Brief Communication

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

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Running title: Overdominant QTL Causing Heterosis

Abstract

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 F_2 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.

Keywords

body weight, heterosis, mice, overdominance, QTL

Heterosis, or hybrid vigor, is an important genetic nature in animal and plant breeding. There are two prominent genetic hypotheses explaining heterosis: dominance and overdominance, but it is not clear whether specific loci exhibit overdominance effects or whether heterozygosity itself confers heterosis in a genome-wide manner (reviewed by Birchler et al. 2006; Lippman and Zamir 2006; Hochholding and Hoecker 2007). In some cases, epistatic interactions among alleles at different loci appear to have a role in heterosis (Kusterer et al. 2007). Semel et al. (2006) mapped many quantitative trait loci (QTLs) for heterosis of yield and fitness in tomato by use of a series of introgression lines, each carrying a small chromosomal segment derived from a wild relative. This approach can largely eliminate the genome-wide epistasis that makes it difficult to dissect the complex nature of heterosis into individual components in segregating populations such as F_2 and backcross. However, very few approaches of this kind have been employed in animals, including laboratory rodents.

Recently, I have developed a congenic mouse strain, named B6.Cg-*Pbwg1*, with an approximately 44-Mb genomic region of proximal chromosome 2 containing the allele derived from wild *Mus musculus castaneus* captured in the Philippines at *Pbwg1*, by recurrent backcrossing to the common inbred strain C57BL/6J (Ishikawa et al. 2007). *Pbwg1* is one of the most potent QTLs affecting body weight and growth from 3 to 10 weeks after birth and it was previously identified in an intersubspecific backcross population between the wild *castaneus* and C57BL/6J mice; it exerts an effect linearly with increasing age and explains ~12% of the phenotypic variance, and the wild-derived allele decreases body weight (Ishikawa et al. 2000, 2005; Ishikawa and Namikawa 2004). In this study, to fine-map and characterize body weight QTLs on the congenic region harboring *Pbwg1*, I performed QTL analysis of body weight data previously obtained from F_2 intercross mice of the B6.Cg-*Pbwg1* congenic strain and its background strain C57BL/6J (Ishikawa et al. 2007).

Materials and Methods

A total of 269 F_2 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 F_2 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 F_2 mice were tested using a linear model of the statistical discovery software JMP release 7.0.2J (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 two-QTL model were also performed with R/qtl to assess epistatic and additive interactions between two QTLs. To save computation time, the two-dimensional scans were performed with 1-cM steps within each interval.

Using R/qtl, significance thresholds for the above single- and two-QTL models were determined by performing a permutation test 1000 times, and the 1.5-LOD support interval of the QTL was computed. The percentage of total phenotypic variance explained by the QTL

was calculated as 100 ($1-10^{-2LOD/N}$), where N is the number of individuals. Phenotypic means of three possible genotypes at the marker closest to the QTL were computed using a one-way ANOVA of JMP. The degree of dominance, i.e., the ratio of dominance effect (difference in body weight between heterozygote and the average of two homozygotes) to additive effect (half the difference in body weight between two homozygotes), was determined by a one-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 wild *castaneus* 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 two significant QTLs affecting body weight at 6 and 10 weeks of age in the F_2 intercross mice between B6.Cg-*Pbwg1* 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, two-QTL models did not fit significantly better than single-QTL models ($P > 0.05$), showing no

evidence of second QTLs for the two body weights. The parameter estimates of the two significant QTLs detected are shown in Table 1. The 6-week body weight QTL peaked at 36.9 cM (64.8 Mb) from the centromer 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 ($P > 0.05$).

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 ($P < 0.05$), 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.

Although the pleiotropic effect of the two 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 two body weights ($r = 0.66$, $P = 1.8 \times 10^{-63}$). I thus named this QTL *Pbwg1.10* to distinguish it from the names of nine QTLs, *Pbwg1.1* - *Pbwg1.9*, previously mapped to the present congenic region (Ishikawa et al. 2007).

To investigate the contribution of the *Pbwg1.10* QTL to heterosis for body weight, three possible diplotype configurations were reconstructed using the F_2 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-*Pbwg1* congenic strain revealed a single overdominant QTL, named *Pbwg1.10*, affecting body weight at 6 and 10 weeks of age within an approximately 21-Mb support interval of proximal chromosome 2. Several overdominant QTLs have been identified by genome-wide analyses of growth, reproductive and other traits in mice (for example, Cheverud et al. 1996; Kenney-Hunt et al. 2006; Rocha et al. 2004; 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 *Pbwg1.10* provided a convincing reason why the *Pbwg1* QTL for body weight at 4-10 weeks of age identified in original genome-wide QTL analyses (Ishikawa et al. 2000; 2004; 2005) 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 *castaneus* males) F_1 females and wild *castaneus* males was used (Ishikawa et al. 2000). In that backcross, two 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 *Pbwg1* 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 *Pbwg1* in the subsequent study on comparison of body weights at 1-10 weeks of age between B6.Cg-*Pbwg1* 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 F_1 hybrids being superior to the average value of the two parental inbred strains or the value of the best parental strain (Hochholding and Hoecker 2007). Although body weight at 10 weeks of age for F_1 hybrids between wild *castaneus* and C57BL/6J mice was previously reported to be slightly greater than the mid-parental value (Ishikawa et al. 2000), no body weight data were recorded for the present F_1 mice between B6.Cg-*Pbwg1* and C57BL/6J strains. Furthermore, QTLs for maternal genetic effects on growth have been reported in mice (Hager et al. 2008). Since the F_1 mice and the two 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, three diplotype configurations were reconstructed using F_2 mice that were reared by F_1 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-*Pbwg1* congenic mouse. Thus, the body weight comparison among the three diplotypes must provide evidence that a single overdominant QTL contributes to heterosis for body weight.

Within the 1.5-LOD support interval of *Pbwg1.10,* seven QTLs for lean body weight, white fat pad weight and body length have been reported (Ishikawa et al. 2007). Among them, three 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 pseudo-overdominance caused 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 ultra fine-scale mapping of *Pbwg1.10* will be needed to reject the pseudo-overdominance hypothesis completely.

In conclusion, the present result supports the overdominance hypothesis explaining heterosis (Birchler et al. 2006; Lippman and Zamir 2006). Since 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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Figure Legend

Figure 1. LOD score plots of significant QTLs for body weight at 6 weeks (solid line) and 10 weeks (dashed line) of age identified in 269 F_2 intercross mice of the B6.Cg-*Pbwg1* congenic strain and its background strain C57BL/6J. Maximum-likelihood interval mapping was performed with the computer package R/qtl version 1.07-12 (Broman et al. 2003). The significance threshold level ($\alpha = 0.05$) was determined by performing a permutation test 1000 times. A sex-averaged, genetic linkage map was constructed from the F_2 segregation data using Kosambi map function. Average maker spacing was 1.6 cM. Arrows indicate the positions of the QTLs. The vertical lines on the x-axis indicate the linkage map positions of the 13 microsatellite markers on mouse chromosome 2 genotyped in this study, and the number on the vertical line is the name of the marker (*D2Mit#*).

Table 1. Parameter estimates of significant QTLs for body weight at 6 and 10 weeks of age detected in an F_2 intercross mouse population of B6.Cg-*Pbwg1* congenic and C57BL/6J strains

Parameter	**6-week body weight QTL**	**10-week body weight QTL**
Maximum LOD score	3.1	2.2
Map position in cM (Mb)[a]	36.9 (64.8)	36.1 (63.6)
Support interval in cM (Mb)[b]	14.1 (21)	28.0 (44)
%Variance	5.2	3.7
Nearest microsatellite marker	*D2Mit205*	*D2Mit205*
Body weight (g) for three genotypes at the nearest marker[c]		
Cas/*Cas*	19.5 ± 0.2[d] (63)	23.8 ± 0.2[de] (63)
Cas/*B6*	20.1 ± 0.1[e] (140)	24.1 ± 0.2[d] (141)
B6/*B6*	19.3 ± 0.2[d] (64)	23.4 ± 0.2[e] (65)
Degree of dominance	6.6	3.0

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

[b] Length of the 1.5-LOD support interval.

[c] 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 7.0.2J (SAS Institute Inc., Cary, NC). *Cas* denotes the allele derived from wild *M. m. castaneus* on the congenic genome region of B6.Cg-*Pbwg1*, and *B6* shows the allele derived from C57BL/6J. The number of individuals is in parenthesis.

[d, e] Means with the same superscript letter in each QTL were not significantly different between genotypes at $P > 0.05$ (Tukey HSD test).

Table 2. Body weights (g) at 6 and 10 weeks of age for three diplotype configurations obtained form an F_2 population of B6.Cg-*Pbwg1* congenic and C57BL/6J strains

Body weight	Diplotype[a]	*N*	Mean ± SE	Degree of dominance
6 weeks	CAS/CAS	40	19.3 ± 0.2 [bc]	
	CAS/B6	86	19.8 ± 0.2 [b]	4.1
	B6/B6	43	19.0 ± 0.2 [c]	
10 weeks	CAS/CAS	40	23.5 ± 0.2 [bc]	
	CAS/B6	86	23.9 ± 0.2 [b]	2.7
	B6/B6	44	23.1 ± 0.2 [c]	

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 two body weights ($P > 0.05$). *N* is the number of individuals.

[a] CAS denotes the haplotype where all alleles at loci on the congenic genome region of B6.Cg-*Pbwg1* 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.

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