	3.962	3.961*	0.0059
17	2	0.63	-0.048	4.663	4.666*	0.0085
17	3	0.629	-0.066	8.453	8.49*	0.0169
17	4	0.631	-0.092	16.868	17.081***	0.0323
17	5	0.632	-0.079	12.571	12.676***	0.0242
17	7	0.633	-0.057	6.607	6.624*	0.0128
18	2	0.633	-0.051	4.947	4.952*	0.0096
19	2	0.631	-0.08	14.288	14.433***	0.0275
19	3	0.639	-0.138	40.905	42.414***	0.0766
19	4	0.638	-0.123	31.546	32.409**	0.0596
19	5	0.637	-0.121	31.219	32.063***	0.0584

Table 3. Summary of the number of QTLs detected for each trait. Most significant markers for fat pads were localized on Chromosomes 17 and 19. Putative QTLs for body weight were found on Chromosomes 2, 5, and 6. Single marker analysis provided the preliminary framework for further analysis.

Trait	Chromosome	Number of putative QTLs
Ing	17, 19	2
Gon	5,12,17,19	4
Peri	17, 19	2
Mes	17, 19	2
Total	17, 19	2
Bwt	2,5,6	2

Ing = Inguinal fat pad, Gon = Godanal fat pad Peri=Peritoneal fat pad,
Mes=Mesenteric fat pat and total = total fat pad, bwt =body weight at 4weeks

For an in-depth analysis, Composite Interval Mapping (CIM) was conducted for independent traits. A summary of

significant QTL, their positions and flanking markers is presented in Table 4. CIM revealed a total of three significant (P = 0.05, LOD threshold =3.4) QTLs affecting body weight, and these QTLs also had significant additive genetic effects. The body weight QTLs were distributed in 3 of the 19 linkage groups (LGs), and individual QTLs accounted for 3-30 % of the phenotypic variance observed. The three QTLs were located on LGs 5, 6 and 17, at the 58cM, 40cM, and 40cM positions, respectively.

Two non-significant but potential QTLs, associated with body weight were also detected on LG 18 and 15, and were 20cM and 38cM near D18Mit12 and D15Mit71 respectively. One significant QTL on LG 19 flanked by the marker D19Mit71 was associated with three of the fat pads: total fat, mesenteric and gonadal. Another significant QTL shared by body weight, as well as mesenteric, peritoneal, and inguinal fat pads, was found on LG 17, at 40cM position near the marker D17Mit88. The QTLs controlling the fat pads also displayed larger additive than dominance genetic effects, and these QTLs explained 2 to 7% of the total variation in body fat composition.

Additional minor QTLs that were likely but below the test threshold and associated with the five fat pads are also shown in Table 4, and the genome-wide distribution of all the detected QTLs is shown in Figure 4.

Table 4. QTLs for fat pads and body weight traits in an F2 population of mouse derived from a cross between SM/J x NZB/BINJ. Threshold for declaring significant QTL is LOD = 3.4, determined by 1000 permutation testing (P = 0.05). R2 shows proportion of phenotypic variation explained by the QTL, "a" and "d" are the additive and dominance genetic effects. The negative signs on "a" and "d" only show the direction of the gene effects.

Trait	LG	QTL	Nearest marker	Position	LOD	R^2	a	d
Bwt	18	1	D18mit12	20	2.5	0.04	-1.06	0.55
	17	1	D17mit88	40	3.5	0.04	-0.52	0.47
	15	1	D15mit71	38	2.5	0.03	-0.87	0.33
	6	1	D6mit149	40	3.5	0.3	-1.0	0.00
	5	1	D5mit200	58	3.9	0.06	-1.27	0.72
totalf	19	1	D19mit71	68	10	0.05	-0.48	-0.44
	17	1	D17mit20	39	5	0.06	-0.54	0.00
	5	1	D5mit200	59	2.9	0.04	-0.42	0.14
Mes	19	1	D19mit71	68	6.5	0.04	-0.08	-0.06
	17	1	D17mit88	30	4	0.04	-0.08	0.03
	1	1	D1mit87	58	5.2	0.05	0.09	0.02
Peri	19	1	D19mit27	56	5.8	0.02	0.06	0.07
	17	1	D17mit88	30	3	0.02	0.06	0.00
Gon	19	1	D19mit71	68	10	0.04	-0.22	-0.21
	17	1	D17mit20	39	5.5	0.07	-0.15	0.08
	12	1	D12mit85	9	3	0.03	0.21	0.06
	5	1	D5mit81	50	4	0.04	-0.12	0.07
Ing	19	1	D19mit27	55	9	0.05	-0.12	-0.1
	17	1	D17mit88	30	3	0.04	-0.05	0.00

Ing = Inguinal fat pad, Gon = Godanal fat pad, Peri=Peritoneal fat pad, Mes=Mesenteric fat pat, total = total fat pad, and bwt =body weight at 4 weeks.

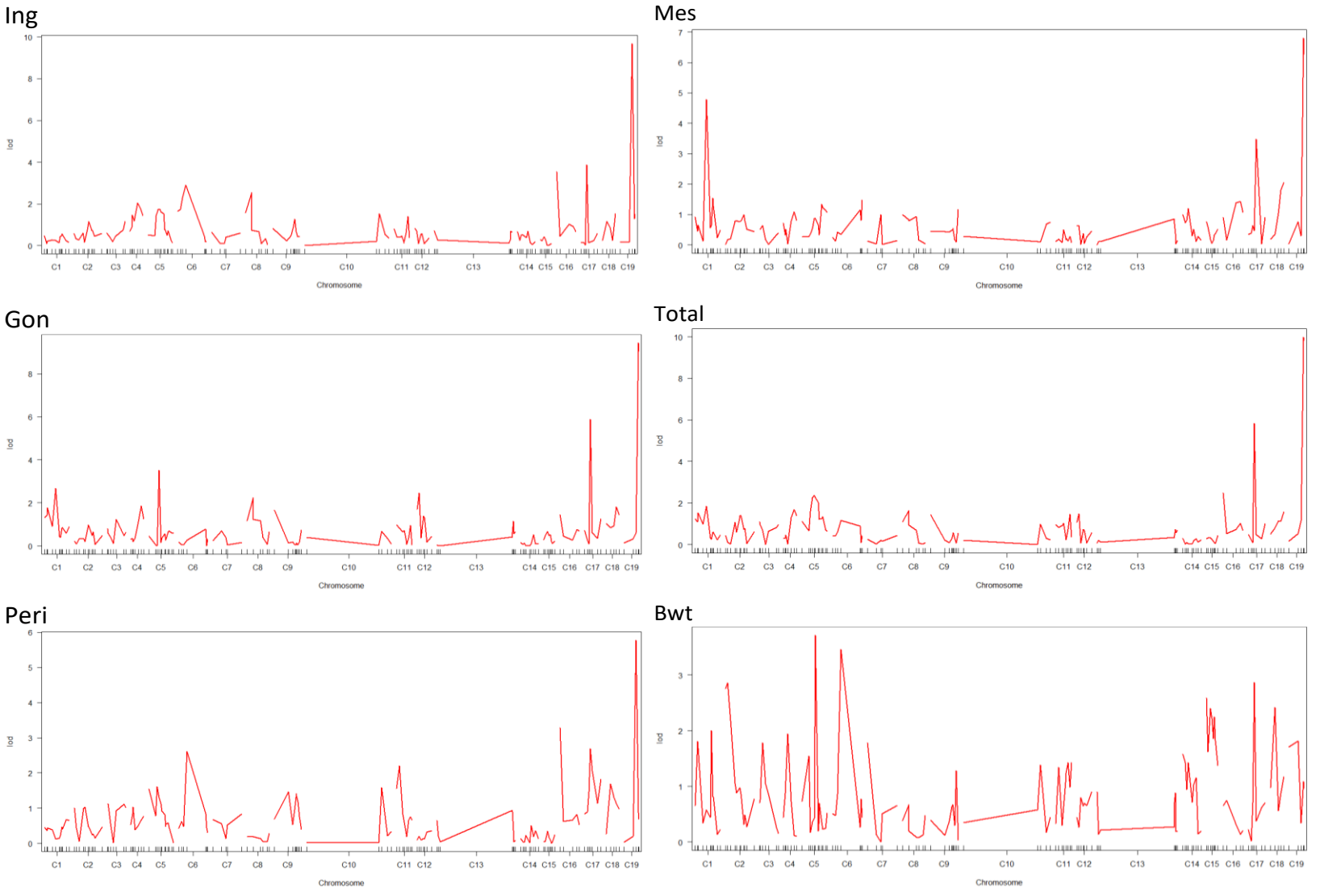

Figure 4. Result of CIM showing genome-wide distribution of QTLs asscoiated with fat pads and body weight in mice. QTLs were mapped using the EM algorith, where 3 markers were used as covariates, mapping function set to Kosambi, with 1000 permutations, alpha=0.05. In this figure, Ing = inguinal fat pad, Gon = godanal fat pad, Peri=retroperitoneal fat pad, Mes=mesenteric fat pat, total = total fat pad, and bwt=body weight at 4 weeks.

Multiple trait analysis (MTA) for the four fat pads known to be most highly correlated was conducted in WinQTL Cartographer version 2.5 (Wang and Zeng 2007). These traits were: inguinal, gonadal, retroperitoneal, and total fat pad weights. CIM was also used during the MTA, with the standard model 6, walking speed set at 2 cM and a window size of 10, run with 100 permutations (P= 0.05). A threshold of LOD =3 was used to declare significant QTL. Results of MTA uncovered new significant QTLs on linkage groups 1, 4, 5, 6 and 8 (Figure 5). These QTLs did not show up in the CIM results when the traits were considered independently.

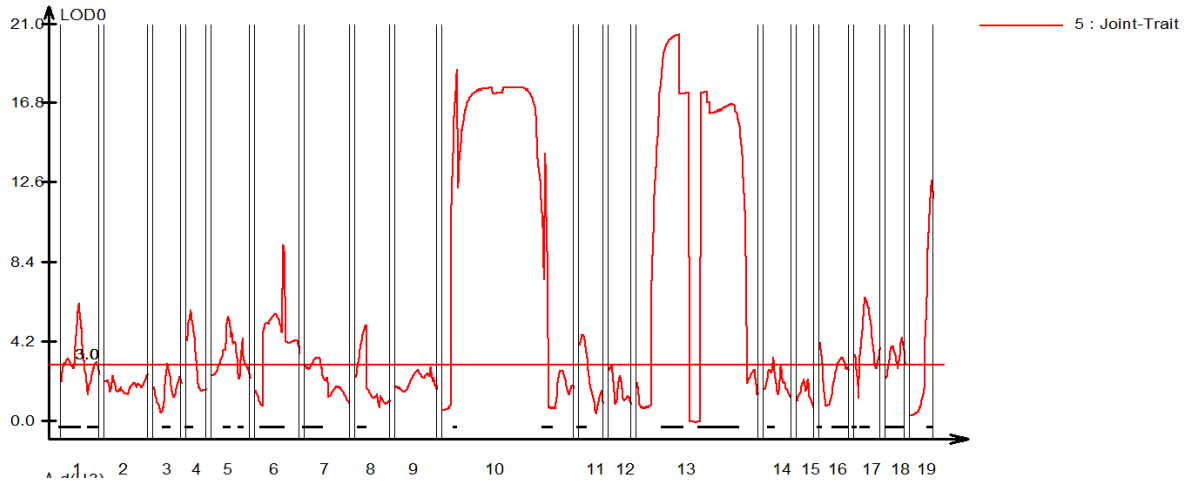

Figure 5. Results of multiple trait analysis showing QTLs detected based on joint analysis of the four fat pads: inguinal, gonadal, retroperitoneal, and total fat pad weight. Analysis was run using CIM method, Model 6, walking speed of 2 cM, and window size of 10. Novel QTLs for the joint analysis were identified on linkage groups 1, 4, 5, 6, and 8, where traits had not previously been identified by independent analysis.

4. Discussion and Conclusion

Segregation distortion of molecular markers has previously been reported in mouse (Schimenti, 2000). In this study, segregation distortion occurred on linkage group 3 for one marker, and it is noted that this distortion could have been inflated by missing data information as well as genotyping errors. It is also possible that segregation distortion existed in the original data set developed by Stylianou et al. (2006), but was removed prior to publication. Lyttle (1991) noted that segregation distorters are genetic elements that exhibit the phenomenon of meiotic drive. That is, the mechanics of the meiotic divisions cause one member of a pair of heterozygous alleles or heteromorphic chromosomes to be transmitted to progeny in excess of the expected Mendelian proportion of 50%. This phenomenon often causes difficulty in construction genetic maps and may also lead to detection of false positive QTLs. It is therefore important to test markers for normal segregation before furthering in to QTL mapping.

The quantitative traits used in this study were examined and because there was an observed departure from normality for the measured traits, there was an indication of the presence of major QTLs. According to Lynch and Walsh (1998), one would expect the phenotypic distribution to be skewed when a major gene is segregating. They note that most tests for deviation from normality are not powerful, and as such even a small non-significant departure from normality cannot be used to declare absence of a QTL.

Correlation analysis results showed a weak relationship between all the five fat pads and body weight, while fat pads themselves tended to co-vary. This result guided the decision in this study to conduct MTA during QTL mapping. Jiang and Zheng (1995) observed that multiple traits that are correlated can add information to each other, and as such, information from correlated traits can reduce the effect of error variance, therefore making it more powerful to detect QTL. Not only is the power of QTL detection is increased, the precision of the QTL map position is superior.

The initial scan of the genome with single marker analysis detected 24 significant QTLs associated with the fat pads and body weight across the genome. This result indicated that fat pad QTLs were clustered on linkage groups different from those of body weight. This outcome is consistent with the correlation results and suggests that body weight genes may be independent of those of the fat pads in mouse while those for fat pads cluster with each other.

Independent analysis using CIM revealed most QTLs as shared among the fat pads and only one QTL for body weight found on linkage group 17 was shared with other fat pads. This corroborated the SMA and correlation

results, suggesting that the five fat pads do show covariance. Interestingly, a joint analysis form MTA identified QTLs that were previously not detectable with independent trait analysis. This confirmed the power of a joint analysis of correlated traits in QTL mapping.

The study concluded that body weight and fat composition in mouse is governed by a few major additive genes making additional research and potential medical practices simpler. As such, further study into the genetic control and management of these traits is suggested, as well as research into potential methods of genetic therapy and targeted means of identifying individuals more at risk for obesity. Comparative mapping across species would also be a potential avenue for determining how obesity in mice translates to the genetics of the disease in humans and other animals.

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