FULL PAPER *Laboratory Animal Science*

Quantitative Trait Loci that Control Body Weight and Obesity in an F_2 Intercross between C57BL/6J and DDD.Cg-A^y Mice

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(Received 23 December 2010/Accepted 6 March 2011/Published online in J-STAGE 18 March 2011)

ABSTRACT. I have developed a congenic mouse strain for the A^y allele at the agouti locus in an inbred DDD/Sgn strain, DDD.Cg-A^y. DDD.Cg-A^y females are extremely obese and significantly heavier than B6.Cg-A^y females. The objectives of this study were to determine the genetic basis of obesity in DDD.Cg-A^y mice, and to determine whether or not their high body weight was due to the presence of DDD background-specific modifiers. I performed quantitative trait locus (QTL) analyses for body weight and body mass index in two types of F_2 mice [F_2 A^y (F_2 mice carrying the A^y allele) and F_2 non-A^y (F_2 mice without the A^y allele)] produced by crossing C57BL/6J females and DDD.Cg-A^y males. The results of the QTL analysis of F_2 A^y mice were very similar to those obtained for F_2 non-A^y mice. It was unlikely that the high body weight of DDD.Cg-A^y mice was due to the presence of specific modifiers. When both F_2 datasets were merged and analyzed, four significant body weight QTLs were identified on chromosomes 6, 9, and 17 (2 loci) and four significant obesity QTLs were identified on chromosomes 1, 6, 9, and 17. Although the presence of DDD background-specific modifiers was not confirmed, a multifactorial basis of obesity in DDD.Cg-A^y females was thus revealed.

KEY WORDS: A^y allele, body weight, DDD mice, obesity, quantitative trait locus (QTL).

J. Vet. Med. Sci. 73(7): 907–915, 2011

In normal mice, the agouti gene is expressed only in the skin [2, 15] and regulates pigmentation by serving as an inverse agonist of the melanocortin 1 receptor (MC1R) [9, 20]. However, in A^y mice, the agouti gene is ectopically overexpressed [4, 15] because the A^y allele is associated with a large deletion and agouti gene expression is controlled by an unrelated *Raly* gene promoter [13, 14]. Obesity in A^y mice is thought to be a consequence of the fact that the agouti protein serves as a constitutive antagonist of the melanocortin 3 receptor (MC3R) and melanocortin 4 receptor (MC4R) by mimicking the action of the agouti-related protein [3, 6, 19]. In particular, MC4R exerts its physiologic effects on feeding by mediating signals from melanocortin peptides downstream of leptin signaling. At present, details of the molecular signaling mechanisms leading to obesity are not yet fully understood.

The magnitude of phenotypic effects of the A^y allele appears to depend on the genetic background, i.e., the location of the A^y allele, suggesting the presence of genes that can modify the action of the A^y allele. For example, KK.Cg-A^y/Ta Jcl (KK-A^y) mice are significantly heavier than B6.Cg-A^y/J (B6-A^y) mice, and we previously identified a quantitative trait locus (QTL), body weight QTL 2 (*Bwq2*) [23, 24]. *Bwq2* is located on the middle of chromosome 6; many gene loci that are relevant to body weight and obesity have been mapped to this chromosome as plausible candidate genes. Identifying causative nucleotide changes is a challenging task; nevertheless, it appears to be a promising approach for searching modifier gene loci and testing their candidacy as QTL genes, because such modifier genes will interact with a known gene whose *in vivo* relevance has been proven.

I have developed a congenic strain for the A^y allele in an inbred DDD/Sgn (DDD) strain, DDD.Cg-A^y (DDD-A^y). Because DDD strain genetically differs from many other inbred mouse strains and is significantly heavier than B6 strain, it is expected that the obesity caused by the A^y allele is altered or enhanced in DDD background. Indeed, female DDD-A^y mice are extremely obese, weighing more than 60 g at 19 weeks onward. These DDD-A^y females are significantly heavier than B6-A^y females. The objectives of this study were (1) to determine the genetic basis of obesity in DDD-A^y mice, and (2) to determine whether or not the high body weight of DDD-A^y mice is due to the presence DDD background-specific modifiers that act similar to *Bwq2*. I performed QTL analyses in two types of F_2 mice [F_2 A^y (F_2 mice carrying the A^y allele) and F_2 non-A^y (F_2 mice without the A^y allele)] produced by crossing C57BL/6J (B6) females and DDD-A^y males. If significant loci are identified only in F_2 A^y mice, these would be regarded as modifier QTLs.

MATERIALS AND METHODS

Mice: The inbred mouse DDD/Sgn ("original" DDD) strain was maintained at the National Institute of Agrobiological Sciences (NIAS, Tsukuba, Ibaraki). The inbred mouse B6 strain was purchased from Clea Japan (Clea Japan Inc., Tokyo). The congenic mouse B6-A^y strain was purchased from the Jackson laboratory (Bar Harbor, ME). The A^y allele was introgressed into the "original" DDD background for 12 generations. During the backcrossing, mice with the A^y allele were identified visually by their yel-

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low coat color. The DDD-A^y strain was thus developed as a congenic strain for the A^y allele. Because "original" DDD strain has an albino coat, congenic mice were further intercrossed between yellow (A^y) and agouti (A) littermates to eliminate the Tyr^c allele. In this study, these agouti littermates were used as the DDD strain. Hereafter, when DDD-A^y and B6-A^y are referred to together, they will be designated "A^y mice". Likewise, their control littermates, DDD and B6 will be designated "non-A^y mice".

Two types of F_2 mice, F_2 A^y (F_2 mice carrying the A^y allele) and F_2 non-A^y (F_2 mice without the A^y allele), were produced by crossing B6 females and DDD-A^y males. Briefly, DDD-A^y males were crossed with B6 females to produce the F_1 generation and F_1 A^y mice were intercrossed with F_1 non-A^y mice to produce the F_2 generation. F_2 females were weaned at 4 weeks and 4 or 5 mice were housed together during the experimental period.

All mice were maintained in a specific-pathogen-free facility with a regular light cycle (12 hr light and 12 hr dark) and controlled temperature (23 ± 1 °C) and relative humidity (50%). Food [CRF-1 (Oriental yeast Co., Ltd., Tokyo)] and water were freely available throughout the experimental period. All the animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of NIAS.

Experimental measurements: At the age of 16 weeks, body weight was determined by an electric balance to the nearest 0.01 g after 4 hr of fasting. The mice were then killed by an overdose of ether. In F_2 mice, the anal-nasal length of an individual mouse was also measured with a pair of calipers to calculate the body mass index {BMI, body weight (g)/[body length (mm)]$^2 \times 10^3$}. BMI was used for estimating the degree of obesity.

Genotyping: Genomic DNA was isolated from the tail of mice with a commercial DNA extraction kit (Wizard Genomic DNA Purification Kit, Promega, Madison, WI). Microsatellite sequence length polymorphism was identified after PCR amplification of genomic DNA by 10% polyacrylamide gel electrophoresis and visualized with ethidium bromide straining.

QTL analysis: QTL analysis was performed only in the females. A total of 96 selected F_2 mice, including the 24 heaviest A^y mice, 24 lightest A^y mice, 24 heaviest non-A^y mice, and 24 lightest non-A^y mice, were genotyped for the following microsatellite marker loci: *D1Mit231*, *D1Mit303*, *D1Mit10*, *D1Mit102*, *D1Mit16*, *D1Mit291*, *D2Mit312*, *D2Mit296*, *D2Mit92*, *D3Mit203*, *D3Mit25*, *D3Mit212*, *D3Mit351*, *D4Mit1*, *D4Mit178*, *D4Mit166*, *D4Mit234*, *D5Mit267*, *D5Mit113*, *D5Mit239*, *D5Mit161*, *D5Mit221*, *D6Mit116*, *D6Mit224*, *D6Mit188*, *D6Mit39*, *D6Mit108*, *D6Mit256*, *D7Mit74*, *D7Mit250*, *D7Mit362*, *D8Mit191*, *D8Mit205*, *D8Mit183*, *D9Mit59*, *D9Mit191*, *D9Mit207*, *D9Mit198*, *D9Mit212*, *D10Mit188*, *D10Mit183*, *D10Mit95*, *D11Mit236*, *D11Mit36*, *D11Mit124*, *D11Mit61*, *D12Mit136*, *D12Mit172*, *D12Mit156*, *D12Nds2*, *D13Mit207*, *D13Mit110*, *D13Mit213*, *D13Mit171*, *D14Mit64*, *D14Mit193*, *D14Mit165*, *D15Mit174*, *D15Mit184*, *D15Mit193*, *D16Mit131*, *D16Mit136*, *D16Mit139*, *D16Mit49*, *D17Mit164*, *D17Mit176*, *D17Mit139*, *D17Mit93*, *D17Mit123*, *D18Mit21*, *D18Mit149*, *D18Mit152*, *D18Mit25*, *D19Mit32*, *D19Mit91*, *D19Mit35*, *DXMit119*, and *DXMit64*. All F_2 mice (n = 298) were genotyped for the underlined microsatellite markers. Due to the design of the cross, chromosomal regions surrounding the agouti locus (*a*) on chromosome 2 and the tyrosinase locus (*Tyr*) on chromosome 7 could not be fully analyzed.

Normality of the trait data distribution in each F_2 type was tested using the Shapiro–Wilk W test (JMP 8, SAS Institute Japan, Tokyo). If the trait values did not follow a normal distribution, they were appropriately normalized by Box-Cox transformation.

Initially, F_2 A^y and F_2 non-A^y mice were analyzed independently for single QTL using the "Marker regression" and "Interval mapping" functions of Map Manager QTX b20 software [11]. The threshold likelihood ratio statistics (LRS) for suggestive ($P<0.63$), significant ($P<0.05$), and highly significant ($P<0.001$) linkages at the genome-wide 5% level was determined by performing 1,000 permutations for each trait. The LRS was converted to a likelihood of odds (LOD) score by dividing by 4.605. For chromosomal regions showing LOD scores exceeding the threshold for significant linkage, all microsatellite makers were genotyped for the remaining mice in both F_2 types. For chromosomal regions showing LOD scores exceeding the threshold for suggestive linkage, microsatellite markers locating such regions were genotyped for the remaining mice in each F_2 type. After simple interval mapping, the potential interaction between marker loci was evaluated pairwise. For this purpose, the following 2-stage test was used: (1) The threshold for significance at the genome-wide 5% level was determined by performing 1,000 permutations on the interaction model of Map Manager QTX; then the significance of the total effect of the two loci was tested (2) When pairs of loci showed a LOD score exceeding the threshold (approximate LOD score ≥8.6), the significance of the pairwise interaction was evaluated. According to the instruction manual of Map Manager QTX, the interaction effect itself must have a P value less than 0.01. In this study, I also employed a stringent criterion that only those pairs of loci showing interaction LOD scores of ≥3 would be recognized as indicating a significant interaction [10, 18].

F_2 A^y and F_2 non-A^y data sets were then combined and analyzed to improve the overall power to detect QTL. To merge the data from the F_2 non-A^y and F_2 A^y mice, transformed trait values were standardized to a mean 0 and variance 1 by subtracting the F_2 type-specific mean from each individual value and dividing each difference by the standard deviation of its respective F_2 type. The threshold LOD scores were then calculated again. After the single QTL scan, when the combined F_2 (F_2 non-A^y plus F_2 A^y) dataset were analyzed, the presence or absence of possible statistical interactions between the genotype of the microsatellite marker nearest the QTL (DDD/DDD, DDD/B6, and B6/B6)

and the agouti genotype (non-A^y and A^y) was evaluated by 2-way ANOVA.

cDNA sequencing of histamine receptor H1 (Hrh1): Because *Hrh1* is a plausible candidate gene for body weight QTL on chromosome 6 [28], the *Hrh1* cDNA sequence was determined in the DDD strain. For *Hrh1* sequencing, mRNA was extracted from the brain using the QuickPrep micro mRNA purification kit (GE Healthcare UK Ltd., UK). Reverse transcription was performed using the TaKaRa RNA PCR kit (AMV) Ver. 3.0 (Takara Bio Japan Inc., Shiga, Japan). The ORF was amplified and directly sequenced using the following sets of PCR primers: 5'-GAGTGAAACCCGATGCTTGT-3' and 5'-AGAGGT-CACCTTGGGGTCTT-3', and 5'-AAGCTGAGGTCG-GAG-GATG-3' and 5'-GAATGCCTTCACACTGCTCA-3'.

2.6. Other statistics: Statistical analysis between 2 groups was performed using Student's or Welch's *t*-test. $P<0.05$ was considered to be statistically significant.

RESULTS

Comparison of body weight among DDD, DDD-A^y, B6, and B6-A^y strains: Figure 1A shows scatter plots of body weight in the DDD males (n=22), DDD-A^y males (n=12), B6 males (n=14), B6-A^y males (n=15), DDD females (n=13), DDD-A^y females (n=12), B6 females (n=14), and B6-A^y females (n=13). Statistical comparisons were separately performed for A^y mice and non-A^y mice. There was no significant difference in body weight between DDD-A^y males and B6-A^y males ($P>0.1$). DDD males were significantly heavier than B6 males ($P<8.3\times10^{-7}$). DDD-A^y females were significantly heavier than B6-A^y females ($P<2.0\times10^{-11}$), and DDD females were significantly heavier than B6 females ($P<9.6\times10^{-9}$).

Body weight and BMI in B6 × DDD-A^y F_1 and F_2 mice: Figure 1A shows scatter plots of body weight in F_1 non-A^y (n=24), F_1 A^y (n=14), F_2 non-A^y (n=148), and F_2 A^y (n=150) mice. Similar to the observation in the parental strains, F_1 A^y and F_2 A^y mice were significantly heavier than F_1 non-A^y and F_2 non-A^y mice, respectively ($P<2.2\times10^{-18}$ and $P<2.6\times10^{-92}$, respectively).

Histograms showing the distribution of BMI in F_2 non-A^y and F_2 A^y mice are shown in Figs. 2A and 2B (the number of mice is not equal to that used for body weight plots because I failed to measure the body length in several mice). The wide spectrum of distribution was similar to that observed for body weight. The distribution in F_2 A^y mice followed a normal distribution, but that in F_2 non-A^y did not. Mean and standard error of BMI was 3.28 ± 0.03 in F_2 non-A^y and 4.85 ± 0.04 in F_2 A^y mice. Thus, BMI was significantly higher in F_2 A^y than in F_2 non-A^y mice ($P<1.8\times10^{-90}$). BMI showed strong positive correlation with body weight in both F_2 types (Pearson correlation coefficient was 0.89 in F_2 non-A^y mice and 0.90 in F_2 A^y mice).

Genome-wide scans for single QTL in B6 × DDD-A^y F_2 mice: First, F_2 non-A^y and F_2 A^y mice were analyzed separately. Table 1 shows the results of single QTL scans. No significant QTLs were identified in F_2 non-A^y mice, whereas four significant QTLs were identified in F_2 A^y mice. Two significant QTLs for body weight were identified on chromosomes 6 and 17 (proximal locus) (Figs. 3A and 3B). I named these loci Body weight in DDD QTL no.2 (*Bwdq2*, because *Bwdq1* was previously assigned [22]) and *Bwdq3*, respectively. Two significant QTLs for BMI were identified on chromosomes 6 and 9 (Figs. 3A and 3C). I named these loci Obesity in DDD QTL no. 1 (*Obdq1*) and *Obdq2*, respectively.

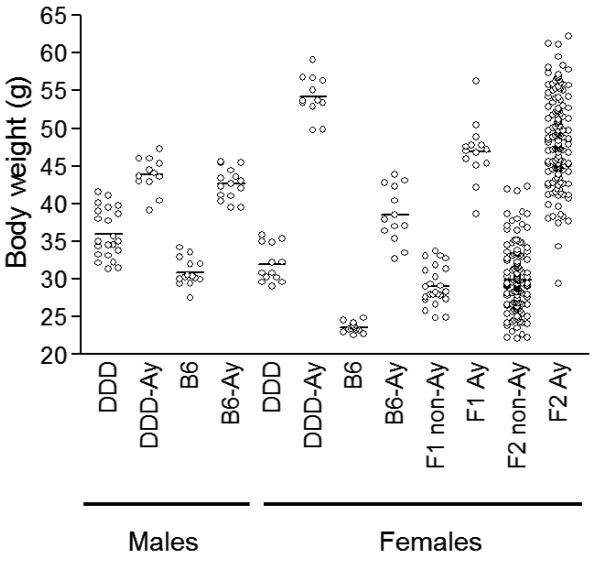

Fig. 1. Plots of body weight in parental, F_1, and F_2 mice. Each point represents the body weight of an individual mouse. Horizontal bar indicates the average for the strain.

Next, data on F_2 non-A^y and F_2 A^y mice were merged and reanalyzed and eight significant QTLs were identified (Table 2). With regard to these QTLs, no statistically significant interactions were identified between the genotype of the microsatellite marker nearest to the QTL and the agouti genotype (see below), i.e., allele effects of QTLs were in the same direction in F_2 non-A^y and F_2 A^y mice. For body weight, four significant QTLs were identified on chromosomes 6, 9, and 17 (proximal and distal loci) (Figs. 3A–C). I named the locus on chromosome 9 as *Bwdq4*, and the locus on chromosome 17 (distal locus) as *Bwdq5*. For BMI, four significant QTLs were identified on chromosomes 1, 6, 9, and 17 (distal locus) (Figs. 3A–D). I named the locus on chromosome 1 as *Obdq3*, and the locus on chromosome 17 (distal) as *Obdq4*. LOD score plot curves were similar between F_2 non-A^y and F_2 A^y mice; allele effects of QTLs were in the same direction in F_2 non-A^y and F_2 A^y mice, and therefore the maximum LOD score increased in the merged F_2 dataset.

Genome-wide scans for interacting QTLs: Potential interactions were analyzed pairwise only in the separate F_2 datasets (not in the merged F_2 dataset). The permutation-

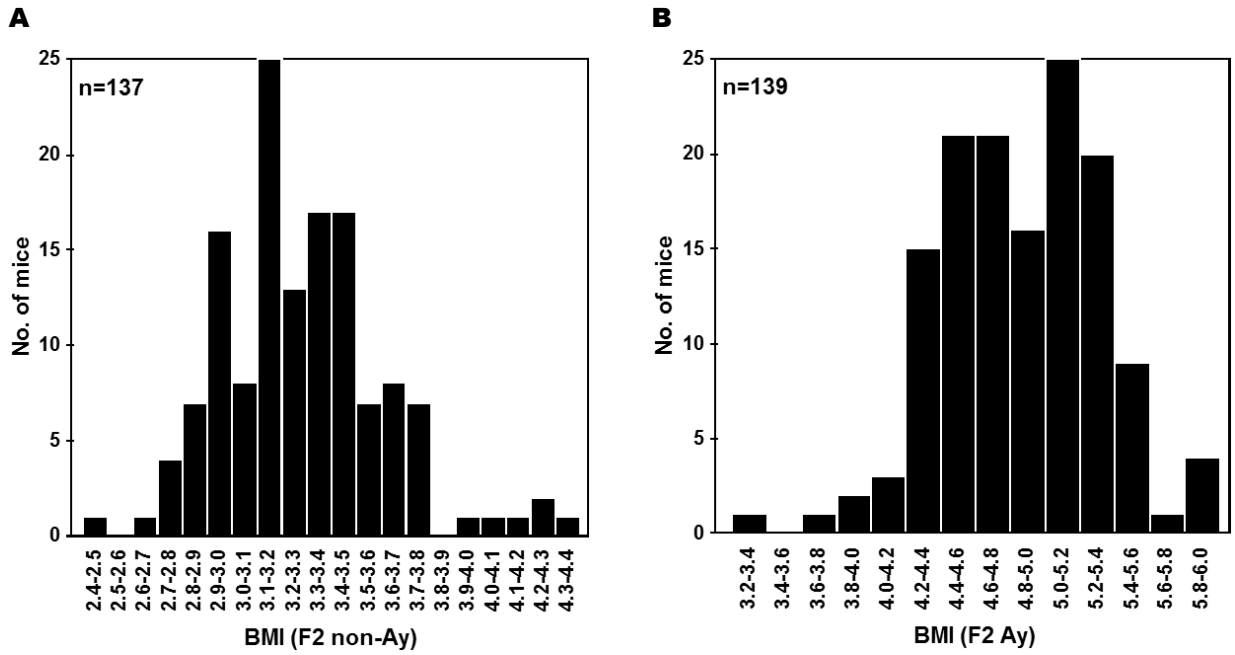

Fig. 2. Histograms showing distribution of BMI in F_2 non-A^y (A) and F_2 A^y (B) mice. The mean ± standard error is 3.28 ± 0.03 in F_2 non-A^y and 4.85 ± 0.04 in F_2 A^y mice.

Table 1. QTL identified in F_2 non-A^y and F_2 A^y mice by single QTL scans

Traits	F_2	Chr[a)]	Location (cM)[b)]	95% CI (cM)[c)]	Max LOD[d)]	Nearest marker	High allele[e)]	Name[f)]
Body weight	non-A^y	1	89		2.8	*D1Mit291*	DDD	
		6	41		3.4	*D6Mit39*	B6	
		15	5		2.3	*D15Mit174*	DDD	
		17	61		3.3	*D17Mit123*	DDD	
	A^y	6	46	35–52	5.6 **	*D6Mit39*	B6	*Bwdq2*
		9	1		2.5	*D9Mit59*	DDD	
		14	31		2.1	*D14Mit64*	B6	
		17	6	[g)] –16	3.8 *	*D17Mit164*	B6	*Bwdq3*
		17	61		2.8	*D17Mit123*	DDD	
BMI	non-A^y	1	54		2.8	*D1Mit102*	DDD	
		6	41		2.6	*D6Mit39*	B6	
		9	1		2.7	*D9Mit59*	DDD	
		17	61		3.4	*D17Mit123*	DDD	
	A^y	6	42	27–53	4.7 *	*D6Mit39*	B6	*Obdq1*
		9	1	[g)] –20	3.6 *	*D9Mit59*	DDD	*Obdq2*
		17	61		3.3	*D17Mit123*	DDD	

a) Chromosome.
b) Location means a chromosomal position showing a peak LOD score in cM units.
c) 95% CI is defined by a 1.5-LOD support interval and is determined only for significant QTLs.
d) Maximum LOD score for QTL. Significant, and highly significant QTLs are indicated by * and **, respectively (suggestive QTLs are shown without an asterisk).
e) Allele that is associated with higher trait values.
f) Assignment of a QTL name was limited to significant and highly significant QTLs.
g) A proximal end of CI cannot be determined because it extends proximally.

derived threshold LOD score for the significance of the total LOD score for association was 8.6. In F_2 non-A^y mice, no significant interactions were identified. In F_2 A^y mice, one significant interaction was identified between *D9Mit59* and *D17Mit164* for body weight (Fig. 4). The total LOD score for association was 9.7 and the interaction LOD score was 3.7. Both loci were the nearest markers for significant body weight QTLs, and therefore, I did not assign novel symbols

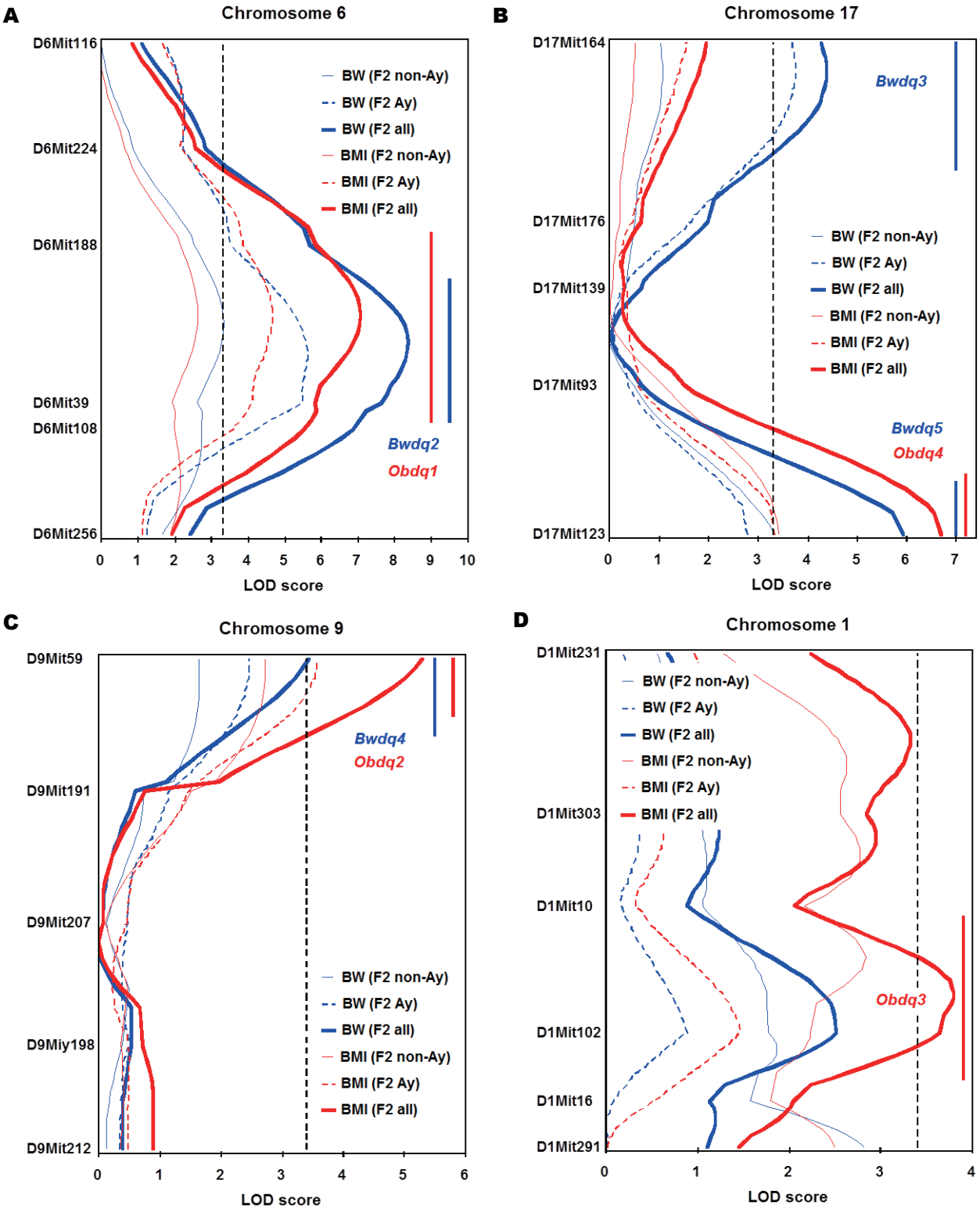

Fig. 3. LOD score plots for body weight (BW) and BMI on chromosomes 6 (**A**), 17 (**B**), 9 (**C**), and 1 (**D**). X-axis represents LOD score. Y-axis represents microsatellite marker localization: the upper part of the graph is in the direction of the centromere, and the lower part is in the direction of the telomere. LOD score plots for BW are drawn in blue, while those for BMI are in red. Vertical short solid lines indicate 95% confidence intervals (CIs) for the plot of the merged F_2 dataset (BW: blue, BMI: red). A broken black line indicates a threshold LOD score obtained by a permutation test in the merged F_2 dataset.

Table 2. QTLs identified in the merged F_2 dataset by single QTL scans

Traits	Chr[a)]	Location (cM)[b)]	95% CI (cM)[c)]	Max LOD[d)]	Nearest marker	High allele[e)]	Name[f)]
Body weight	1	63		2.5	*D1Mit102*	DDD	
	5	74		2.3	*D5Mit221*	DDD	
	6	43	35–52	8.4 **	*D6Mit39*	B6	*Bwdq2*
	9	1	[g)] –15	3.4 *	*D9Mit59*	DDD	*Bwdq4*
	14	29		2.0	*D14Mit64*	B6	
	15	2		2.0	*D15Mit174*	DDD	
	17	5	2–15	4.4 *	*D17Mit164*	B6	*Bwdq3*
	17	61	56–[h)]	5.9 **	*D17Mit123*	DDD	*Bwdq5*
BMI	1	60	47–73	3.8 *	*D1Mit102*	DDD	*Obdq3*
	6	40	32–51	7.1 **	*D6Mit39*	B6	*Obdq1*
	9	1	[g)] –12	5.3 *	*D9Mit59*	DDD	*Obdq2*
	17	2		2.0	*D17Mit164*	B6	
	17	61	56– [h)]	6.7 **	*D17Mit123*	DDD	*Obdq4*

a) Chromosome.
b) Location means a chromosomal position showing a peak LOD score in cM units.
c) 95% CI is defined by a 1.5-LOD support interval, and is determined only for significant QTLs.
d) Maximum LOD score for QTL. Significant, and highly significant QTLs are indicated by * and **, respectively (suggestive QTL are shown without an asterisk).
e) Allele that is associated with higher trait values.
f) Assignment of a QTL name was limited to significant and highly significant QTLs.
g) A proximal end of CI cannot be determined because it extends proximally.
h) A distal end of CI cannot be determined because it extends distally.

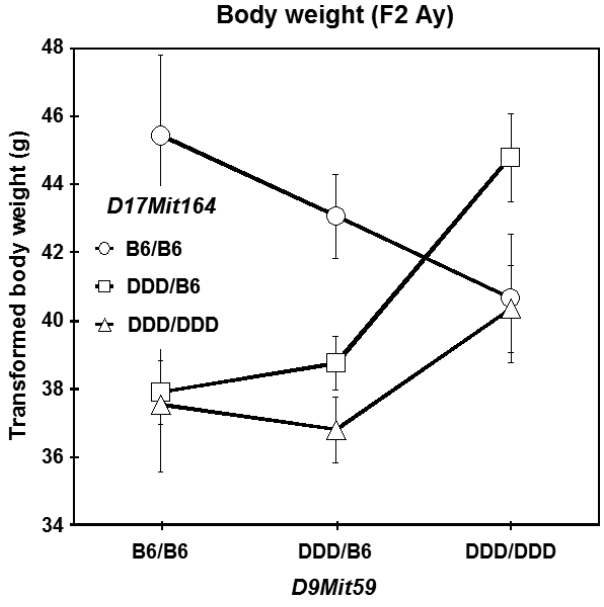

Fig. 4. Effects of gene interactions on body weight in F_2 A^y mice detected by a pairwise genome scan. Homozygous DDD alleles are denoted by DDD/DDD, homozygous B6 alleles by B6/B6, and a heterozygous allele by DDD/B6.

to them.

Hrh1 cDNA sequencing: Because *Hrh1* is a plausible candidate gene for *Bwdq2* on chromosome 6, the *Hrh1* cDNA sequence was determined in the DDD strain and compared with that in the B6 strain. A nucleotide substitution was identified at nucleotide 1,430 (nucleotide numbering was based on GenBank entry NM_008285); B6 strain had T (CTT), while DDD strain had C (CTC). This nucleotide substitution was not accompanied by an amino acid change.

DISCUSSION

A multifactorial basis of obesity in DDD-A^y strain females was revealed. It is unlikely that the high body weight of DDD-A^y mice was due to the presence of specific modifiers, although some genomic regions, including mitochondria and parts of chromosomes 2, 7, and X, were only poorly analyzed.

I speculate the reason why only females are obese in DDD-A^y strain may be related to a difference in hormonal conditions between males and females. DDD strain is known to have higher blood testosterone levels than does B6 strain [5]. According to Moulana *et al*. [17], obesity is associated with reductions in testosterone levels in men, whereas obesity is associated with increases in testosterone levels in women. Therefore, it appears to be possible to consider that a part of reason for the female-specific obesity in DDD-A^y strain is related to a difference in hormonal conditions between males and females.

For body weight, two significant QTLs were identified on chromosomes 6 and 17 (proximal) in F_2 A^y mice, whereas no significant QTLs were identified in F_2 non-A^y mice. For BMI, two significant QTLs were identified on chromosomes 6 and 9 in F_2 A^y mice, whereas no significant QTLs were identified in F_2 non-A^y mice. Furthermore, one significant interaction was identified between *D9Mit59* and *D17Mit164* for body weight but only in F_2 A^y mice. Therefore, these QTLs were suggested as modifier loci for the A^y allele. However, all significant QTLs identified in F_2 A^y mice, except for the locus on chromosome 17, were also

suggestive QTLs in F_2 non-A^y mice. Thus, it appeared inadequate to regard these QTLs as modifier loci. Furthermore, except for QTL on chromosome 9, the B6 allele was associated with increased body weight and/or BMI at all loci. It was not surprising that the lean strain-derived allele was associated with increased body weight [26, 27], but it was troublesome that the B6 allele was associated with increased body weight at majority of the loci. Nevertheless, there are explanations to account for this discrepancy. First, the DDD allele was associated with increased trait values at all suggestive loci except for the locus on chromosome 14 for body weight. Thus, the cumulative contribution of these suggestive loci should not be underestimated. Indeed, the suggestive QTLs on distal chromosome 17 (Table 1) were identified as significant QTLs for both body weight and BMI with the second highest LOD scores in merged F_2 dataset (Table 2). Second, the contribution of the mitochondrial genome of the DDD strain was lost in F_2 analysis because the F_2 generation was derived from B6 females and DDD-A^y males. Likewise, X-linked genes were not fully analyzed in this study. The F_2 mice were either homozygous for the B6 allele or heterozygous for the X-linked loci; mice homozygous for the DDD allele were absent. Thus, if a putative X-linked QTL from the DDD strain is associated with increased body weight and a recessive inheritance mode, it could not be detected. Third, it should be noted that portions of chromosomes 2 (surrounding the A^y allele at the agouti locus, 76.83 cM) and 7 (surrounding the *Tyr* locus, 49.01 cM) in DDD-A^y mice were contributed by the B6 genome. Because the DDD strain is homozygous for the *A* allele (*A*/*A*) and Tyr^c allele (Tyr^c/Tyr^c), the A^y and wild-type *Tyr* alleles, and their neighboring genome were introgressed from the B6 strain (with regard to chromosome 7, the introgressed region was limited to a maximum of 45–64.2 cM based on the estimation of microsatellite polymorphisms). Thus, the high body weight of the DDD-A^y strain may be intrinsically sustained by the B6 genome on chromosomes 2 and 7 in combination with the rest of the DDD genome. I cannot deny the possibility that the B6-derived genes in these chromosomal regions contain the principal QTL for enhanced obesity in DDD-A^y mice. It should be noted that unlike the "original" DDD strain, DDD mice also had a B6-derived genome on chromosome 7.

It was noted that the results of QTL analysis from two separate F_2 types showed high similarity; for example, loci for body weight on chromosomes 6 and 17 (distal) and those for BMI on chromosome 6, 9, and 17 (distal) were common for the two F_2 types (Table 1). Therefore, data of both F_2 types were merged and reanalyzed. As expected, statistical power to detect significant QTLs increased, i.e., LOD scores for some suggestive QTLs identified in the separate F_2 dataset exceeded the significance threshold in the merged F_2 dataset. In addition, no significant interactions were identified between the genotypes of the microsatellite marker nearest to the QTL and the agouti genotype in the merged F_2 dataset. This in turn justifies the QTL analyses performed in the merged F_2 dataset. As a result, four significant QTLs were identified for body weight and BMI, respectively. Taken together, the high body weight of DDD-A^y mice was probably because DDD strain is heavier than B6, and not because of specific modifiers. Differences of body weight between DDD-A^y and B6-A^y as well as between DDD and B6 strains were explained by the same set of loci. It is thus possible to regard the DDD-A^y female as a mouse strain of multifactorial obesity.

Many QTLs that are coincidental with the present ones have been reported. For example, *Bwq2* [24] and *Tabw2* [8] apparently overlap with *Bwdq2* and *Obdq1* on chromosome 6. We previously identified *Bwq2* as a modifier QTL for the A^y allele in an F_2 intercross between B6 and obese KK-A^y strains [23, 24]. Similar to *Bwq2*, the effects of *Bwdq2* and *Obdq1* were stronger in F_2 A^y than in F_2 non-A^y mice. However, in contrast to *Bwq2*, the lean strain (B6)-derived allele at *Bwdq2* and *Obdq1* was associated with increased body weight and obesity. *Tabw2* was identified in the TallyHo strain, which is a multigenic mouse model of non-insulin-dependent diabetes mellitus with obesity [8]. At *Tabw2*, the TallyHo-derived allele was associated with increased body weight. Allelism among these loci is unclear. *Hrh1* is a plausible candidate gene for body weight QTL identified in the middle of chromosome 6 because *Hrh1* constitutes part of the leptin signaling pathway in a way similar to MC4R, and because central administration of histamine decreases body weight and adiposity in A^y mice [12]. I could not find any mutations in the coding sequence of *Hrh1* in DDD and KK mice [23]. Kim *et al.* [8] also analyzed *Hrh1* as a candidate gene, and found no nucleotide changes between TallyHo and B6 alleles. Needless to say, this chromosome 6 region contains many other candidate genes relevant to body weight and obesity; for example, peroxisome proliferator activated receptor gamma, thyrotropin releasing hormone, and ghrelin [28]. Their candidacy remains to be investigated.

Proximal chromosome 17 is also known to contain several obesity QTLs. For example, *Obq4a* [25], *Obq4b* [27], and *Obq19* [7] apparently overlap with *Bwdq3*. *Bwdq3* was a significant QTL for body weight, but not for obesity (BMI). In contrast, *Obq4a*, *Obq4b*, and *Obq19* were identified for obesity using an adiposity index or BMI. Results for body weight, BMI, and adiposity should not be simply compared with one another, and indeed, the effects of *Obq4a* on body weight and BMI were modest (i.e., LOD scores were 1.5 for body weight and 1.6 for BMI). The effects of *Obq4b* and *Obq19* on body weight were not described. Thus, it is unclear whether *Bwdq3* was allelic with one or more of the remaining QTLs. Nevertheless, it is interesting and important to note that at *Obq4a*, *Obq4b*, *Obq19*, and *Bwdq3* loci, the allele from the lean strain was associated with increased adiposity and/or body weight. In contrast to the proximal locus, only a few coincidental QTLs have been reported for the locus on distal chromosome 17. Allan *et al.* [1] analyzed growth and body composition in a large F_2 population resulting from an intercross between M16 and ICR and identified several QTLs for body weight, growth, and fat-

ness. The M16 line is more obese than ICR, and the M16 allele is associated with increases in these trait values, in a way similar to *Bwdq5* and *Obdq4*.

Other significant QTLs for body weight (*Bwdq4*) and BMI (*Obdq2*) were mapped to the centromeric end of chromosome 9 (Fig. 3C). *Obq5*, identified in an F_2 intercross between KK/HlLt and B6, was mapped to the proximal portion of chromosome 9 [26]. Based on the LOD score, effect of *Obq5* was stronger for adiposity than for body weight in females. However, the location of the 1.0-LOD support interval appeared to be slightly distal to *Bwdq4* and *Obdq2* in females. Su *et al.* [21] identified the body fat QTL *Obq32* on proximal chromosome 9 in an F_2 intercross between B6 and 129S1/SvlmJ, but its effect was significant only in males. Thus, sex specificity was noted for QTL on chromosome 9. *Bwdq4* as well as *Obdq2* may be novel QTLs in mice.

Several studies have reported the presence of obesity QTL on chromosome 1. Moody *et al.* [16] identified *Fatq1* for fat weight in an F_2 intercross between MH and B6. Taylor *et al.* [27] identified *Obq8* for adiposity in an F_2 intercross between NZO and SM. Ishimori *et al.* [7] identified *Obq17* for fatness in a F_2 intercross between B6 and 129S1/SvlmJ. Vogel *et al.* [29] identified *Nob3* for obesity in an F_2 intercross between NZO and B6. *Obq8* and *Nob3* were identified in crosses containing the obese NZO strain, thus suggesting that they were allelic. *Nob3* was also significant for body weight. In this study, *Obdq3* was not significant for body weight, but significant for obesity. Thus, *Obdq3* may not be allelic with *Obq8* and *Nob3*.

Pairwise searches for interacting QTLs identified one significant interaction between *D9Mit59* and *D17Mit164* for body weight in F_2 A^y mice. *D9Mit59* and *D17Mit164* were the nearest markers for *Bwdq4* and *Bwdq3*, respectively. Therefore, the interaction immediately suggests functional relationships between these QTLs. These loci were fully genotyped in all F_2 mice. However, many other loci were genotyped in a limited number of mice. Hence, I cannot deny the possibility that I missed additional interacting QTLs.

In conclusion, although the presence of DDD background-specific modifiers was not confirmed, a multifactorial basis of obesity in DDD-A^y strain females was revealed.

ACKNOWLEDGMENT. This study was supported in part by a grant from Kieikai Research Foundation.

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