Confirmation and Characterization of Murine Body Weight QTLs, \textit{Bwq1} and \textit{Bwq2}, Identified in C57BL/6J × KK-\textit{A}\textit{\textasciitilde}1/\textit{a} F\textsubscript{2}\textit{-A}\textit{\textasciitilde}1/\textit{a} Mice

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ABSTRACT. Body weight quantitative trait loci (QTLs), \textit{Bwq1} and \textit{Bwq2}, identified previously in C57BL/6J × KK-\textit{A}\textit{\textasciitilde}1/\textit{a} F\textsubscript{2}\textit{-A}\textit{\textasciitilde}1/\textit{a} mice, were further confirmed and characterized. Body weight measurement was done from 21 days after birth (Day 21) through Day 100, at 10-day intervals. \textit{Bwq1} was statistically significant only on Days 40, 50, and 60, whereas \textit{Bwq2} was statistically significant on and after Day 40. When body weight gain (WG) between two successive weight measurements was evaluated, both \textit{Bwq1} and \textit{Bwq2} were statistically significant only for WG between Days 30 and 40. The results suggest that variations in body weight among F\textsubscript{2} individuals in later life have been determined by variations in WG during the period shortly after weaning. The results also suggest that \textit{Bwq1} is related to increased body weight in the KK strain, because the effect of \textit{Bwq1} on the body weight is observed not only in F\textsubscript{2}-\textit{A}\textit{\textasciitilde}1/\textit{a}, but also in F\textsubscript{2}-\textit{a}\textit{\textasciitilde}1/\textit{a}. On the other hand, it is suggested that \textit{Bwq2} is related to enhanced obesity caused by \textit{A}\textit{\textasciitilde}1 mutation and therefore is a genetic modifier that specifically interacts with the \textit{A}\textit{\textasciitilde}1 allele, because the effect of \textit{Bwq2} is only observed in F\textsubscript{2}-\textit{A}\textit{\textasciitilde}1/\textit{a}. There are two candidate genes, \textit{Pparg} and \textit{Hhrl}, which are located near the 95% confidence interval of \textit{Bwq2}, and which are expressed in the adipose tissue; however, we could not find any nucleotide differences in both cDNAs between KK and C57BL/6J strains.

KEY WORDS: \textit{A}\textit{\textasciitilde}1 allele, body weight, KK mouse, obesity, quantitative trait locus (QTL).

Obesity is defined as a physiologic state of overweight with excessive fat accumulation. Prevalence of obesity is a common health problem in modern nations, not only because of morbidity, but also because an obese state is a serious risk factor in increasing susceptibility to non-insulin-dependent diabetes mellitus, coronary heart disease, and other metabolic complications (e.g., hyperlipidemia) [29]. A familial tendency and a high concordance rate in twin studies suggest that the establishment of the obese phenotype has a genetic basis [3]. With the exception of extremely rare morbid cases, the mode of inheritance underlying obesity is based on multiple genes under the influence of environmental stimuli [3]. To dissect genetic basis, quantitative trait locus (QTL) analyses have been carried out extensively during the last decade, particularly on mice, and more than seventy obesity QTLs and more than eighty body weight QTLs have been identified to date [4] (hereafter, we use term “body weight QTL” for both traits). Because body weight QTLs have been mapped on every chromosome (Chr) except for the Y with broad and coarse confidence intervals (CIs), one can easily postulate several candidate genes for a QTL; however, most of these candidates are unlikely to be correct [12]. An alternative way to identify body weight QTLs is to map modifier loci for major obese mutations, such as \textit{A}\textit{\textasciitilde}1, \textit{Lep}\textasciitilde\textit{ob}, \textit{Lepr}\textasciitilde\textit{ob}, \textit{Cpe}\textasciitilde\textit{fat}, and \textit{tub} [15]. Because causative genes for these mutations have been identified, it is practicable to prove direct interactions between molecules from mutant and from candidate gene for modifiers [20]. Furthermore, identification of modifiers gives a better understanding of the whole picture of metabolic pathways underlying these obese mutations molecularly [18].

We previously carried out a QTL analysis of body weight and obesity in C57BL/6J × KK-\textit{A}\textit{\textasciitilde}1/\textit{a} F\textsubscript{2} mice [24]. We used two sets of F\textsubscript{2} mice, F\textsubscript{2}-\textit{a}\textit{\textasciitilde}1/\textit{a} and F\textsubscript{2}-\textit{A}\textit{\textasciitilde}1/\textit{a}, with regard to the agouti locus genotype, to identify QTLs underlying body weight in the KK strain, and to identify modifiers for the obese phenotype caused by the \textit{A}\textit{\textasciitilde}1 allele at the agouti locus [24]. Because KK-\textit{A}\textit{\textasciitilde}1/\textit{a} mice (\textit{A}\textit{\textasciitilde}1 congenic strain, see Materials and Methods) were much heavier than C57BL/6J-\textit{A}\textit{\textasciitilde}1/\textit{a} at 60 days after birth [25], and because the F\textsubscript{2}-\textit{A}\textit{\textasciitilde}1/\textit{a} exhibited a wide spectrum of variations in body weight, as did F\textsubscript{2}-\textit{a}\textit{\textasciitilde}1/\textit{a}, it was strongly suggested that there are modifier genes that differ between KK and C57BL/6J. By comparison of the QTL results from F\textsubscript{2}-\textit{A}\textit{\textasciitilde}1/\textit{a} with that from F\textsubscript{2}-\textit{a}\textit{\textasciitilde}1/\textit{a}, it will be possible to identify the modifiers that specifically interact with obese phenotype caused by the \textit{A}\textit{\textasciitilde}1 allele. Consequently, we have identified two body weight QTLs, \textit{Bwq1} (body weight QTL 1) on Chr 4 and \textit{Bwq2} (body weight QTL 2) on Chr 6. The effect of \textit{Bwq1} on the body weight was observed in both F\textsubscript{2}-\textit{a}\textit{\textasciitilde}1/\textit{a} and F\textsubscript{2}-\textit{A}\textit{\textasciitilde}1/\textit{a}. On the other hand, the effect of \textit{Bwq2} was observed only in F\textsubscript{2}-\textit{A}\textit{\textasciitilde}1/\textit{a}. Therefore \textit{Bwq2} can be regarded as a genetic modifier that specifically interacts with the \textit{A}\textit{\textasciitilde}1 allele. Because the agouti peptide is overexpressed ectopically in \textit{A}\textit{\textasciitilde}1 mice and serves as a constitutive antagonist for α-melanocortin stimulating hormone (α-MSH) at the melanocortin 4 receptor (MC4R) in the hypothalamus [19], \textit{A}\textit{\textasciitilde}1 mice become obese as a consequence of hyperphagia [9, 11, 16, 22]. Therefore, molecule encoded by \textit{Bwq2} is expected to serve in the MC4R metabolic pathway, which, in turn, is situated in the lower course of the leptin metabolic pathway.
In the present study, we confirmed the presence of these QTLs in a larger F2 population, and at the same time, we employed a new trait variable, body weight gain (WG), to specify their physiologic roles in the control of body weight.

MATERIALS AND METHODS

Mice, genetic cross and diet: Inbred strain C57BL/6J (aaBBCC) females and KK-A^a (A^aBBCc) males were purchased from Clea Japan (Tokyo). KK-A^a is a congenic strain, in which the A^a allele at the agouti locus had been introduced onto the inbred KK (aaBBcc) strain. The KK strain was established by Kondo and his colleagues in Japan, and later was found to have a diabetic tendency [21]. Hereafter, we define the C57BL/6J strain as having B alleles, and the KK-A^a strain as having K alleles, throughout the genome.

The C57BL/6J females were crossed with the KK-A^a males to produce C57BL/6J×KK-A^a F1. Yellow F1 (A^a/la) females and males were intercrossed to produce C57BL/6J × KK-A^a/la F2. The F2 mice were randomly culled to six to eight pups per dam on the day of birth. Albino F2 mice were discarded because it was uncertain if they possessed the A^a allele. Therefore, the F2 mice comprised black-pigmented (F2-a/a) and yellow-pigmented mice (F2-A^a/la). In this study, we analyzed 147 F2-A^a/la mice (71 males and 76 females), of which 99 had been analyzed previously, and 48 were newly analyzed. These 147 mice were produced in 1996 as a cohort. All mice were weaned at 21 days after birth and housed individually in cages thereafter. The mice were maintained in a specific-pathogen-free facility with a 12 hr light:12 hr dark, with controlled temperature and humidity. Rodent pellet chow [CE-2 (342.2 kcal/100 g, containing 4.4% crude fat), Clea Japan] was fed ad libitum, and tap water were freely available.

Body weight measurement: The body weight of each individual was measured with an electronic balance from the day of weaning (Day 21, 21 days after birth) to Day 100, at intervals of 10 days (except for a shorter initial interval between Days 21 and 30). We defined an increase in body weight between each two successive weight measurements as WG.

Genotyping: Ninety-seven informative loci, including microsatellites and PCR-RFLP for the Apoa2 locus, were genotyped in the previously analyzed 99 F2-A^a/la mice, and six loci on Chr 4, six loci on Chr 6, and four loci on Chr 16 were genotyped in the additional 48 F2-A^a/la mice. A list of the microsatellite markers used in the present study is available upon request.

Genomic DNA was prepared from the tail by standard procedures. Microsatellite sequence polymorphisms were detected by electrophoresis after PCR. Amplifications were carried out under the following conditions: 1 cycle at 94°C for 5 min; 35 cycles at 94°C for 30 s, 55°C for 1 min, and 72°C for 45 s; 1 cycle at 72°C for 7 min. PCR products were electrophoresed on 10% polyacrylamide gels for a minimum of 60 min and visualized by ethidium bromide staining.

QTL mapping and statistical analysis: In order to merge body weight and WG data from F2-A^a/la males and F2-A^a/la females, trait variables for the males and females were standardized to a mean 0 and a variance 1 by subtracting the gender-specific mean from each individual value and dividing each difference by the standard deviation of its respective gender.

QTL analysis was carried out with the Mapmaker/EXP version 3.0b and the Mapmaker/QTL version 1.1b computer programs [13]. The chromosomal region with a logarithm of odds (LOD) score of more than 4.3 (threshold of statistical significance at α=0.05) was recognized as indicating significant linkage, and the region with a LOD score between 2.8 and 4.3 was recognized as indicating suggestive linkage [14]. The α level for suggestive linkage implies the expectation that there will be one false positive in a genome-wide search. Once a significant QTL was identified, the 95% CI was determined as an approximate indicator for postulating candidate genes. The 95% CI is defined by a 1.5 LOD decrease; therefore, it is the equivalent of a 1.5-LOD support interval.

Candidate cDNA sequencing: As plausible candidate genes for the Bwq2 on Chr 6, peroxisome proliferator activated receptor gamma (Pparg) and histamine receptor H1 (Hrh1) are the only known genes that are located near the 95% CI of Bwq2, and that are expressed in adipose tissues. Therefore, we determined and compared these cDNAs between KK and C57BL/6J mice. [i] Pparg: The DNA sequence that corresponds to the Pparg1 ORF was amplified on the basis of cDNA from the liver mRNA with the following sets of PCR primers: 5'-gacaggttgcagcaaga3' and 5'-tcacgaaattcttagttgca3', and 5'-gggtgatggcctgtgct3' and 5'-ccctggcaaagcatttgtat3'. A 30-bp sequence that is added specifically to the 5' side of the Pparg cDNA by alternative splicing was amplified by use of genomic DNA with the following set of PCR primers: 5'-ttgaaactttggcttcggtt-3' and 5'-ccacagaaatgcaaggatt3'. [ii] Hrh1: The ORF of the Hrh1 cDNA was amplified on the basis of the mRNA from the brain with the following sets of PCR primers: brain RNA: 5'-gactgaaacccgtctg-3' and 5'-agaggctacggtgctt-3', and 5'-aagtgagctgctga-3' and 5'-aatgtcctcaagctca-3'. All PCR products were directly sequenced with these oligonucleotides as sequence primers.

RESULTS

We show body weight data on Day 60 from KK-A^a/la males (n=15), C57BL/6J (n=15) females, F2-A^a/la males (n=7), F2-A^a/la females (n=7), F2-A^a/la males (n=71), and F2-A^a/la females (n=76) in Fig. 1a. Except on Day 21, all the above strains possessing the A^a allele were apparently heavier than C57BL/6J at every time of body weight measurement. Although body weight data from two strains were not contemporary, KK-A^a/la was apparently heavier than C57BL/6J-A^a/la [An average body weight in KK-A^a/la
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Figure 1a

Fig. 1. a: Plots of body weight on Day 60 in KK-A\(^{y}\)a males (n=15), C57BL/6J females (n=15), F1-A\(^{y}\)a males (n=7), F1-A\(^{y}\)a females (n=7), F2-A\(^{y}\)a males (n=71), and F2-A\(^{y}\)a females (n=76). Each circle represents body weight of an individual mouse. b: Plots of WG between Days 30 and 40 in parental KK-A\(^{y}\)a females, C57BL/6J females, F2-A\(^{y}\)a males, and F2-A\(^{y}\)a females. Each circle represents body weight gain in an individual mouse.

females (n=12) at 66–71 days after birth was 37.0 g, while that in C57BL/6J-A\(^{y}\)/a females (n=16) at 76–82 days was 27.9 g.\[25\]. The F2-A\(^{y}\)a mice exhibited a wide spectrum of body weights on Day 60 (Fig. 1a), in a similar way as the F1-A\(^{y}\)a mice did.\[24\]. These results suggested that identification of modifier loci in F2-A\(^{y}\)a can be achieved successfully. A gender difference was noted in F1, where the body weight on Day 60 ranged from 35.14 g to 50.52 g (mean, 42.17 g) in males, and 24.52 g to 47.25 g (mean, 37.98 g) in females. As a rule, differences in body weight among individuals were larger than in females than in males (Fig. 1a). This was a consequence that the coefficient of variation in F1-A\(^{y}\)a females was significantly larger than that in F1-A\(^{y}\)a males. In addition, the coefficient of variation in body weight on Day 60 in F1-A\(^{y}\)a was as large as that in F2-a/a [F2-A\(^{y}\)a males: 0.024, F2-A\(^{y}\)a females: 0.102, F1-a/a males: 0.050, F1-a/a females: 0.034].

Scatter plots for WG in F2-A\(^{y}\)a males and F2-A\(^{y}\)a females are shown in Fig. 1b, together with those in KK-A\(^{y}\)a males and C57BL/6J females. Similarly to the finding for body weight, the F2-A\(^{y}\)a mice exhibited a wide spectrum of WG. A gender difference was noted again in the F2, where the body weight between Days 30 and 40 ranged from 4.72 g to 12.49 g (mean, 8.05 g) in males, and 1.27 g to 8.89 g (mean, 5.75 g) in females. At variance with the result for body weight, however, some of the F2-A\(^{y}\)a females had WG similar to that of C57BL/6J females.

A genome-wide linkage curve for body weight on Day 60 in an initial 99 F2-A\(^{y}\)a mice is shown in Fig. 2. Regions on Chrs 4 (proximal part, Bwq1, peak LOD score 3.7), 6 (mid part, Bwq2, 5.0) and 16 (proximal part, 3.4) reached suggestive QTL levels (LOD ≥ 2.8). The K allele was associated with increased body weight at Bwq1 and Bwq2, but was associated with decreased body weight at the locus on Chr 16. Next, we analyzed Bwq1 and Bwq2 as well as the locus on Chr 16 in 147 F2-a/a mice. Subsequent to genotyping of 6 loci on Chr 4, 6 loci on Chr 6, and 4 loci on Chr 16 in 48 F2-a/a mice, all 17 traits (body weight from Day 21 to Day 100, and WG between each successive body weight measurement) were analyzed. The Bwq1 and Bwq2 exhibited significant linkages for several traits (Fig. 3a, b, see below), whereas the locus on Chr 16 exhibited only suggestive linkages for body weight on Day 40 (peak LOD score 3.7) and on Day 50 (peak LOD score 3.3). We therefore did not take the locus on Chr 16 into further consideration.

LOD scores for Bwq1 and Bwq2 obtained from all traits examined in this study are presented in Fig. 3a (Bwq1) and 3b (Bwq2). Bwq1 and Bwq2 were significant QTLs (i.e., LOD score ≥ 4.3) at several times. With regard to body weight, Bwq1 was statistically significant only on Days 40, 50, and 60 (Fig. 3a), whereas Bwq2 was statistically significant on and after Day 40 (Fig. 3b). With regard to the WG, both Bwq1 and Bwq2 were suggestive QTLs for WG between Days 21 and 30, and were significant QTLs for WG between Days 30 and 40, but they did not show any indications of linkage thereafter (Fig. 3a, b).

Chromosomal localizations of Bwq1 and Bwq2 are shown in Fig. 4, with the 95% CI. The highest peak LOD score for Bwq1 was obtained for body weight on Day 60 (LOD score 5.5, Fig. 3a) at D4Mit111 (21.9 cM) (Fig. 4a). This was an about 15.6 cM distal position to the previous result, where the peak LOD score was obtained at D4Mit1\[24\]. On the other hand, the highest peak LOD score for Bwq2 was obtained for body weight on Day 70 (LOD score 8.8, Fig. 3b) at D6Mit361 (35.2 cM) (Fig. 4b). For both loci, LOD score curves for WG were very similar to those for body weight on Day 60 (peak LOD score 8.8, Fig. 3b) at D6Mit361 (35.2 cM) (Fig. 4b).
Results of ANOVA in several representative traits are listed in Table 1. Two-way ANOVA confirmed that there were no statistical interactions between gender and genotype at the markers closest to the QTLs. Particularly, the effect of \textit{Bwq2} on body weight on Day 70 was significant even when the males and females were examined separately. This was confirmed by use of the Mapmaker software on males and females independently. Although the data are not shown, patterns of LOD score plot curves for males and females were very similar, and the highest LOD score was obtained near \textit{D6Mit361}.

Although several similar QTLs have been mapped to a region where \textit{Bwq1} is located (see Discussion), we could not postulate candidates for \textit{Bwq1}. On the other hand, there are two genes that are located near the 95% CI of \textit{Bwq2}, and that are expressed in adipose tissue, on the basis of the Mouse Genome Informatics (http://www.informatics.jax.org/searches/). One is peroxisome proliferator activated receptor gamma (\textit{Pparg}, 52.7 cM), the other is histamine receptor \textit{H1} (\textit{Hrh1}, 49.0 cM). We therefore determined and compared nucleotide sequences of these cDNAs between C57BL/6J and KK strains to address their suitability as candidate genes for \textit{Bwq2}. We sequenced \textit{Pparg} in both strains, but we sequenced the \textit{Hrh1} gene only in the KK strain. The \textit{Hrh1} sequence in C57BL/6J was extracted from the GenBank database (AK038480). Consequently, we could not find any nucleotide substitutions in ORFs of both \textit{Pparg} and \textit{Hrh1} cDNAs between the two strains.

**Fig. 2.** Genome-wide linkage curve for body weight on Day 60 obtained in an initial 99 F\textsubscript{2} \textit{A/y} mice. As seen, suggestive QTLs (LOD score ≥ 2.8) were identified on Chrs 4 and 16, and a significant QTL (LOD score ≥ 4.3) was identified on Chr 6.

**Fig. 3.** a: LOD scores at \textit{D4Mit111} (\textit{Bwq1}, Chr 4) for all traits examined in the present study. WG a-b: body weight gain between Days a and b. b: LOD scores at \textit{D6Mit361} (\textit{Bwq2}, Chr 6) for all traits examined in the present study. WG a-b: body weight gain between Days a and b.
In contrast to the traditional view of genetic diseases caused by single-gene mutations, increasing numbers of experimental results suggest that there are "modifier genes" which modulate phenotypes in combination with causative genes, as exemplified in numerous genetic studies in mice [18, 20]. Thus, the variability of mutant phenotypes

![Fig. 4](image_url)
depends on the genetic background on which the causative gene is placed. In this respect, quite a few Mendelian traits share bases with a qualitative trait that is determined by association of multiple genes with environmental factors. For example, diabetes caused by the Lepr<sup>ab</sup> and Lepr<sup>ab</sup> genes is expressed more severely on the C57BL/KsJ background than on the C57BL/6J background [8, 10]. Indeed, Chung et al. [7] have identified three modifier loci for diabetes and obesity traits caused by Lepr<sup>ab</sup> on Chr 1, 12, and 16 in rats. Thus, it may be straightforward to consider that there will be several modifier loci for traits induced by the A<sup>y</sup> allele, and as a result, we have identified a modifier locus, Bwq2, on Chr 6. The fact that the allele from the KK strain was associated with increased body weight and WG at this locus may explain why obese phenotype caused by the A<sup>y</sup> mutation is more severely expressed in KK-A<sup>y</sup>/a than in C57BL/6J-A<sup>y</sup>/a.

In our previous QTL results in 99 F<sub>2</sub>-A<sup>y</sup>/a mice [24], two QTLs, Bwq1 and Bwq2, were identified for body weight on Day 60, on Chr 4 and 6, respectively. The effect of Bwq1 on the body weight was observed not only in F<sub>2</sub>-A<sup>y</sup>/a, but also in F<sub>2</sub>-a/a; therefore, Bwq1 does not specifically interacts with the A<sup>y</sup> allele. On the other hand, the effect of Bwq2 was only observed in F<sub>2</sub>-A<sup>y</sup>/a; therefore, Bwq2 is a genetic modifier that specifically interacts with the A<sup>y</sup> allele. For body weight at 6 months, the effect of Bwq1 was observed in F<sub>2</sub>-a/a, but not in F<sub>2</sub>-A<sup>y</sup>/a (LOD score was less than 2.8, a threshold of suggestive linkage). On the other hand, the effect of Bwq2 was still only observed in F<sub>2</sub>-A<sup>y</sup>/a. In particular, the effect of Bwq2 was observed only not in body weight (LOD score 3.4), but also in carcass lipid weight (LOD score 4.2) and in adiposity (LOD score 4.1) [24]. We consider that Bwq1 is primarily related to increased lean body mass in the KK strain; therefore, the effect of Bwq1 on mere body weight in F<sub>2</sub>-A<sup>y</sup>/a is too small to be detected at 6 months, because A<sup>y</sup> mice at this age possess extensive adipose tissue mass. Thus, Bwq1 does not modify obese phenotype caused by the A<sup>y</sup> allele. On the other hand, we consider that Bwq2 is primarily related to enhanced obesity/adiposity in A<sup>y</sup> mice, and specifically modify obese phenotype caused by the A<sup>y</sup> allele.

It may be straightforward to search candidates for Bwq2 into genes that are known to be expressed in adipose tissues. On the basis of the Mouse Genome Informatics (http://www.informatics.jax.org/searches/) database search, there are two known genes that are located near the 95 % CI of Bwq2, and that are expressed in adipose tissue. One is peroxisome proliferator activated receptor gamma (Ppar<sub>γ</sub>, 52.7 cM), and the other is histamine receptor H<sub>1</sub> (Hrh<sub>1</sub>, 49.0 cM). Ppar<sub>γ</sub> has been known to promote adipocyte differentiation [28]. On the other hand, Hrh<sub>1</sub> is known to constitute a part of the leptin signaling pathway in the brain [17]. Masaki et al. [17] recently showed that neuronal histamine has an antiobese effect in A<sup>y</sup>/a mice. Although there were no differences in cDNA ORF sequences in these two genes between the KK and C57BL/6J strains, we cannot deny that other explanations are possible; for example, there may be differences in 5′- or 3′- untranslated sequences that affect transcriptional efficiency or mRNA stability.

QTLs for similar traits have been mapped to a neighboring region of Bwq1 as well as Bwq2. On proximal Chr 4, Cheverud et al. [5, 6] have identified Bglq4 (body growth late QTL 4, 6.5 cM) and Wta1 (adult bw, 7.0 cM) in F<sub>2</sub> mice between LG/J and SM/J. On the other hand, in the mid-part of Chr 6, Anunciado et al. [1] have mapped Bw18 (body weight QTL 18, 35.0 cM) to a neighboring region of Bwq2. Bw18 is identified as a suggestive QTL in an SMXA recombinant inbred strains. Anunciado et al. [2] also identified a suggestive body weight QTL on Chr 6 (55.0 cM) in F<sub>2</sub> mice between SM/J and A/J. Taylor et al. [27] mapped Obq14 (obesity QTL 14, 43.5 cM) in F<sub>2</sub> mice between NZO/HILt and SM/J. Cheverud et al. [5, 6] identified Adip2 (adiposity 2, 46.3 cM) and Bgeq4 (body growth early QTL 4, 46.3 cM) in F<sub>2</sub> mice between LG/J and SM/J.

It is noteworthy that the effects of Bwq1 and Bwq2 on WG were significant only between Days 30 and 40. The results suggest that the body weight variations among F<sub>2</sub>-A<sup>y</sup>/a individuals in later life have been determined by the growth during the period shortly after weaning, long before the establishment of an obese phenotype with excessive fat. This cautions us to interpret the results of QTL studies on body weight.

Further modifier loci of the obese phenotype caused by the A<sup>y</sup> allele will be identified by use of different strain combinations of mice. We have identified different modifier loci for yellow pigmentation caused by the A<sup>y</sup> allele in different sets of F<sub>2</sub> combinations [25, 26]. Mice possessing the A<sup>y</sup> allele are predominantly veiled by yellow fur as a consequence of a constitutive antagonism of α-MSH action at the melanocortin 1 receptor (MC1R) in melanocytes by the ubiquitous agouti peptide [23], in a manner analogous to the case in MC4R antagonism. Therefore, a considerable portion of genes or loci that are involved in establishing obesity in A<sup>y</sup> mice may be disclosed when further QTL studies are carried out again and again in different F<sub>2</sub> sets. Identification of modifier genes will provide insight into clarifying definitive physiologic processes.

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