Fine Mapping and Candidate Gene Search of Quantitative Trait Loci for Growth and Obesity Using Mouse Intersubspecific Subcongenic Intercrosses and Exome Sequencing

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Abstract

Although growth and body composition traits are quantitative traits of medical and agricultural importance, the genetic and molecular basis of those traits remains elusive. Our previous genome-wide quantitative trait locus (QTL) analyses in an intersubspecific backcross population between C57BL/6J and wild Mus musculus castaneus mice revealed a major growth QTL (named Pbwg1) on a proximal region of mouse chromosome 2. Using the B6.Cg-Pbwg1 intersubspecific congenic strain created, we revealed 12 closely linked QTLs for body weight and body composition traits on an approximately 44.1-Mb wild-derived congenic region. In this study, we narrowed down genomic regions harboring three (Pbwg1.12, Pbwg1.3 and Pbwg1.5) of the 12 linked QTLs and searched for possible candidate genes for the QTLs. By phenotypic analyses of F2 intercross populations between B6 and each of four B6.Cg-Pbwg1 subcongenic strains with overlapping and non-overlapping introgressed regions, we physically defined Pbwg1.12 affecting body weight to a 3.8-Mb interval (61.5–65.3 Mb) on chromosome 2. We fine-mapped Pbwg1.3 for body length to an 8.0-Mb interval (57.3–65.3) and Pbwg1.5 for abdominal white fat weight to a 2.1-Mb interval (59.4–61.5). The wild-derived allele at Pbwg1.12 and Pbwg1.3 uniquely increased body weight and length despite the fact that the wild mouse has a smaller body size than that of B6, whereas it decreased fat weight at Pbwg1.5. Exome sequencing and candidate gene prioritization suggested that Gcg and Grb14 are putative candidate genes for Pbwg1.12 and that Ly75 and Igb6 are putative candidate genes for Pbwg1.5. These genes had nonsynonymous SNPs, but the SNPs were predicted to be not harmful to protein functions. These results provide information helpful to identify wild-derived quantitative trait genes causing enhanced growth and resistance to obesity.

Introduction

Body weight and body composition traits, including fat and organ weight, are quantitative in nature and are controlled by multiple genetic loci, referred to as QTLs (quantitative trait loci), environmental factors and their interactions. They are important economic traits in livestock [1]. For example, modern broiler chickens have been intensively selected for rapid growth rate, but they display excessive deposition of body fat. Since fat is a by-product of little economic value and often causes a decrease in feed efficiency, it is now an important selection criterion in chicken breeding programs [2]. In humans, obesity is characterized by excessive abdominal fat deposition and is now a main health concern worldwide because it is a predisposing factor of complex metabolic diseases such as type-2 diabetes and cardiovascular diseases [3]. The laboratory mouse has been long and widely used as the premier model animal for elucidating the genetic and molecular basis of these traits and other quantitative traits in livestock and humans because of its small body size, cost-effective rearing, easy development of genetically engineered mice (e.g., knockouts and transgenics) and large amount of genomic information that is freely available [1,4]. Thousands of QTLs affecting various quantitative traits have been mapped to many chromosomal regions of the mouse and have been deposited in the Mouse Genome Database (MGD, release 5.19, August 2014) [5].

However, the genetic and molecular basis of quantitative traits remains elusive because it is not an easy task to pinpoint causative genes underlying QTLs, particularly for QTLs with small phenotypic effects on the traits. Most of the QTLs have small effects and only a few loci have moderate to large effects [4]. In mice, initial genome-wide QTL analysis is usually performed with a backcross or F2 intercross population between two inbred strains and it provides a large confidence interval (10–50 cM) for a mapped QTL [6], where hundreds or thousands of genes are possibly located. Next, to reduce the confidence interval of the QTL to a level amenable to positional cloning, fine mapping is performed using a congenic mouse strain and subsequently

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developed subcongenic strains [7]. Then phenotypic values are compared between homozygous congenic/subcongenic strains and the background strain and/or among homozygous congenic/subcongenic strains with overlapping and non-overlapping introgressed regions. Often, the phenotypic effect of the QTL fails to be confirmed, illustrating the difficulty of identifying a causative gene for the QTL. If the QTL successfully is fine-mapped to a small region, the road from a QTL to a causative gene is still long [8]. In the present study, to overcome the problem of frequent failure in traditional congenic/subcongenic analyses, we used several F2 populations obtained from intercrosses between each of the subcongenic strains and the background strain. In each of the F2 populations, three possible diplootypes for the introgressed region are segregating: two are homozygous for either haplotype derived from the donor mouse or from the background mouse and the other is heterozygous for both haplotypes. Hence, using the F2 mice can randomize environmental effects such as litter size and effects of contaminating donor and recipient alleles on unwanted small regions, both of which are produced by double recombination during recurrent backcrossing for development of subcongenic strains, as previously documented [9,10]. Moreover, the F2 mice produced have genetically identical F1 dams and F1 sires. That is, the F1 mice are heterozygotes for all loci on the introgressed region. Hence, using the F2 mice can minimize genomic imprinting effects of alleles inherited from either F1 dams or F1 sires [11] and maternal genetic effects exerted from the F1 dams [12] and epigenetic effects such as histone modification [13–15] exerted from either or both F1 parents. Probably, some of these effects result in the missing QTL effect seen in traditional congenic/subcongenic analyses.

We previously discovered many QTLs for postnatal body weight and growth from an untapped resource of wild M. m. castaneus mice captured live in the Philippines, by genome-wide QTL analyses in an interspecific backcross population between C57BL/6J and B6.Cg-Pbwg1 congenic strain on the B6 genetic background with an approximately 44.1-Mb wild-derived genomic region between D2Mit33 and D2Mit38 microsatellite markers, on which Phag1, a prominent growth QTL on a proximal region of mouse chromosome 2, is located. We developed more than 20 subcongenic strains derived from B6.Cg-Pbwg1. By phenotypic analysis of the F2 intercross between B6.Cg-Pbwg1 and B6 strains and by congenic/subcongenic analyses, we revealed 12 closely linked QTLs for body weight and body composition traits within the 44.1-Mb congenic region. These linked QTLs explained a small fraction of phenotypic variances [9,19–22]. Among the linked loci, several have unique QTL effects and are located on the distal half of the congenic region. For example, the wild-derived allele at Phag1.12 and Phag1.3 QTLs increases body weight and total body length, respectively, despite the fact that wild mice have a smaller body size than that of B6 [19,9]. In contrast, the allele at Phag1.5 QTL decreases abdominal white fat weight [19] and prevents obesity in mice fed both standard and high-fat diets [21].

In this study, we fine-mapped the three unique QTLs (Phag1.12, Phag1.3 and Phag1.5) mentioned above by phenotypic analysis of F2 mice obtained from intersubspecific subcongenic intercrosses. To search for possible candidate genes of the QTLs, we performed exome sequencing of genes on the congenic region and also prioritized candidate genes using bioinformatics analysis. Sequence data are not available for our wild M. m. castaneus mice captured in the Philippines, in contrast to the CAST/Ej inbred strain derived from wild M. m. castaneus mice trapped in Thailand, for which the whole genome has been already sequenced [23].

Materials and Methods

Ethics Statement

This study was carried out in accordance with the guidelines for the care and use of laboratory animals of the Graduate School of Bioagricultural Sciences, Nagoya University, Japan. The protocol
was approved by the Animal Research Committee of Nagoya University.

**Animals**

The B6.Cg-PhagI congenic strain was previously constructed [19]. Many subcongenic strains, named B6.Cg-PhagI/#Nga (old name: B6.Cg-PhagI/SR#, called SR# hereafter), were previously developed from descendants of B6.Cg-PhagI [9]. Previously developed SR1, SR3 and SR12 subcongenic strains and a newly developed SR21 subcongenic strain were used in this study (Figure 1). The background C57BL/6/Jcl (B6) mice were purchased from Clea Japan (Tokyo, Japan). To develop four F2 segregating populations, males of each subcongenic strain were crossed with B6 females to generate F1 mice. The F1 mice obtained were mated inter se. In total, the following F2 individuals were produced: 273 (138 males and 135 females) for B6×SR1, 236 (113 males and 123 females) for B6×SR2, 132 (58 males and 74 females) for B6×SR12 and 291 (151 males and 140 females) for B6×SR21. Litter size was not standardized at birth to maximize the number of F2 mice reared.

All mice were weaned at 3 weeks of age. Littermates of the same sex were housed in groups of up to four mice per cage. Standard chow (CA-1, Clea Japan), containing 27% crude protein, 5% crude fat, 3% crude fiber, 8% crude ash and 3.5 kcal/g, and tap water were provided *ad libitum*. The mice were reared in an environment with a temperature of 23±3°C, 55% relative humidity, and a light/dark cycle of 12:12.

**Genotyping**

Genomic DNAs were extracted with a standard method from ear clips of the F2 mice. Microsatellite markers located within each of the subcongenic intervals (Figure 1) were genotyped as described previously [19]. To reduce as short as possible the gray regions flanking the subcongenic intervals, where recombination occurred, we newly developed two PCR-RFLP markers based on two SNPs identified by exome sequencing in this study (Figure S1). Each of the F2 mice had one of three diplotypes (B/B, B/C and C/C), where B is the haplotype derived from B6 mice and C is the haplotype derived from wild *castaneus* mice. Diplotype configuration was determined for each mouse of the four F2 populations produced. F2 mice having recombination within the subcongenic interval were excluded from this study.

**Phenotyping**

Body weight of F2 mice was measured to the nearest 0.01 g at 1, 3, 6, 10 and 14 weeks of age. Four body weight gains at 1–3 weeks, 3–6 weeks, 6–10 weeks and 10–14 weeks of age were calculated. After overnight fasting, mice were sacrificed under anesthesia. Total body length (from the tip of the nose to the end of the tail) and tail length (from the anus to the end of the tail) were immediately measured to the nearest 0.01 cm. Head-body length was obtained by subtracting tail length from total body length. After taking a blood sample by cardiopuncture, the lungs, spleen, liver, kidneys and testes were dissected and weighed to the nearest 0.001 g. In addition, the weights of two-sided inguinal and gonadal (epidydimal in males and parametrical in females) fat pads were recorded. In mice, the weight of white fat depots such as gonadal (epidydimal in males and parametrical in females) and perirenal fat pads has been long and widely used as an indicator of fatness because the fat depots can be easily dissected out and are highly correlated with total body fat [1].

Body weight, weight gain and body composition data obtained for the F2 populations were analyzed with a linear mixed model of the statistical discovery software JMP version 11.1.1 (SAS Institute, Cary, NC) in which diplotype, sex, parity, litter size and their possible two-way interactions were treated as fixed effects and dam was treated as a random effect. The fixed effects and interaction effects that were significant at the nominal 5% level were included in the final model. Phenotypic differences among diplotypes were determined by one-way analysis of variance followed by Tukey’s HSD test. To adjust for multiple testing, Bonferroni-corrected 5% level was finally used as a significant threshold.

To estimate additive and dominance effects of diplotypes, a linear mixed model was fitted, with additive, dominance, sex, parity, litter size and their possible two-way interactions being included as fixed effects and dam being included as a random effect. The fixed effects and interaction effects that were significant at the nominal 5% level were finally included in the model. As previously defined for allelic effects of a QTL [24], additive diplotype effect is half of the difference between B/B and C/C homozygous diplotypes, and dominance diplotype effect is the difference between B/C heterozygous diplotypes and the average of B/B and C/C homozygous diplotypes. To estimate the additive
Table 1. Additive and dominance diplotype effects for body weight at # weeks of age and weight gain between # and # weeks of age in the F2 populations obtained from B6×SR1 and B6×SR21 intercrosses.

| Sex   | F2 population | Trait  | Additive | P value | Dominance | P value | Degree of dominance | Inheritance b |
|-------|---------------|--------|----------|---------|-----------|---------|---------------------|---------------|
| Male  | B6×SR1        | Wt1    | 0.039±0.046 | 0.40   | 0.049±0.069 | 0.47   | -                   | -             |
|       |               | Wt3    | 0.041±0.111 | 0.71   | 0.193±0.170 | 0.26   | -                   | -             |
|       |               | Wt6    | 0.525±0.173 | 0.0030 | 0.376±0.264 | 0.16   | 0.72                | Add           |
|       |               | Wt10   | 0.788±0.167 | 0.0000076 | 0.582±0.256 | 0.025 | 0.73                | Dom           |
|       |               | Wt14   | 0.874±0.178 | 0.0000034 | 0.586±0.271 | 0.033 | 0.67                | Dom           |
|       |               | Gain1−3| 0.023±0.095 | 0.81   | 0.151±0.142 | 0.29   | -                   | -             |
|       |               | Gain3−6| 0.395±0.128 | 0.0026 | 0.206±0.196 | 0.30   | 0.52                | Add           |
|       |               | Gain6−10| 0.267±0.123 | 0.031 | 0.040±0.185 | 0.83   | 0.15                | Add           |
|       |               | Gain10−14 | 0.160±0.102 | 0.12 | −0.021±0.153 | 0.89   | -                   | -             |
|       | B6×SR21       | Wt1    | −0.001±0.040 | 0.98   | −0.034±0.062 | 0.59   | -                   | -             |
|       |               | Wt3    | −0.066±0.097 | 0.49   | −0.319±0.140 | 0.024 | −4.83               | Overrec        |
|       |               | Wt6    | 0.198±0.164 | 0.23   | −0.379±0.238 | 0.11   | -                   | -             |
|       |               | Wt10   | 0.635±0.172 | 0.000032 | −0.483±0.249 | 0.055 | −0.76               | Add           |
|       |               | Wt14   | 0.833±0.192 | 0.000030 | −0.552±0.279 | 0.050 | −0.66               | Rec           |
|       |               | Gain1−3| −0.074±0.070 | 0.29   | −0.282±0.102 | 0.0065 | −3.81               | Overrec        |
|       |               | Gain3−6| 0.277±0.144 | 0.016 | −0.045±0.166 | 0.78   | −0.16               | Add           |
|       |               | Gain6−10| 0.440±0.121 | 0.00039 | −0.099±0.176 | 0.57   | −0.23               | Add           |
|       |               | Gain10−14 | 0.155±0.072 | 0.033 | −0.080±0.105 | 0.45   | −0.52               | Add           |
| Female| B6×SR1        | Wt1    | −0.069±0.047 | 0.15   | −0.146±0.063 | 0.023 | −2.12               | Overrec        |
|       |               | Wt3    | −0.156±0.091 | 0.089 | −0.278±0.120 | 0.023 | −1.78               | Overrec        |
|       |               | Wt6    | 0.006±0.136 | 0.97   | −0.092±0.185 | 0.62   | -                   | -             |
|       |               | Wt10   | 0.060±0.122 | 0.62   | −0.423±0.164 | 0.011 | −7.05               | Overrec        |
|       |               | Wt14   | 0.099±0.136 | 0.47   | −0.379±0.182 | 0.040 | −3.83               | Overrec        |
|       |               | Gain1−3| −0.079±0.061 | 0.19   | −0.160±0.081 | 0.051 | −2.03               | Overrec        |
|       |               | Gain3−6| 0.010±0.111 | 0.93   | −0.030±0.152 | 0.84   | -                   | -             |
|       |               | Gain6−10| 0.177±0.096 | 0.069 | −0.143±0.130 | 0.27   | -                   | -             |
|       |               | Gain10−14 | 0.033±0.079 | 0.68 | 0.058±0.107 | 0.59   | -                   | -             |
|       | B6×SR21       | Wt1    | 0.039±0.042 | 0.36   | 0.003±0.058 | 0.96   | -                   | -             |
|       |               | Wt3    | 0.094±0.096 | 0.33   | 0.207±0.132 | 0.12   | -                   | -             |
|       |               | Wt6    | 0.271±0.114 | 0.019 | −0.103±0.157 | 0.51   | −0.38               | Add           |
|       |               | Wt10   | 0.301±0.119 | 0.013 | −0.201±0.165 | 0.22   | −0.67               | Add           |
|       |               | Wt14   | 0.208±0.120 | 0.086 | −0.204±0.116 | 0.22   | -                   | -             |
|       |               | Gain1−3| 0.058±0.068 | 0.39   | 0.216±0.094 | 0.023 | 3.72                | Overdom       |
diplotype effect, diplotypes were assigned quantitatively as $-1$ for B/B homozygotes, zero for B/C heterozygotes and $+1$ for C/C homozygotes. To estimate the dominance diplotype effect, diplotypes were assigned as zero for two types of homozygotes and $+1$ for heterozygotes. The degree of dominance was calculated as the ratio of dominance diplotype effect to additive diplotype effect.

Exome Sequencing

Genomic DNA was extracted with a standard method from the tail of a B6.Cg-Plbwg1 congenic mouse. Enrichment of exon regions in the 44.1-Mb congenic interval on mouse chromosome 2 was performed using Roche NimbleGen sequence capture arrays that were custom-made on the basis of UCSC Mouse Genome Browser NCBI37/mm9 assembly (RefSeq mm9). Enrichment experiments and exome sequencing with the next-generation sequencer Roche GS FLX were outsourced to Hokkaido System Science Co., Ltd (Sapporo, Japan). Sequence reads obtained were mapped to RefSeq mm9, and then synonymous SNPs (sSNPs), nonsynonymous SNPs (nsSNPs), indels (insertions and deletions) and nonsense mutations were investigated.

Candidate Gene Search

Endevour is a web-based computational software program that prioritizes candidate genes with respect to their biological processes or diseases of interest [25]. Genes on target regions carrying QTLs for body weight and obesity were prioritized on the basis of similarity to training genes that have already been shown to be involved in body weight and obesity regulation (Table S1). The training genes used were searched using Online Mendelian Inheritance in Man (OMIM) database (http://www.omim.org).

Effects of nsSNPs identified by exome sequencing on protein functions were investigated with two web-based software programs, SIFT [26] and PolyPhen-2 [27]. SIFT predicts tolerated and deleterious substitutions for nsSNPs based on the evolutionary conservation of amino acids within protein families [26]. PolyPhen-2 predicts possible impact of an amino acid substitution on the structure and function of a protein using straightforward physical and comparative considerations [27]. Since PolyPhen-2 was developed for human proteins, this software was implemented after converting the positions of amino acid substitutions in our mouse study to the corresponding positions of the human protein.

Results

Intersubspecific Subcongenic Intercross Analyses

Most growth and body composition traits examined in F2 segregating populations between each of the four subcongenic strains (Figure 1) and the background B6 strain showed significant interactions between sex and trait (data not shown). We thus performed statistical comparisons of these traits among mice with three diplotypes in each sex separately.

Figure 2 shows measurements of body weight and body weight gain in the four F2 segregating populations. In the B6×XR1 intercross, body weight of male mice with C/C diplotype at 6 weeks of age was significantly higher than that of mice with the B/B diplotype ($P = 0.0041$, Tukey’s HSD test) at the Bonferroni-corrected 5% significance level. However, it was not different from that of B/C males throughout ages examined. From 6 weeks onwards, the weight difference between C/C and B/B males remained significant ($P = 0.0000036$ at 10 weeks and $0.0000017$ at 14 weeks of age). Additive diplotype effects for body weights at 6, 10 and 14 weeks of age exceeded the Bonferroni-corrected 5% level, whereas dominance diplotype effects were not significantly
Table 2. Body length and fat pad weight not adjusted for body weight at 14 weeks of age, and additive and dominance diplotype effects for body length and fat pad weight in the F2 populations obtained from B6xSR1 and B6xSR21 intercrosses.

| Sex | F2 population | Trait | B/B (cm) | B/C (cm) | C/C (cm) | P value | Additive | P value | Dominance | P value | Degree of dominance | Inheritance |
|-----|---------------|-------|----------|----------|----------|---------|----------|----------|------------|---------|---------------------|------------|
| Male| B6xSR1        | No. of mice | 33       | 33–34    | 35–37    |         |          |          |            |         |                     | Add        |
|     |               | Tail length (cm) | 8.33 ±0.04   | 8.42 ±0.04   | 8.39 ±0.04   | 0.13    | 0.028 ±0.024   | 0.24    | 0.060 ±0.038   | 0.12    | -                   | -          |
|     |               | Head-body length (cm) | 9.20 ±0.04    | 9.34 ±0.04    | 9.38 ±0.04    | 0.00079  | 0.088 ±0.025   | 0.0080  | 0.058 ±0.041   | 0.17    | 0.66                | Add        |
|     | B6xSR21       | No. of mice | 32       | 38–39    | 38–39    |         |          |          |            |         |                     |            |
|     |               | Tail length (cm) | 8.38 ±0.04   | 8.38 ±0.03   | 8.45 ±0.03   | 0.13    | 0.034 ±0.021   | 0.11    | −0.039 ±0.033 | 0.24    | -                   | -          |
|     |               | Head-body length (cm) | 9.26 ±0.04    | 9.27 ±0.03    | 9.41 ±0.03    | 0.00021  | 0.078 ±0.021   | 0.0035  | −0.070 ±0.033 | 0.038   | −0.90               | Rec        |
| Female| B6xSR1       | No. of mice | 24       | 34–36    | 32       |         |          |          |            |         |                     |            |
|      |               | Tail length (cm) | 8.14 ±0.03b  | 8.11 ±0.03b  | 8.20 ±0.03a  | 0.38    | 0.030 ±0.021   | 0.16    | −0.063 ±0.031 | 0.048   | −2.10               | Overrec    |
|      |               | Head-body length (cm) | 8.88 ±0.05b  | 8.77 ±0.04b  | 8.91 ±0.04a  | 0.023   | 0.013 ±0.030   | 0.67    | −0.122 ±0.045 | 0.0081  | −9.38               | Overrec    |
|      | B6xSR21       | No. of mice | 28       | 38–39    | 33       |         |          |          |            |         |                     |            |
|      |               | Tail length (cm) | 8.00 ±0.03a  | 8.09 ±0.03b  | 8.16 ±0.03b  | 0.0024  | 0.077 ±0.021   | 0.00052 | 0.011 ±0.032 | 0.73    | 0.14                | Add        |
|      |               | Head-body length (cm) | 8.85 ±0.04ab | 8.88 ±0.03b  | 8.98 ±0.03b  | 0.012   | 0.065 ±0.023   | 0.0064  | −0.035 ±0.036 | 0.33    | −0.54               | Add        |
|      |               | Inguinal fat pad weight (g) | 16.85 ±0.06a | 16.99 ±0.05a | 17.15 ±0.05b | 0.00051 | 0.150 ±0.004   | 0.00011 | −0.013 ±0.057 | 0.82    | −0.87               | Rec        |

Additive effect on body length and fat pad weight in F2 populations obtained from B6xSR1 and B6xSR21 intercrosses.
Owing to space limitation, we provide in Tables S1 and S2 the detailed information on the traits studied, as well as the diplotype frequencies and heritability estimates.

In Table 2, we present the results of a linear model analysis including fixed and random effects to evaluate the diplotype effect on body composition traits. The results show that there were significant differences among the three diplotypes, indicating the presence of QTLs with different modes of inheritance.

The C haplotype derived from wild mice increased body weight, despite the fact that the wild mice have approximately 60% of the body weight of B6 mice [16]. The mode of inheritance of this haplotype was additive or dominant (Table 1). In females, however, there were no significant differences in body weight at any age (Figure 2). For body weight gains at 1–3 weeks, 3–6 weeks, 6–10 weeks and 10–14 weeks, both sexes of mice with three diplotypes did not show significant differences at the Bonferroni-corrected 5% level. Although body weight gain in males at 3–6 weeks ($P = 0.0058$) was on the border of that level, its additive diplotype effect surpassed it (Table 1).

In B6×SR2 and B6×SR12 intercrosses, body weights at any age did not differ significantly among mice with three diplotypes in both sexes. Similarly, there were no significant diplotype differences in body weight gains at any age in both sexes (Figure 2 and Table S2).

On the other hand, in the B6×SR21 intercross, body weights of C/C males were significantly higher than those of B/C and B/B males at 10 weeks ($P = 0.00028$) and 14 weeks ($P = 0.000029$) of age (Figure 2). The wild-derived C haplotype was transmitted as different modes of inheritance in males (additive) and females (overrecessive) for an unknown reason. For inguinal fat pad weight and gonadal fat pad weight, C/C mice had the lowest values among mice with three diplotypes in both sexes. The C haplotype was inherited in an additive or recessive fashion depending on the sex. A significant difference in kidney weight was observed only in females (Table S3).

In B6×SR21 intercross, unadjusted body length traits were significantly different in both sexes at the Bonferroni-corrected 5% level. C/C mice had the longest length in both sexes. Similarly, there were no significant diplotype differences at any age in both sexes (Figure 2). For body weight and body weight gain in males at 3–6 weeks ($P = 0.0058$) was on the border of that level, its additive diplotype effect surpassed it (Table 1).

Tables 2 and S3 show measurements of body composition traits not adjusted for final body weight at 14 weeks of age in two kinds of F2 populations obtained from B6×SR1 and B6×SR21 intercrosses. In the B6×SR1 intercross, head-body length and total body length of C/C males were both significantly larger than those of B/B males at the Bonferroni-corrected 5% level (Table 2). The same tendency was observed for those traits in females, but the C haplotype was transmitted as different modes of inheritance in males (additive) and females (overrecessive) for an unknown reason. For inguinal fat pad weight and gonadal fat pad weight, C/C mice had the lowest values among mice with three diplotypes in both sexes. The C haplotype was inherited in an additive or recessive fashion depending on the sex. A significant difference in kidney weight was observed only in females (Table S3).

On the other hand, in both B6×SR2 and B6×SR12 intercrosses, no unadjusted traits were significantly different among mice with three diplotypes at the Bonferroni-corrected 5% level as well as the nominal 5% level (Table S4).

Tables 3 and S5 show measurements of body composition traits adjusted for final body weight at 14 weeks of age, which are body-size-free traits, in two F2 populations obtained from B6×SR1 and B6×SR21 intercrosses. In the B6×SR1 intercross, significant diplotype differences were observed in inguinal and gonadal fat pad weights for both sexes and in testis weight for males at the Bonferroni-corrected 5% level. Both of the fat weights in C/C mice were lowest in each sex. Testis weight in C/C males was also lowest. The C haplotype for these three traits indicated an additive mode of inheritance. In contrast, in the B6×SR21 intercross, inguinal and gonadal fat pad weights were significantly lower in C/C females than in B/B females at the nominal 5% level, but those fat weights were not significantly different in males.
Table 3. Body length and fat pad weight adjusted for body weight at 14 weeks of age, and additive and dominance diplotype effects for body length and fat pad weight in the F2 populations obtained from B6×SR1 and B6×SR21 intercrosses.

| Sex          | F2 population | Trait                  | Diploype | B/B | B/C | C/C | P value | Additive | P value | Dominance | P value | Degree of dominance | Inheritance |
|--------------|---------------|------------------------|----------|-----|-----|-----|---------|----------|---------|------------|---------|--------------------|-------------|
| Male         | B6×SR1        | No. of mice            | 33       | 33–34 | 33–37 |         |         |         |           |           |        |                   |             |
|              |               | Tail length (cm)       |          | 8.37±0.04<sup>a</sup> | 8.40±0.04<sup>a</sup> | 8.37±0.04<sup>a</sup> | 0.70 | -0.003±0.024 | 0.91 | 0.032±0.037 | 0.39 | - | Add |
|              |               | Head-body length (cm)  |          | 9.27±0.03<sup>a</sup> | 9.30±0.03<sup>a</sup> | 9.32±0.03<sup>a</sup> | 0.52 | 0.022±0.021 | 0.31 | 0.010±0.033 | 0.76 | - | Add |
|              |               | Total body length (cm) |          | 17.64±0.05<sup>a</sup> | 17.71±0.05<sup>a</sup> | 17.69±0.05<sup>a</sup> | 0.56 | 0.021±0.032 | 0.51 | 0.042±0.051 | 0.41 | - | Add |
|              |               | Inguinal fat pad weight (g) |      | 0.287±0.01<sup>a</sup> | 0.249±0.01<sup>a</sup> | 0.198±0.01<sup>a</sup> | 0.00000049 | 0.042±0.008 | 0.00000097 | 0.013±0.012 | 0.28 | 0.31 | Add |
|              |               | Gonadal fat pad weight (g) |      | 0.290±0.01<sup>a</sup> | 0.269±0.01<sup>a</sup> | 0.203±0.01<sup>a</sup> | 0.0000028 | 0.042±0.009 | 0.0000036 | 0.023±0.013 | 0.088 | 0.55 | Add |
| Female       | B6×SR1        | No. of mice            | 24       | 34–36 | 32 |         |         |         |           |           |        |                   |             |
|              |               | Tail length (cm)       |          | 8.10±0.03<sup>a</sup> | 8.14±0.03<sup>a</sup> | 8.18±0.03<sup>a</sup> | 0.096 | 0.008±0.002 | 0.69 | -0.016±0.032 | 0.63 | - | Add |
|              |               | Head-body length (cm)  |          | 8.86±0.03<sup>a</sup> | 8.82±0.03<sup>a</sup> | 8.87±0.03<sup>a</sup> | 0.43 | 0.017±0.016 | 0.28 | -0.013±0.024 | 0.58 | - | Add |
|              |               | Total body length (cm) |          | 16.96±0.04<sup>a</sup> | 16.96±0.034<sup>a</sup> | 17.05±0.036<sup>a</sup> | 0.40 | 0.035±0.024 | 0.14 | -0.056±0.036 | 0.13 | - | Add |
|              |               | Inguinal fat pad weight (g) |      | 0.227±0.01<sup>a</sup> | 0.200±0.01<sup>a</sup> | 0.178±0.01<sup>a</sup> | 0.0030 | -0.025±0.007 | 0.00066 | -0.003±0.011 | 0.82 | -0.12 | Add |
|              |               | Gonadal fat pad weight (g) |      | 0.173±0.01<sup>a</sup> | 0.145±0.008<sup>a</sup> | 0.127±0.009<sup>a</sup> | 0.0019 | -0.021±0.006 | 0.00071 | -0.003±0.009 | 0.71 | -0.14 | Add |
| B6×SR21      |               | No. of mice            | 28       | 38–39 | 33 |         |         |         |           |           |        |                   |             |
|              |               | Tail length (cm)       |          | 8.03±0.03<sup>a</sup> | 8.10±0.03<sup>a</sup> | 8.13±0.03<sup>a</sup> | 0.027 | 0.050±0.018 | 0.0076 | 0.014±0.027 | 0.60 | - | Add |
|              |               | Head-body length (cm)  |          | 8.88±0.03 | 8.89±0.03 | 8.95±0.03 | 0.093 | 0.039±0.020 | 0.054 | -0.026±0.030 | 0.38 | - | Add |
|              |               | Total body length (cm) |          | 16.91±0.04<sup>a</sup> | 16.97±0.041<sup>a</sup> | 17.08±0.044<sup>a</sup> | 0.011 | 0.086±0.028 | 0.0035 | -0.017±0.042 | 0.69 | -0.20 | Add |
|              |               | Inguinal fat pad weight (g) |      | 0.385±0.01<sup>a</sup> | 0.372±0.012<sup>a</sup> | 0.341±0.013<sup>a</sup> | 0.016 | -0.022±0.008 | 0.0068 | 0.009±0.012 | 0.45 | - | Add |
On the other hand, in B6×SR2 and B6×SR12 intercrosses, neither males nor females showed significant diplotype differences in any adjusted body composition traits at the Bonferroni-corrected 5% level (Table S6).

Taking all results together, a growth QTL, the wild-derived allele of which increased body weight and body weight gain, was localized to an interval between D2Mit433 (57.3 Mb) and D2Mit205 (65.3), as summarized in Table 4. In a previous study using congenic strains [9], the growth QTL Phag1.12 was physically localized to a maximum interval between D2Mit472 (61.5) and D2Mit327 (69.5) (see Figure 1), which overlapped with the interval of the growth QTL identified in this study. The wild-derived allele at Phag1.12 increased body weight [9], which was exactly the same allelic effect of the growth QTL identified in this study. Therefore, in this study, we succeeded in confirming the presence of Phag1.12 and in narrowing it down to a 3.8-Mb interval between D2Mit472 (61.5) and D2Mit205 (65.3).

Likewise, we were able to confirm the presence of the Phag1.3 QTL affecting total body length that was previously revealed by interval mapping with an F2 intercross population between the B6.Cg-Phag1 original congenic and B6 strains [19]. Phag1.3 was physically defined to an 8.0-Mb interval between D2Mit433 (57.3) and D2Mit205 (65.3) (Table 4).

In the B6×SR1 intercross, a QTL for which the wild-derived allele decreased inguinal and gonadal fat pad weights was clearly identified. In the B6×SR21 intercross, however, the presence of the obesity QTL was ambiguous, because P values for diplotype comparisons marginally exceeded the nominal 5% level but did not reach the Bonferroni-corrected 5% level. Previously, the Phag1.5 QTL for resistance to obesity was physically mapped to an interval between D2Mit270 (52.9) and D2Mit472 (61.5) [21] (see Figure 1). Therefore, we were able to confirm the presence of Phag1.5 in this study and localize it to a 2.1-Mb interval between D2Mit123 (59.4) and D2Mit472 (61.5) (Table 4).

Exome Sequencing

Since no sequence data have so far been reported for the Philippine wild castaneus mice used in this study, we performed sequencing of 2,205 exons for 153 genes on the 44-Mb original congenic region of chromosome 2. According to RefSeq mm9, target bases for the exons were 767,440 bp. The NimbleGen sequence capture covered 97.1% of the target bases, i.e., 745,515 bp. Individual sequence coverage was 11.2 fold on average and ranged from 3 to 36 fold. As expected, some kinds of sequence variants, such as SNPs and indels, were observed in most genes derived from the wild mouse (Table S5). In total, 840 sSNPs and 334 nsSNPs were identified. Nine deletions and 10 insertions were detected in 13 genes. In addition, five nonsense mutations were identified within three genes. On the QTL regions narrowed by the above intersubspecific subcongenic intercross analyses, many SNPs and a few indels were identified, but no nonsense mutation was detected (Tables 5 and S7).

Candidate Gene Search

As shown in Table 5, there were 11 genes on the 3.8-Mb region between D2Mit472 and D2Mit205, where the growth QTL Phag1.12 was located. Using training genes related to body weight (Table S1), Endeavour prioritized Gcg (glucagon) and Grb14 (growth factor receptor-bound protein 14) as the top two candidate genes for Phag1.12. Gcg had one nsSNP and Grb14 had two nsSNPs. Both SIFT and PolyPhen-2 predicted that none of these nsSNPs inflicted possible damage on protein functions (Table 5).

On the 2.1-Mb region between D2Mit123 and D2Mit472 harboring the obesity QTL Phag1.5, 12 genes were located
Table 4. Summary of QTLs for growth and body composition confirmed in the four F2 segregating populations.

| Trait                        | F2 population* | QTL                      |
|------------------------------|----------------|--------------------------|
|                              | B6 × SR1       | B6 × SR2                 | B6 × SR12                | B6 × SR21                |
| Body weight & weight gain    | Increased      | ND                       | ND                       | Increased                |
| Total body length (unadjusted)| Increased      | ND                       | ND                       | Increased                |
| Fat pad weight (unadjusted & adjusted) | Decreased | ND                       | ND                       | MG                       |
|                             | Symbol         | Genomic interval (Mb)    |
|                             | Phbg1.12       | D2Mit472-D2Mit205        |
|                             | Phbg1.3        | D2Mit433-D2Mit205        |
|                             | Pbwg1.5        | D2Mit123-D2Mit472        |

*The effect of the QTL allele derived from the wild mouse is shown: Increased, increased the trait value; Decreased, decreased the trait value; ND, decreased the trait value but the QTL effect was marginal; ND, QTL was not detected.

Table 5. Endeavour ranked Ly75 (lymphocyte antigen 75) and Igh6 (integrin beta 6) as the top two candidate genes for Phbg1.3 using training genes related to obesity (Table S1). Ly75 had nine nsSNPs and these were predicted to have no affect on protein function. In contrast, Igh6 had three nsSNPs. PolyPhen-2 predicted that none of the three nsSNPs caused possible damage to protein function, whereas SIFT predicted that one of them, Igh6, at the position of 260491216 leading to amino acid substitution of Sel302Ala, is harmful to protein function. This nsSNP has previously been reported as dbSNP rs28025203.

Discussion

Previously we discovered the Phbg1.12 QTL for growth [9], the Phbg1.3 QTL for body length [19] and the Phbg1.5 QTL for obesity [21] from an unassigned resource of wild M. m. castaneus mice caught in the Philippines. In this study, we were able to confirm the presence of these three QTLs by interspecific intercross analyses using four newly or previously constructed subcongenic strains with overlapping and/or non-overlapping genomic intervals. The unique effects of the wild-derived QTL allele at the QTLs revealed in previous studies [9,19,21] were duplicated in the present independent study. That is, allele at the QTLs revealed in previous studies [9,19,21] were uniquely enhanced growth at Phbg1.12 and increased body length at Phbg1.3, despite the fact that the wild mouse has approximately 60% of the body weight of B6 [16], whereas it decreased fat weight at Phbg1.5. Furthermore, we were able to reduce the genomic interval harboring Phbg1.12 from 8.9 Mb [9] to 3.8 Mb in length and to narrow the interval of Phbg1.5 from 8.8 Mb [21] to 2.1 Mb. Although Phbg1.3 was previously localized to a 20-Mb confidence interval [19], it was physically mapped to an 8.0-Mb interval in this study.

Although Phbg1.3 and Phbg1.5 exerted phenotypic effects on both sexes, Phbg1.12 exhibited male-specific effect on body weight. In our previous study [9], the sex-specificity of Phbg1.12 on chromosome 2 was not tested because the sample size was small. Sex-specific QTLs for body weight are revealed on different chromosomal regions in our previous genome-wide QTL analysis [18] and in different mouse crosses [5]. In addition, sex-specific QTLs have commonly been observed in different quantitative traits of mice and other species, as mentioned previously [16,18]. However, the molecular mechanisms underlying sex-specific QTLs remain unclear. It is reported that androgen control of growth hormone secretion induces male-specific gene expression in the liver of mice [28]. We thus consider that the male-specific effect of Phbg1.12 may be mediated by sex hormones through male-specific expression of the causative gene of Phbg1.12.

According to MGD [5], several QTLs affecting growth, body length and obesity were previously mapped to mouse chromosome 2 regions that are overlapped with our 8.0-Mb region between D2Mit443 (57.3) and D2Mit205 (65.3). Since the previous QTLs were mapped by genome-wide QTL analyses, confidence intervals of the QTLs are generally very large, spanning approximately 100 Mb or more. Thereafter, few map positions of QTLs have been determined physically, with a few exceptions. Four growth and obesity QTLs, named Wg2n-Wg2d, have been fine-mapped to the interval from D2Urd15 (74.7) to D2Mit196 (160.5) by phenotypic analyses of subcongenic strains that possess the introgressed regions of the CAST/EiJ strain established from wild M. m. castaneus mice on the B6 genetic background [10]. However, this interval is outside of our QTL regions. The Nidd5 QTL affecting adiposity has been fine-mapped by phenotypic analysis of congenic strains with donor regions derived from the BALB/cA strain on the genetic background of the obese/diabetic TSOD strain, and Acvr1c encoding activin receptor-like kinase 7 at the position of 58.1 Mb was very recently identified as a responsible gene for Nidd5 [29]. The obese/diabetic TSOD strain unexpectedly has the wild-type allele at Acvr1c, whereas the normal BALB/cA strain has a nonsense mutation resulting in decreased fat mass phenotype [29]. In contrast, our exome sequencing analysis indicated that the Acvr1c gene derived from our wild M. m. castaneus mouse had neither nsSNPs nor nonsense mutations. Furthermore, Acvr1c lies outside the interval containing our Phbg1.12 for increased body weight and Phbg1.5 for decreased fat weight. Acvr1c is thus unlikely to be a candidate gene for our QTLs. In addition, as no nonsense mutation was identified for any of the genes located in the QTL interval, this kind of mutation could not become a sequence variant causing the differences in body weight and fat weight shown in this study.

In this study, exome sequencing and candidate gene prioritization strongly suggested that Gcg and Grb14 are putative candidate genes for the Phbg1.12 QTL for enhanced growth. Gcg encodes proglucagon, a precursor of glucagon, glucagon-like peptide-1 (GLP-1) and several other components. Glucagon is generated in pancreatic α-cells and GLP-1 is yielded in intestinal L-cells, and these peptides play key roles in glucose metabolism and homeostasis [30]. Mice lacking glucagon and GLP-1 are born normally without gross abnormalities and display α-cell hyperplasia and increased body weight [31]. It has very recently been revealed in rats fed a high-fat diet that hypothalamic glucagon signaling can suppress hepatic glucose production, suggesting that hypothalamic glucagon resistance may contribute to the hyperglycemia observed in obesity and diabetes [32]. Grb14 encodes an adapter protein belonging to the GRB7 family and it plays an important role in receptor-tyrosine kinase signaling pathways and...
### Table 5. Variants detected by exome sequencing of genes on the genomic regions harboring the growth QTL Pbwg1.12 and the obesity QTL Pbwg1.5, prioritization of candidate genes and damage of protein functions caused by nsSNPs found in the candidate genes.

| Gene symbol | Position (bp) | Number of variants | Candidate gene ranking | Damage to protein function |
|-------------|---------------|--------------------|------------------------|---------------------------|
|             | Start End     | sSNP nsSNP Deletion Insertion | Body weight Obesity SIFT PolyPhen-2 |
| Dapl1       | 59322709 59343075 | 1 0 0 0 | NA |
| Tanc1       | 59450100 59684206 | 21 4 0 1 | NA |
| Walub1      | 59690423 59720663 | 6 1 0 0 | NA |
| Baz2b       | 59737419 59963797 | 15 6 1 0 | NA |
| March7      | 60047992 60086442 | 6 0 0 0 | NA |
| Cd302       | 60090049 60122475 | 1 0 1 0 | NA |
| Ly75        | 60131816 60221288 | 27 9 0 0 | NA 1 Tolerated Benign |
| Pka2r1      | 60257095 60391318 | 18 8 0 0 | NA |
| Igb6        | 60436349 60511750 | 11 3 0 0 | NA 2 Affected Benign |
| Rbms1       | 60590009 60801261 | 2 0 0 0 | NA |
| Tank        | 61416642 61492224 | 1 5 0 0 | NA |
| Psmd14      | 61549750 61638433 | 1 0 0 0 | NA |
| Tbr1        | 61642509 61652170 | 2 1 0 0 | NA |
| Sflda10     | 61864959 62164800 | 6 0 0 0 | NA 1 Tolerated Benign |
| Dpp4        | 62168131 62250288 | 6 0 0 0 | NA |
| Gcg         | 62312586 62321710 | 0 1 0 0 | 1 NA Tolerated Benign |
| Fap         | 62339001 62412078 | 2 2 0 0 | NA |
| Ihh1        | 62438489 62484312 | 17 5 0 0 | NA |
| Gca         | 62502383 62532166 | 3 1 0 0 | NA |
| Kchh7       | 62541002 63022344 | 6 1 0 0 | NA |
| Fign        | 63815417 63936064 | 4 1 0 0 | NA |
| Gnb14       | 64750539 64860823 | 7 2 0 0 | 2 NA Tolerated Benign |
| Cobbll      | 64926395 65076683 | 14 18 0 0 | NA |

*aSNP, synonymous SNP; nsSNP, nonsynonymous SNP; NA, not applicable because the QTL in question was not located on the region including the genes.

**The top two genes were prioritized as candidate genes for growth and obesity QTLs by the web-based software program Endevour [25].**

*Damage caused by nsSNPs was investigated for the ranked genes by two software programs, SIFT [26] and PolyPhen-2 [27].*
insulin signaling [33]. Grib14 knockout mice are born normally, show a small reduction in body weight and exhibit improved glucose homeostasis and enhanced insulin signaling in the liver and skeletal muscle [34]. Judging from the phenotypic similarity between Plagl1.12 and knockout mice, Gegr is very likely to become a candidate gene for Plagl1.12, although further studies such as pancreatic islet characterization and Gegr expression analysis will be needed in our subcongenic mice.

For the obesity Plagl1.5 QTL, Ly75 and Igt66 were suggested to be putative candidate genes in this study. Ly75 encodes DEC-205, a 205-kD integral membrane protein homologous to the macrophage mannose receptor, and DEC-205 is a novel endocytic receptor used by dendritic cells and thymic epithelial cells to direct captured antigens from the extracellular space to a specialized antigen-processing compartment [35]. Ly75 knockout mice exhibit abnormalities in CD8-positive T cell morphology and cytotoxic T cell physiology [36]. Igt66 encodes the integrin β6 subunit, a member of the integrin family. This subunit heterodimerizes with the αv subunit to bind and/or activate latent transforming growth factor β. The expression of αvβ6 integrin is largely restricted to a subset of epithelial cells [37,38]. Igt66 knockout mice are born and grow normally but exhibit juvenile baldness associated with macrophage infiltration of the skin and accumulation of activated lymphocytes around conducting airways in the lungs, suggesting that alterations in this integrin may contribute to the development of inflammatory diseases of epithelial organs including the skin, lungs and kidney [39]. A previous microarray analysis revealed that 259 genes are differentially expressed in the liver between SM/J and LG/J mouse strains fed a high-fat diet, where SM/J is more responsive than LG/J for many obesity and diabetes traits. Most of these genes are associated with immune function, and 62 genes are located within intervals of QTLs previously mapped for obesity, diabetes and related traits [40]. High-fat diets are known to trigger an immune response through inflammation in many organs and tissues such as the liver and adipose tissue [41,42]. Hence, the genes associated with immune function can become candidate genes for obesity and related QTLs. Therefore, the Ly75 and Igt66 genes with immune function may be good candidate genes for our Plagl1.5 that shows prevention of obesity when mice are fed both low-fat standard and high-fat diets [21].

The Igt66 gene on the Plagl1.5 region derived from a wild castaneus mouse caught in the Philippines had the msSNP of g.260491216A>C, leading to amino acid substitution of Sel302Ala that was predicted to be harmful to protein function by SIFT but not by PolyPhen-2. In fact, the Sel residue is conserved among many mammals including humans, dogs, bovines, horses and rats [43]. In mice, both Sel and Ala residues are segregating among common inbred strains and also among wild-derived inbred strains [5]. It is noteworthy that the CAST/EiJ strain established from wild castaneus mice in Thailand has the same base substitution (C: Ala) as that of our wild castaneus mouse in the Philippines. In addition, QTLs for obesity and related traits identified from CAST/EiJ and other strains have so far not been fine-mapped to the Plagl1.5 region on mouse chromosome 2, as discussed earlier. These facts thus suggest that the A>C msSNP might not act as a sequence variant causing our phenotypic variation.

Next-generation sequencing of 13 classical inbred mouse strains and four wild-derived inbred strains has recently revealed that QTLs with small effects on 100 phenotypes of disease and physiological traits, which were identified in more than 2,000 heterogeneous stock mice, are more likely to arise from intergenic sequence variants lying outside genes and are less likely to arise from nsSNPs and structural variants (indels, inversions, copy number gains and others) lying within genes. In contrast, it has been shown that QTLs with large effects are more likely to arise from structural variants and are less likely to arise from intergenic variants [23,44]. We therefore consider that, since our QTLs have small effects on growth and obesity, their causative variants may be intergenic variants rather than nsSNPs and structural variants identified by exome sequencing in this study. As the next step, we will need to perform expression analysis of the four putative candidate genes searched in this study. Gene expression results will provide information helpful for identifying causative genes and further causative variants underlying our QTLs on chromosome 2.

In conclusion, by analysis using intersubspecific subcongenic intercrosses, we precisely fine-mapped three unique QTLs for enhanced growth, prevention of obesity and increased body length, which were discovered from a wild M. m. castaneus mouse, to small genomic intervals ranging from 2.1 to 8.0 Mb on mouse chromosome 2. By combined analysis of exome sequencing and bioinformatics, we identified four genes as putative candidate genes for the unique growth and obesity QTLs. We furthermore predicted that nsSNPs found in the candidate genes would not be harmful to protein functions.

Supporting Information

Figure S1 Two PCR-RFLP markers on mouse chromosome 2 developed in this study. (A) The rs13476521 PCR-RFLP marker was constructed on the basis of the rs13476521 SNP located at 58,131,026 bp on the Ctyt gene. B6 has the nucleotide base T and our exome sequencing revealed that our wild mouse has the base C being the same as that of CAST/EiJ. A pair of primers, 5’-CTGCGGGAATGGA-TAAAGT-3’ and CCTGACTCGGACACTGGAAT, amplified a 364-bp fragment including this SNP. The restriction enzyme EcoRv cut the 364-bp fragment derived from B6 in two (195 and 169 bp), whereas it did not cut the 364-bp fragment derived from the wild mouse. (B) The rs48690987 PCR-RFLP marker was developed on the basis of the rs48690987 SNP at 62,606,536 bp on the Ifih1 gene. B6 has the nucleotide base T, whereas our wild mouse has the base C being the same as that of CAST/EiJ. A pair of primers, AAATTAGCCGTTTCTGGCACC, and GGA-TAGTTTTCGGCCCTTTGGC, amplified a 306-bp fragment. The enzyme EcoT22I generated two B6-derived fragments (160 and 146 bp), whereas it did not cut a wild-derived fragment. PCR was performed as described previously [19], and 2.0–2.5% agarose gels were used for electrophoresis.

Table S1 Training genes used in prioritization of candidate genes.

Table S2 Additive and dominance diplotype effects for body weight and weight gain in the F2 populations obtained from B6 × SR2 and B6 × SR12 intercrosses.

Table S3 Organ weight not adjusted for body weight at 14 weeks of age, and additive and dominance diplotype effects for organ weight in the F2 populations obtained from B6 × SR1 and B6 × SR21 intercrosses.

Table S4 Measurements of body composition traits not adjusted for body weight at 14 weeks of age, and additive and dominance diplotype effects for body composition traits in the F2 populations obtained from B6 × SR2 and B6 × SR12 intercrosses.
Table S5  Organ weight adjusted for body weight at 14 weeks of age, and additive and dominance diplotype effects for organ weight in the F2 populations obtained from B6 × SR1 and B6 × SR21 intercrosses.

Table S6 Measurements of body composition traits adjusted for body weight at 14 weeks of age, and additive and dominance diplotype effects for body composition traits in the F2 populations obtained from B6 × SR2 and B6 × SR12 intercrosses.

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Author Contributions
Conceived and designed the experiments: AI. Performed the experiments: AI, SO. Analyzed the data: AI. Contributed reagents/materials/analysis tools: AI. Wrote the paper: AI.