Mapping an Overdominant Quantitative Trait Locus for Heterosis of Body Weight in Mice

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The genetic basis of heterosis has not been elucidated. Previously, a congenic mouse strain with a 44-Mb genomic region of proximal chromosome 2 containing the allele derived from wild Mus musculus castaneus at Pbwg1, a quantitative trait locus (QTL) for body weight and growth, has been developed. In this study, to fine-map and characterize body weight QTLs on the congenic region, QTL analysis of body weight at 1, 3, 6, and 10 weeks after birth was performed on a population of 265 F2 intercross mice between the developed congenic strain and its background strain C57BL/6J. A significant QTL (named Pbwg1.10) affecting body weight at 6 and 10 weeks of age was identified within an approximately 21-Mb support interval. Surprisingly, Pbwg1.10 had an overdominance effect and caused heterosis for body weight. This result supported the overdominance hypothesis explaining heterosis.

Key words: body weight, heterosis, mice, overdominance, QTL

Materials and Methods

A total of 269 F2 individuals (143 males and 126 females) between B6.Cg-Pbwg1 congenic and C57BL/6J strains were used. Litter size was not standardized at birth to maximize the number of F2 mice reared. Body weight was measured at 1, 3, 6, and 10 weeks of age. Genomic DNA was extracted from mouse ear or tail clips, and 13 microsatellite markers on the 44-Mb introgressed region of the B6.Cg-Pbwg1 congenic strain were genotyped. Detailed body weight data, husbandry conditions for mice, and genotyping methods were described by Ishikawa et al. (2007).

Before QTL analysis, the effects of sex, dam, parity, and litter size on body weight in the F2 mice were tested using a linear model of the statistical discovery software JMP release 7.0.2] (SAS Institute Inc, Cary, NC). The covariates that were significant at the nominal 5% level were included in the QTL model as additive covariates.

Two simple interval mapping methods, maximum likelihood (Lander and Botstein 1989) and Haley–Knott regression
(Haley and Knott 1992), based on a single-QTL model were implemented with the computer package R/qtl version 1.07-12 that can include covariates directly in the QTL model to improve the power to detect QTLs (Broman et al. 2003). These interval mappings were carried out with 0.2-cM steps within each interval. An interaction effect between sex and QTL was tested with R/qtl as described by Solberg et al. (2004). Two-dimensional genome scans with a 2-QTL model were also performed with R/qtl to assess epistatic and additive interactions between 2 QTLs. To save computation time, the 2-dimensional scans were performed with 1-cM steps within each interval.

Using R/qtl, significance thresholds for the above single- and 2-QTL models were determined by performing a permutation test 1000 times, and the 1.5—logarithm of the odds (LOD) support interval of the QTL was computed. The percentage of total phenotypic variance explained by the QTL was calculated as 100(1 − 10−2LOG10/N), where N is the number of individuals. Phenotypic means of 3 possible genotypes at the marker closest to the QTL were computed using a 1-way analysis of variance (ANOVA) of JMP. The degree of dominance, that is, the ratio of dominance effect (difference in body weight between heterozygote and the average of 2 homozygotes) to additive effect (half the difference in body weight between 2 homozygotes), was determined by a 1-way ANOVA. As described by Kenney-Hunt et al. (2006), the mode of inheritance of the QTL was determined by use of the degree of dominance. That is, when the value for the degree of dominance was greater than 1.5, the Cas allele derived from wildCASTaneus mice on the congenic genomic region was considered overdominant to the B6 allele from C57BL/6J. When that value was between 1.5 and 0.5, Cas was considered dominant to B6.

Results

The covariates included in QTL analysis models were dam for 1-week body weight; dam and litter size for 3-week body weight; sex, dam, parity, and litter size for 6-week body weight; and sex and dam for 10-week body weight. Experiment-wide 5% significance thresholds determined by permutation tests ranged from 1.77 to 1.92 in LOD score units. Two simple interval mappings based on maximum likelihood and Haley–Knott regression methods provided nearly the same results. Only the results obtained by using the maximum likelihood method are presented here.

Simple interval mapping revealed 2 significant QTLs affecting body weight at 6 and 10 weeks of age in the F2 intercross mice between B6.Cg-<i>Pbwg1</i> congenic and C57BL/6J strains at experiment-wide 5% levels (Figure 1). Second peaks exceeding the 5% levels were seen for both body weights on the distal end of the congenic region. However, 2-QTL models did not fit significantly better than single-QTL models (<i>P > 0.05</i>), showing no evidence of second QTLs for the 2 body weights. The parameter estimates of the 2 significant QTLs detected are shown in Table 1. The 6-week body weight QTL peaked at 36.9 cM (64.8 Mb) from the centromere with a 1.5-LOD support interval of 14.1 cM in length, approximately 21 Mb between D2Mit270 (53 Mb) and D2Mit38 (74 Mb). The 10-week body weight QTL peaked at 36.1 cM (63.6 Mb) with a support interval of 28.0 cM, approximately 44 Mb between D2Mit33 (30 Mb) and D2Mit38 (74 Mb). For both QTLs, no statistical evidence for sex-by-QTL interactions was observed (<i>P > 0.05</i>).

The wild CASTaneus mouse has 60% of the body size of C57BL/6J (Ishikawa et al. 2000); thereby additive or recessive inheritance is usually expected for the QTLs identified. Surprisingly, body weight at 6 weeks of age for mice heterozygous for Cas and B6 alleles was significantly greater than those of Cas/Cas and B6/B6 homozygotes (<i>P < 0.05</i>), and the degree of dominance was 6.6 (Table 1). This clearly indicated that the 6-week body weight QTL was inherited in an overdominance manner. Body weight at 10 weeks of age for the Cas/B6 heterozygote was significantly greater than that of the B6/B6 homozygote but not that of Cas/Cas. However, the degree of dominance was 3.0, indicating that the Cas allele at the 10-week body weight QTL was overdominant to the B6 allele.
Table 1. Parameter estimates of significant QTLs for body weight at 6 and 10 weeks of age detected in an F2 intercross mouse population of B6.Cg-Plng1 congenic and C57BL/6J strains

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6-week body weight QTL</th>
<th>10-week body weight QTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum LOD score</td>
<td>3.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Map position in cM (Mb)</td>
<td>36.9 (64.8)</td>
<td>36.1 (63.6)</td>
</tr>
<tr>
<td>Support interval in cM (Mb)</td>
<td>14.1 (21)</td>
<td>28.0 (44)</td>
</tr>
<tr>
<td>%Variance</td>
<td>5.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Nearest microsatellite marker</td>
<td>D2Mit205</td>
<td>D2Mit205</td>
</tr>
</tbody>
</table>

* Distances from the centromere according to the database Mouse Genome Informatics (http://www.informatics.jax.org/, March 2008, Release 4.0).

* Length of the 1.5-LOD support interval.

* Least-squared mean ± standard error (SE) was computed by including fixed effects of genotype, sex, dam, parity, and litter size for 6-week body weight and genotype, sex, and dam for 10-week body weight in a linear model of the statistical discovery software JMP release 7.0.2. Cas denotes the allele derived from wild Mus musculus castaneus on the congenic genome region of B6.Cg-Plng1, and B6 shows the allele derived from C57BL/6J. The number of individuals is in parenthesis.

* Means with the same superscript letter in each body weight were not significantly different between genotypes at P > 0.05 (Tukey HSD test).

Although the pleiotropic effect of the 2 QTLs on body weight at 6 and 10 weeks of age was not able to be tested with R/qtl, the QTL detected was considered to be a single locus due to the following circumstantial evidence: nearly the same map positions, the same mode of inheritance, and a highly significant phenotypic correlation between the 2 body weights (r = 0.66, P = 1.8 × 10^{-6}). I thus named this QTL Plng1.10 to distinguish it from the names of 9 other QTLs, Plng1.1–Plng1.9, previously mapped to the present congenic region (Ishikawa et al. 2007).

To investigate the contribution of the Plng1.10 QTL to heterosis for body weight, 3 possible diplotype configurations were reconstructed using the F2 mice after removing recombinant individuals. As shown in Table 2, body weight at 6 and 10 weeks of age for the CAS/B6 diplotype was not significantly different from that of the best CAS/CAS diplotype. However, it was significantly greater than that of the B6/B6 diplotype (P < 0.05) and exceeded the average value of CAS/CAS and B6/B6 diplotypes. The value of the degree of dominance ranged from 2.7 to 4.1, meeting the definition of overdominance inheritance.

**Discussion**

The present fine-mapping using the B6.Cg-Plng1 congenic strain revealed a single overdominant QTL, named Plng1.10, affecting body weight at 6 and 10 weeks of age within an approximately 21-Mb support interval of proximal chromo-

**Table 2. Body weights (g) at 6 and 10 weeks of age for 3 diplotype configurations obtained from an F2 population of B6.Cg-Plng1 congenic and C57BL/6J strains**

<table>
<thead>
<tr>
<th>Body weight</th>
<th>Diplotype</th>
<th>N</th>
<th>Mean ± SE</th>
<th>Degree of dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 weeks</td>
<td>CAS/CAS</td>
<td>40</td>
<td>19.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>CAS/B6</td>
<td>86</td>
<td>19.8 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B6/B6</td>
<td>43</td>
<td>19.0 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>10 weeks</td>
<td>CAS/CAS</td>
<td>40</td>
<td>23.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>CAS/B6</td>
<td>86</td>
<td>23.9 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B6/B6</td>
<td>44</td>
<td>23.1 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Least-squared mean ± SE was computed by including fixed effects of diplotype, sex, dam, parity, sex × parity, and dam × parity for 6-week body weight and diplotype, sex, dam, parity, dam × parity, litter size, and dam × litter size for 10-week body weight in a linear model of JMP. There was no significant interaction between sex and diplotype for 2 body weights (P > 0.05). N is the number of individuals.

<sup>a</sup> CAS denotes the haplotype where all alleles at loci on the congenic genome region of B6.Cg-Plng1 are fixed for the wild-derived Cas alleles, and B6 indicates the haplotype on which all alleles are fixed for B6 alleles. Individuals with recombinant haplotypes were all removed from this analysis.

<sup>b</sup>c Means with the same superscript letter in each body weight were not significantly different between diplotypes at P > 0.05 (Tukey HSD test).

some 2. Several overdominant QTLs have been identified by genome-wide analyses of growth, reproductive, and other traits in mice (e.g., Cheverud et al. 1996; Rocha et al. 2004; Kenney-Hunt et al. 2006; Stylianou et al. 2006). However, there have been very few studies in which overdominance effects were confirmed in independent studies, and/or the locations were narrowed down in subsequent congenic studies.

The observed overdominance effect of Plng1.10 provided a convincing reason why the Plng1 QTL for body weight at 4–10 weeks of age identified in original genome-wide QTL analyses (Ishikawa et al. 2000, 2005; Ishikawa and Namikawa 2004) failed to be duplicated in a subsequent independent study (Ishikawa et al. 2007). In the original genome-wide studies, a backcross population obtained from a cross between (C57BL/6J females × wild Castaner males) F1 females and wild Castaner males was used (Ishikawa et al. 2000). In that backcross, 2 QTL genotypes, Cas/Cas and Cas/B6, were segregating. As shown in Table 1, the body weight of the Cas/Cas genotype contrasted with that of the Cas/B6 genotype, leading to detection of Plng1 in the original backcross. On the other hand, the Cas/Cas genotype had nearly the same body weight at 6 and 10 weeks of age as that of the B6/B6 genotype (Table 1), resulting in a failed duplication of Plng1 in the subsequent study on comparison of body weights at 1–10 weeks of age between B6.Cg-Plng1 congenic and C57BL/6J strains that are fixed for the Cas and B6 alleles, respectively (Ishikawa et al. 2007).

Heterosis is defined as the phenomenon of trait performance of F1 hybrids being superior to the average value of the 2 parental inbred strains or the value of the best parental strain (Hochhheading and Hoecker 2007). Although
body weight at 10 weeks of age for F1 hybrids between wild
*Mus musculus castaneus* and C57BL/6J mice was previously reported to be
slightly greater than the midparental value (Ishikawa et al. 2000), no body weight data were recorded for the present F1
mice between B6.Cg-*Plng1* and C57BL/6J strains. Furthermore, QTLs for maternal genetic effects on growth have
been reported in mice (Hager et al. 2008). Because the F1
mice and the 2 parental strains reared by their own strain
mothers have different maternal genetic effects, comparison
of body weights among them is likely to lead to an erroneous
conclusion. In this study, to meet the definition of heterosis
and to remove the maternal genetic effects, 3 diplotype
configurations were reconstructed using F2 mice that were
reared by F1 mothers with the same genotypes. Furthermore,
the genome-wide epistasis effect was eliminated as much as
possible in this study by use of the B6.Cg-*Plng1* congenic
mouse. Thus, the body weight comparison among the 3
diplotypes must provide evidence that a single overdominant
QTL contributes to heterosis for body weight.

Within the 1.5-LOD support interval of *Plng1.10*, 7
QTLs for lean body weight, white fat pad weight, and body
length have been reported (Ishikawa et al. 2007). Among
them, 3 loci for lean body weight and body length obviously
have dominance effects because of the degree of dominance
ranging from 1.2 to 1.4, and they are all in a coupling phase
(Ishikawa et al. 2007). Therefore, the pseudooverdominance
causled by closely linked dominant loci in a repulsion phase
(Birchler et al. 2006) is unlikely to contribute to the heterosis
observed in this study. However, further ultrafine-scale
mapping of *Plng1.10* will be needed to reject the
pseudooverdominance hypothesis completely.

In conclusion, the present result supports the over-
dominance hypothesis explaining heterosis (Birchler et al.
2006; Lippman and Zamir 2006). Because the wild-derived
DNA region of the congenic mouse strain has not been
artificially selected so far, it will be possible to investigate
whether the heterotic phenotype observed here has a role in
the evolution of fitness because body weight is one of
fitness traits.

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