Quantitative Trait Loci for Body Weight in the Intercross between SM/J and A/J Mice

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Abstract: We performed a genome-wide quantitative trait locus (QTL) analysis of body weight at 10 weeks of age in a population of 321 intercross offspring from SM/J and A/J mice, progenitor strains of SMXA recombinant inbred strains. Interval mapping revealed two significant QTLs, Bwq3 (body weight QTL3) and Bwq4, on Chromosomes (Chrs) 8 and 18 respectively, and five suggestive QTLs on Chrs 2, 6, 7, 15 and 19. Bwq3 and Bwq4 explained 6% of the phenotypic variance. The SM/J alleles at both QTLs increased body weight, though the SM/J mouse was smaller than the A/J mouse. On the other hand, four of the five suggestive QTLs detected had male-specific effects on body weight and the remainder was female-specific. These suggestive QTLs explained 5–6% of the phenotypic variance and all the SM/J alleles decreased body weight.

Key words: body weight, interval mapping, mouse, quantitative trait loci (QTLs)

Introduction

Although the genetic analysis of multifactorial traits is complicated, the availability of highly informative genetic markers that span the genome and specifically developed statistical methods has made the dissection of complex traits possible [14, 15, 32]. Quantitative trait locus (QTL) mapping methods have allowed dramatic progress toward the detection of major and minor loci involved in such traits [9]. Recent reports have identified QTLs affecting body weight in different mouse crosses [3, 4, 7, 12, 22].

The SMXA recombinant inbred (RI) strains were derived from systematic inbreeding of randomly selected

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pairs of the F2 generation from a cross between the SM/J and A/J inbred strains [24]. The progenitor strains of the SMXA RI strains differ in their origins [8], and have differences in a variety of phenotypic traits such as susceptibility to pulmonary adenoma, body weight and so on [24]. On susceptibility to pulmonary adenoma, Pataer et al. [26] identified the pulmonary adenoma resistance loci 3 (Par3) in a cross between a highly resistant strain (SMXA-24) and the susceptible parental strain (A/J). Body weight is another contrasting trait between SM/J and A/J mice, and is one of the characteristic features of the SM/J strain. The SM/J strain was selectively bred for small body size at 60 days of age, with about 50% lower body weight than counterparts selected for large size from the same foundation stocks [18]. We used an intercross population derived from the SM/J and A/J mice to search for QTLs affecting body weight at 10 weeks of age; SM/J mice were about 65% lighter than A/J.

Materials and Methods

Animals: All mice used in this study were bred at the Institute for Experimental Animals, Hamamatsu University School of Medicine (Hamamatsu, Japan). SM/J females were mated with A/J males to obtain F1 mice. The F1 progeny were intercrossed to produce 321 F2 mice (157 females and 164 males). The mice were housed in a conventionally conditioned room with 22 ± 2°C, 55 ± 5% humidity and 12L12D light, and were given ad libitum access to commercial diet (MR Breeder, Nihon Nosan Kogyo, KK, Yokohama, Japan) and tap water. The body weight of each individual was measured at 10 weeks of age, and the mice were subsequently sacrificed.

Genotyping: Genomic DNA samples were isolated from spleens or livers with the G NOME™ DNA Isolation Kit (Bio 101 Inc., La Jolla, Calif., USA). Mice were genotyped using ninety microsatellite markers purchased from Research Genetics Inc. (Huntsville, Ala., USA). Amplified products were resolved using 3% NuSieve GTG (FMC Bioproducts) and 1% LO3 agarose gel (TaKaRa) or 3.5% agarose gel (2% Metaphor and 1.5% SeaKem LE, FMC, Rockland, ME), stained with ethidium bromide and visualized with UV fluorescence.

QTL analysis: Values are expressed as mean ± SD. The associations between phenotypes and genetic markers in the F2 population were determined by Map Manager QTb28 [19], a program based on interval mapping, using the free regression model. The significance of each potential association was measured with the likelihood ratio statistic (LRS) of Haley and Knott [10]. LOD scores were obtained by dividing the LRS by 4.605 [17]. The significance threshold was computed by 1,000 permutations as recommended by Doerge and Churchill [6]. Each sex was analyzed separately to identify gender-specific QTLs, and sex-pooled data was analyzed to search for common QTLs in both sexes. Body weight was corrected for fixed effects of sex and litter size with the MIXED procedure of SAS software (SAS Institute, Cary, NC). Reduction of the variance due to environmental effects increases the power to detect QTLs [22]. After correction for these effects, normality of the distribution for body weight was tested with the UNIVARIATE procedure of SAS.

Results

The parental strains showed significant differences in body weight at 10 weeks of age. SM/J males (15.3 g ± 2.9, n=8) were significantly smaller than A/J males (23.6 ± 0.9, n=8) (P=0.0027, Mann-Whitney U test). SM/J females (13.5 g ± 2.2, n=8) were also significantly smaller than A/J females (20.1 ± 0.8, n=8) (P=0.0027). The body weight of F1 mice (24.2 g ± 1.76, n=11 in males; 19.4 ± 0.81, n=18 in females) showed significant differences with the SM/J parental strain (P=0.0009 in males, P=0.0003 in females). There were no significant differences in the body weight of F1 mice compared with the A/J parental strain. The mean body weights of F2 mice in males (23.2 g ± 2.2, n=164; P<0.0001) and females (19.4 ± 1.7, n=157; P<0.0001) were significantly greater than the SM/J parental strains, however there were no significant differences compared with the F1 and A/J mice. The frequency distributions of values for body weight did not deviate significantly from normality, thus the results of nontransformed data are reported here.

To identify QTLs affecting body weight, 321 F2 mice were genotyped at 90 microsatellite markers polymorphic between the SM/J and A/J parental strains. The following LOD scores were used for declaring sugges-
tive, significant and highly significant associations for body weight in the male (2.1, 3.4, 5.5), female (2.1, 3.5, 5.6) and sex-pooled (2.0, 3.6, 5.3) populations. By interval mapping, two significant QTLs for body weight were identified on chromosomes (Chrs) 8 and 18 in the pooled population (Fig. 1). We provisionally named the Chr 8 QTL as Bwq3 (body weight QTL3) and the Chr 18 QTL as Bwq4, because the gene symbols Bwq1 and Bwq2 have already been assigned to the body weight QTLs on Chrs 4 and 6, respectively, identified in the intercross of C57BL/6J and KK-A y mice [29].

Peak LOD score (4.6) in the pooled population for Bwq3 is located 3 cM distal to D8Mit88 (Fig. 1A). The 1-LOD support interval for Bwq3 is 5 cM proximal to D8Mit88 and 2 cM distal to D8Mit14, accounting for 6% of the total phenotypic variation. Separate analysis of body weight data for each sex showed suggestive LOD scores. Peak LOD score (2.6) for Bwq3 is located at D8Mit88 in females. In males, the peak LOD score (2.2) is located 4 cM proximal to D8Mit14. At the Bwq3 locus, the SM/J alleles were associated with increased body weight (Table 1). Bwq3 appeared to show additive or recessive inheritance, however the genetic model is more overrecessive ($h=-1.5$) in females, and recessive in males ($-0.73$) and in sex-pooled data ($-0.88$) as determined using the criteria of Stuber et al. [28].

Peak LOD score (4.8) in the pooled population for Bwq4 is located 14 cM distal to D18Mit15 and 10 cM proximal to D18Mit139 (Fig. 1B). The 1-LOD support interval for Bwq4 is 4 cM distal to D18Mit15 and 17 cM distal to D18Mit139, accounting for 6% of the phenotypic variation. Interestingly, increased body weight is also associated with the SM/J allele at this locus (Table 1). Separate analysis of body weight data for each sex showed suggestive LOD scores. The peak LOD score (3.2) for Bwq4 is located 11 cM distal to D18Mit15 in females. In males, the peak LOD score (2.1) is located 15 cM distal to D18Mit15. The SM/J alleles at this locus were also associated with increased body weight (Table 1). Bwq4 appeared to show dominant inheritance, however the genetic model is more appropriately overdominant in male ($h=1.3$), female (2.6) and sex-pooled data (1.8).

Five suggestive QTLs for body weight were also identified on Chrs 2, 6, 7, 15 and 19 (Table 1). The QTLs

Fig. 1. LOD score plots of QTLs for 10-week body weight on chromosomes 8(A) and 18(B). Map positions (cM) were calculated from the present data by using the Haldane mapping function. Gray bars to the right of the linkage map indicate 1-LOD support intervals of the QTL.
on Chrs 2, 7, 15 and 19 had male-specific effects on body weight. On the other hand, the QTL on Chr 6 was female-specific. All the SM/J alleles at these five suggestive loci were associated with decrease in body weight.

**Discussion**

*Bwq3* on distal Chr 8 is the first significant QTL for body weight identified in this region. Distal Chr 8 showed suggestive linkage with adiposity (adiposity index, gonadal and inguinal fat) in an intercross between C57BL/6J and KK mice, with increased trait values associated with the KK allele [31]. The *Bwq3* region has homology to human 16q23-q24 and 1q41-q42 [25]. There are no candidate genes for body weight within the 1-LOD support interval of the QTL on mouse Chr 8 as well as in the syntenic regions on human Chr 1 and 16. *Bwq4* on middle Chr 18 showed significant linkage to body weight in this study. This region showed suggestive linkage to body weight in the (C57BL/6J × KK-A*)F₂ mice [29]. Taylor and Phillips [30] also reported that this region showed a suggestive linkage to the adiposity index in a cross between 129/Sv and EL/Suz mice. *Bwq4* identified in this study may possibly correspond to these previous QTLs although further studies are necessary to verify whether they are allelic variants of the same locus. There are some interesting genes near *Bwq4*. Early growth response 1 (*Egr1*) and the glucocorticoid receptor-1 (*Grl1*) loci are located within the 1-LOD support interval [23]. Mice bearing an antisense RNA transgene of the glucocorticoid receptor were reported to develop marked obesity [27]. The implicated locus, *Bwq4* on Chr 18 has homology to human 5q21-q35 [25]. In humans, possible linkage between the glucocorticoid receptor (GRL, 5q31-q32) and obesity have been reported [5, 11, 16]. Cheverud *et al.* [4] identified QTLs for murine growth

<table>
<thead>
<tr>
<th>Chr</th>
<th>Population</th>
<th>Position of the QTL</th>
<th>Nearest proximal marker</th>
<th>1-LOD support interval</th>
<th>LOD score</th>
<th>% Variance</th>
<th>Effect</th>
<th>Genetic model</th>
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<td>2</td>
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<td>15</td>
<td><em>D2Mit6</em></td>
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<td>35</td>
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<td>6</td>
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<td>63</td>
<td><em>D6Mit287</em></td>
<td>33</td>
<td>2.6*</td>
<td>6.0</td>
<td>–0.39</td>
<td>0.33</td>
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<td>55</td>
<td><em>D6Mit287</em></td>
<td>35</td>
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<td>0.14</td>
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<td>82.4</td>
<td><em>D7Mit17</em></td>
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<td>2.2*</td>
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<td>–0.13</td>
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<td>–0.03</td>
<td>0.42</td>
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Raw data adjusted for fixed effects were used for the QTL analysis (see Materials and Methods for details). LOD scores, additive and dominant effects are presented for a free model. *Position of the QTL in cM from the centromere with reference to the Mouse Genome Database (1999). Chromosomal regions on the genetic map with values falling within one LOD score of the maximum value. *The maximum LOD scores, exceeding suggestive (*) and significant (**) threshold levels estimated by the Map Manager QT permutation test. The proportion of the phenotypic trait variation explained by the QTL. Additive (Add) and dominance (Dom) effect of the QTL shown in phenotypic standard deviation units. A positive sign for additive effect indicates an increase in the trait value conferred by the SM/J allele. A model in favor of an alternative was rejected when the LOD score under the first model was lower than the LOD score under the second model by at least one unit.
on 16 of the 19 autosomes using F₂ mice from SM/J and LG/J parental strains, with the latter selected for large body size. As expected given the origins of these strains, individuals which inherited the SM/J allele in the majority of the QTLs detected resulted in smaller size and slower growth. The QTLs for body weight at 10 weeks of age on Chr 7, 8, 15 and 18 detected in our cross had regions overlapping with the report of Cheverud et al. [4]. However in this study, the SM/J allele conferred greater body weight on the QTLs on Chr 8 and 18. In addition to the opposite effect conferred by the SM/J allele, the QTL for body weight on Chr 8 identified by Cheverud et al. [4] affected growth rate from 3 to 6 weeks of age whereas Bwq3 affected body weight at a much later age (10 weeks). However, both Bwq4 and the suggestive QTL on Chr 18 identified by Cheverud et al. [4] affected body weight at 10 weeks of age.

A previous QTL mapping study on the SMXA RI strains [1] identified two suggestive QTL for body weight on Chr 1 and 6. In the SMXA RI study, body weights were recorded for a long period from 10 to 20 weeks of age, and after correction for the age effect QTL analysis was performed [1]. As Cheverud et al. [4] have reported many age-specific QTLs affecting body weight in mice, it can be interpreted that the suggestive QTLs detected in the SMXA RI study reflect an average effect of body weights between 10 and 20 weeks. Therefore, there is not much point in comparing the QTL mapping result of the present study with that of the SMXA RI study.

Generally, it would be logical to assume that the SM/J allele would be associated with lower body weight and the A/J allele with the opposite effect. However, the (SM/J × A/J)F₂ mice in this study revealed two significant QTLs for body weight where the SM/J allele conferred an effect opposite from the strain’s phenotype (Table 1). Previous QTL studies on the genetic analysis of body weight [7], hypertension [13] and diabetes [20] have also led to the identification of such “cryptic factors” (alleles with opposite effects). It has clearly been established for several different complex traits that although the combined effect of alleles in “low-value” strains produces low-value phenotype, this does not necessarily imply that alleles for all genes by these strains favor such a phenotype, nor conversely, that each allele of the genes of a “high-value” strain favors the “high-value” phenotype [2]. The presence of certain strains (SMXA-7, SMXA-26, SMXA-21, SMXA-9) among the SMXA RI set with greater body weight than the heavy A/J inbred strain is strong evidence that SM/J mice possess alleles that confer high body weight [1]. Probably, unique genetic combinations among alleles from the parental strains interacted to cause an increase in body weight. All the other suggestive QTLs for body weight identified in this study nevertheless showed the SM/J allele to be associated with decrease in body weight. It is possible that there are some other undetected major QTLs for body weight with increasing effects conferred by the A/J allele.

In this study, major QTLs for body weight with increasing effects conferred by the A/J allele may be overlooked for two reasons: (1) the existence of two QTLs with opposite effects (one with positive effect and the other with negative effect, but both conferred by the A/J allele) on one chromosome may cancel out each other; and (2) the positive effect of the A/J allele may be apparent only when in critical interaction with the SM/J allele. In order to identify other major body weight increasing QTLs associated with the A/J allele, it would be interesting to cross the low body-weight SMXA-12 strain with the higher body-weight SMXA-21 strain [1]. SMXA-12 and SMXA-21 have SM/J alleles on distal Chr 8 and middle Chr 18 [21], the locations of Bwq3 and Bwq4, so these two loci will be fixed for the SM/J allele. These loci will not affect this cross, leading to the possible detection of major A/J-derived QTLs with an increasing effect on body weight.

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References

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