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Quantitative Trait Locus Analysis of Plasma Cholesterol Levels and Body Weight by Controlling the Effects of the *Apoa2* Allele in Mice

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ABSTRACT. Colleagues and I previously performed quantitative trait locus (QTL) analysis on plasma total-cholesterol (T-CHO) levels in C57BL/6J (B6) × RR F_2 mice. We identified only one significant QTL (*Cq6*) on chromosome 1 in a region containing the *Apoa2* gene locus, a convincing candidate gene for *Cq6*. Because *Cq6* was a highly significant QTL, we considered that the detection of other potential QTLs might be hindered. In the present study, QTL analysis was performed in B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice [B6.KK-*Apoa2*^{b} N(8) is a partial congenic strain carrying the *Apoa2*^{b} allele from the KK strain, and RR also has the *Apoa2*^{b} allele] by controlling of the effects of the *Apoa2* allele, for identifying additional QTLs. Although no significant QTLs were identified, 2 suggestive QTLs were found on chromosomes 2 and 3 in place of the effects of the *Apoa2* allele. A significant body weight QTL was identified on chromosome 3 (*Bwq7*, peak LOD score 5.2); its effect on body weight was not significant in previously analyzed B6 × RR F_2 mice. Suggestive body weight QTL that had been identified in B6 × RR F_2 mice on chromosome 4 (LOD score 3.8) was not identified in B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice. Thus, contrary to expectation, the genetic control of body weight was also altered significantly by controlling of the effects of the *Apoa2* allele. The QTL mapping strategy by controlling of the effects of a major QTL facilitated the identification of additional QTLs.

KEY WORDS: *Apoa2* allele, body weight, cholesterol, quantitative trait locus (QTL).

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Altered cholesterol metabolism is frequently associated with obesity and/or non-insulin-dependent diabetes mellitus, and serves as a major risk factor for atherosclerosis. Therefore, proper control of plasma cholesterol levels is clinically important. Plasma cholesterol levels are determined by both genetic and environmental factors, and genetic factors presumably consist of multiple loci. Colleagues and I previously performed quantitative trait locus (QTL) analysis to identify genes influencing variation in plasma total-cholesterol (T-CHO) levels in F_2 mice between C57BL/6J (B6) and RR strains [22]. We identified a highly significant T-CHO QTL (*Cq6*) on distal chromosome 1 in a region containing the apolipoprotein A-II (*Apoa2*) gene locus, a convincing candidate gene for *Cq6*, with a maximum LOD score of 16.3 (this locus explained 33.1% of phenotypic variance). Other QTLs, even suggestive ones, were not identified. Considering the facts that B6 × RR F_2 mice exhibited large variations in T-CHO levels, and that four significant QTLs (*Cq1* through *Cq4*; of these, *Cq2* was colocalized with *Cq6* on chromosome 1) were identified in an essentially similar analysis in F_2 mice between B6 and KK ($-A^y$) strains [21, 24], it seemed somewhat strange and disappointing that there was only one QTL. We considered that the large QTL effect of *Cq6* hindered the detection of other potential QTLs that have small QTL effects compared to that of *Cq6* (the maximum LOD score for *Cq2* was 9.2, whereas that for *Cq6* was 16.3). On the basis of experimental results [8, 22, 24, 26], it is worthwhile to presume that *Apoa2* is causative of *Cq6*. In fact, I previously concluded that the *Apoa2*^{b} allele is unique in its ability to increase cholesterol levels among the commonly observed three *Apoa2* alleles (*Apoa2*^{a}, *Apoa2*^{b}, and *Apoa2*^{c}), and that cholesterol QTL is identified in the *Apoa2* region when either one of two strains of F_2 mice has the *Apoa2*^{b} allele (therefore the other strain must have *Apoa2*^{a} or *Apoa2*^{c}) [7, 18, 22]. The functional property of the *Apoa2*^{b} allele is probably attributable to the Ala-to-Val substitution at amino acid residue 61 [18, 22]. A study by Wang *et al.* [27] reported that Ala at residue 61 in *Apoa2*^{a} and *Apoa2*^{c} strains is conserved in wild-derived mouse strains, humans, chimpanzees, monkeys, horses, cattle, pigs, and rats; they concluded that Val at residue 61 is a mutation in mice with *Apoa2*^{b}. They also carried out haplotype analysis of the strains carrying *Apoa2*^{b}, and excluded the possibility that an unknown gene in linkage disequilibrium with *Apoa2* was underlying QTLs on distal chromosome 1 [27]. Therefore, I designed the present study to control the effects of the *Apoa2* allele by use of partial congenic mice, B6.KK-*Apoa2*^{b} N(8), which had been established by successive backcrossing of the *Apoa2* allele from the KK strain (*Apoa2*^{b}) onto the B6 strain (*Apoa2*^{a}), thereby performing a QTL analysis in B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice. By mapping of QTLs in the absence of a segregating *Apoa2* allele in B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice, and comparing the QTL results with those in B6 × RR F_2 mice, I expect that it is possible to identify additional QTLs, whose detection had been prevented by the strong QTL effects of *Cq6*.

MATERIALS AND METHODS

Mice and genetic crosses: Mice of the inbred B6 and KK strains were purchased from Clea Japan (Tokyo). The inbred mouse RR strain had been maintained in my laboratory. The partial *Apoa2* congenic strain, B6.KK-*Apoa2*^{b}

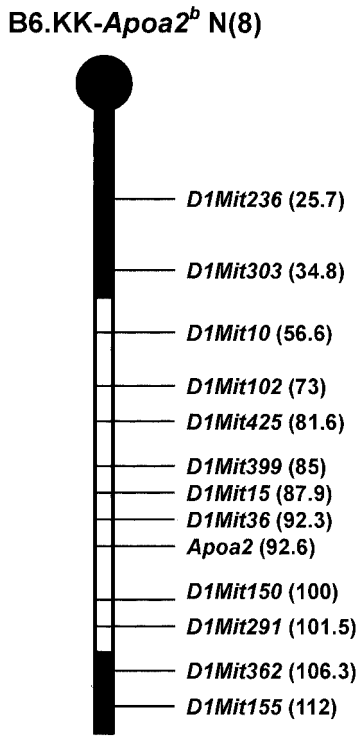

Fig. 1. Molecular mapping of chromosomal break points in the partial *Apoa2* congenic strain, B6.KK-*Apoa2*^{b} N(8). Polymorphisms for 12 microsatellite marker loci and *Apoa2* gene locus on chromosome 1 were identified between the B6 and KK strains. Chromosomal position of each marker locus is indicated in a parenthesis in centi-Morgans (cM) unit from the centromere based on the information from MGI (http://www.jax.org). A minimum of 45 cM (*D1Mit10-D1Mit291*, this is approximately equal to 94 Mb) of B6 genome (pained out by black) has been replaced by KK genome (white box).

N(8), had been established by successive backcrossing of the *Apoa2* allele from the KK strain (*Apoa2*^{b}) onto the B6 strain (*Apoa2*^{a}) for eight generations ([22] and Fig. 1). B6.KK-*Apoa2*^{b} N(8) females were crossed with RR males to produce F_1 mice, which were intercrossed to produce F_2 mice. A total of 152 female F_2 mice were weaned at 30 days after birth, and four or 5 mice were housed together in a large aluminum cage. Hereafter, we define the RR strain as having R alleles, and the B6 strain as having B alleles, throughout the genome. All mice were maintained in a specific-pathogen-free facility, with regular light cycles of 12 hr light/12 hr dark, and with controlled temperature and humidity. They had free access to food [rodent pellet chow, CE-2 (342.2 kcal/100 g, containing 4.4% crude fat), Clea Japan] and tap water. Experiments were approved by the Institutional Animal Care and Use Committee of National Institute of Agrobiological Sciences.

Experimental measurements: Phenotype measurements were done on individual mice at the age of 124 ± 2 days. After 4-hr fasting, individual body weight was determined to the nearest 0.1 g by an electric balance, and then mice were sacrificed with overdoses of ether. Blood was collected from the heart into microtubes, with heparin used as an anticoagulant. For separating the plasma from whole blood, the tubes were centrifuged at 7,000 rpm for 5 min at 4°C. All plasma samples were stored at –70°C until use. Plasma cholesterol levels were determined enzymatically with a spectrophotometer by use of clinical chemical kits (Cholesterol E test WAKO, Wako Pure Chemical Industries, Osaka) according to the instructions of the manufacturer.

QTL analysis and statistics: Genomic DNA was isolated from the tails of mice with a commercial DNA extraction kit (Wizard Genomic DNA Purification Kit, Promega, Madison, WI). Microsatellite sequence length polymorphism was detected by electrophoresis subsequent to PCR. Most microsatellite primers were purchased as MapPairs (Research Genetics, Huntsville, AL), whereas others were synthesized on the basis of information from Mouse Genome Informatics (MGI, http://www.jax.org). Amplification was carried out by use of a Takara PCR thermal cycler MP (TaKaRa Biomedicals, Tokyo) 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. All PCR products were electrophoresed on 10% polyacrylamide gels for 70 min and visualized by ethidium bromide staining.

To identify putative T-CHO QTLs, a total of 48 F_2 mice, including 24 mice showing the highest T-CHO levels and 24 mice showing the lowest T-CHO levels, were genotyped for a total of 64 microsatellite marker loci distributed on all autosomes and on the X chromosome, with an approximate distance between markers of 25 cM (1,600/64). A list of microsatellite markers used in this study was already published, with information on their chromosomal positions [19, 22]. At each locus, one-way ANOVA was carried out. Once a nominal P value of less than 0.05 was identified, the remaining 104 F_2 mice were genotyped for the relevant microsatellite marker. Essentially the same analysis was performed to identify putative body weight QTLs. In this case, a total of 46 F_2 mice, including 22 mice showing the lightest body weight and 24 mice showing the heaviest body weight, were genotyped. In total, 80 F_2 mice were genotyped for all microsatellite loci at the initial stage.

QTL analysis was carried out with Mapmaker/EXP version 3.0b and the Mapmaker/QTL 1.1b computer program [10]. A chromosomal region with a 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 (equivalent to $P=1.6 \times 10^{-3}$) and 4.3 (equivalent to $P=5.2 \times 10^{-5}$) was recognized as showing suggestive linkage [11]. The α level for suggestive linkage implies the expectation that there will be one false positive in a genome-wide search. Once significant QTLs were identified, the 95% confidence interval (CI) for the QTL was defined as a 1.5-LOD score support interval. Potential interaction between marker loci was evaluated

pairwise using Map Manager QTX b20 software [13]. For this analysis, the threshold for significance at genome-wide 5% level was obtained for both traits (T-CHO and body weight) by performing 1,000 permutations on the interaction model of Map Manager QTX b20, and then the significance of the total effect of the two loci was tested.

RESULTS

Comparison of T-CHO levels and body weight between B6.KK-Apoa2^b N(8) × RR F$_2$ mice and B6 × RR F$_2$ mice: T-CHO levels and body weight were determined at the age of 130 ± 5 days after 24-hr fasting in B6 × RR F$_2$ mice, and at the age of 124 ± 2 days after four-hr fasting in B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice. Even though both F$_2$ mice are consisted of only females; there are thus slight differences in the details of the experimental procedures.

Plasma T-CHO levels were higher in RR than in B6 as shown in the previous study [22], and T-CHO levels in RR are probably higher than in B6.KK-*Apoa2^b* N(8) based on the results of a previous congenic analysis [22]. In the present study, T-CHO levels were determined in all B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice (n=152) (Fig. 2A), and were compared with those previously determined in B6 × RR F$_2$ mice (n=187) (Fig. 2B). The average T-CHO value was 102.0 ± 24.3 (mean ± S.D.) mg/dl in B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice, and 90.9 ± 22.8 (mean ± S.D.) mg/dl in B6 × RR F$_2$ mice; the difference was statistically significant ($P<2.4 \times 10^{-5}$). This may be attributed to the fact that B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice have only the *Apoa2^b* allele, which contributes to an increase in T-CHO [22]. Because B6.KK-*Apoa2^b* N(8) had a higher T-CHO than did B6 [22], the difference in T-CHO values between B6.KK-*Apoa2^b* N(8) and RR should be smaller than that between B6 and RR. Nevertheless, there was a large variation in T-CHO values in B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice, suggesting that the observed large variation may be a consequence of the contributions of multiple genes (Fig. 2A).

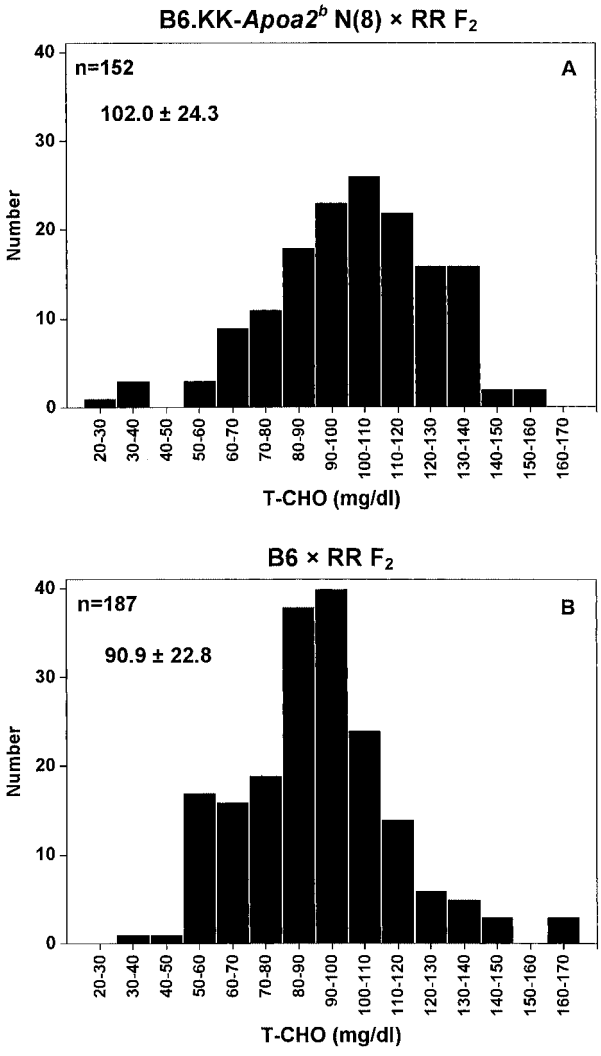

Fig. 2. Histograms showing distributions of T-CHO in B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice (A) and in B6 × RR F$_2$ mice (B). The mean ± S.D. of the trait values and the number of F$_2$ mice (n) are also shown.

Body weight was determined in B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice (n=150; I failed to measure body weight in 2 mice) (Fig. 3A), and was compared with that previously determined in B6 × RR F$_2$ mice (n=187) (Fig. 3B). The average body weight was 26.4 ± 4.4 (mean ± S.D.) g in B6.KK-*Apoa2^b* N(8)× RR F$_2$ mice, and 25.6 ± 3.5 (mean ± S.D.) g in B6 × RR F$_2$ mice; the difference was statistically significant ($P<0.05$). This may be attributed to the fact that B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice invariably have the *Apoa2^b* allele. Indeed, the *Cq6* region (containing *Apoa2*) was shown to have an effect on body weight in B6 × RR F$_2$ mice, and B6.KK-*Apoa2^b* N(8) females were heavier than B6 females [21.7 ± 1.1 (mean ± S.D.) g vs. 19.4 ± 1.7 (mean ± S.D.) g, $P<0.01$]. Because RR females are heavier than B6.KK-*Apoa2^b* N(8) females and B6 females, the difference in body weight between B6.KK-*Apoa2^b* N(8) and RR should be smaller than that between B6 and RR. Nevertheless, there was again a large variation in body weight in B6.KK-*Apoa2^b* N(8) × RR F$_2$ mice, suggesting that the observed large variation may be a consequence of the contributions of multiple genes (Fig. 3A), the same as in the case of the T-CHO.

Linkage and QTL analysis: For T-CHO levels, loci on chromosomes 2 (*D2Mit274* and *D2Mit285*), 3 (*D3Mit25*, *D3Mit230*, and *D3Mit102*), 4 (*D4Mit12*), and 9 (*D9Mit212*) showed evidence for linkage on a single-point statistical basis (i.e., $P<0.05$) (Fig. 4). Therefore, the remaining 104 F$_2$ mice were genotyped for these loci. As a result, nominal P values at loci on chromosomes 4 and 9 did not attain the threshold for suggestive linkage (data not shown). In addition, I genotyped *D1Mit270*, which is located at 92.3 cM on chromosome 1 near *Apoa2* locus (92.6 cM), in 152 F$_2$, with the consequence that there was no significant association with T-CHO levels as expected. Thus, two suggestive T-

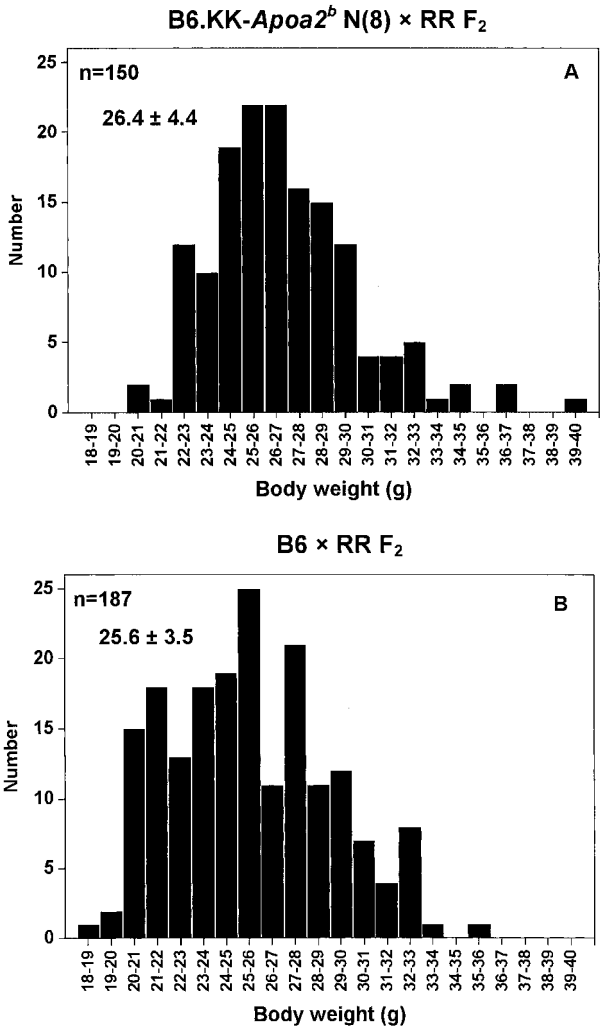

Fig. 3. Histograms showing distributions of body weight in B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice (A) and in B6 × RR F_2 mice (B). The mean ± S.D. of the trait values and the number of F_2 mice (n) are also shown.

CHO QTLs were identified on chromosomes 2 and 3. A suggestive QTL on chromosome 2 was located between *D2Mit274* and *D2Mit285* with a peak LOD score 2.8. This locus explained 9.8% of the F_2 phenotypic variance, with an additive effect of 11.3, and a dominance effect of –1.6 mg/dl T-CHO per R allele [mean ± S.D. T-CHO values (mg/dl) for genotypes at *D2Mit285* in F_2 mice were as follows: R/R (homozygote for R allele): 112.1 ± 24.5 (n=38); R/B (heterozygote): 101.0 ± 24.2 (n=83); B/B (homozygote for B allele): 92.2 ± 20.4 (n=31)]. On the other hand, a suggestive QTL on chromosome 3 (hereafter referred to as 'Chr3 locus') was located near the microsatellite marker *D3Mit25* with a peak LOD score 3.5 (Fig. 5). This locus explained 11.3% of the F_2 phenotypic variance, with an additive effect of –11.7, and a dominance effect of 1.9 mg/dl T-CHO per R allele [mean ± S.D. T-CHO values (mg/dl) for genotypes at *D3Mit25* in F_2 mice were as follows: R/R: 90.7 ± 27.4 (n=37); R/B: 102.8 ± 22.1 (n=78); B/B: 111.6 ± 21.6 (n=37)]. The 95% CI for the Chr 3 locus lay from 8 cM to 44 cM (Fig. 5). Because *D3Mit25* showed the least P value, because *Cq3* (a T-CHO QTL previously identified in B6 × KK-*A*^{y} F_2 mice [19]) was mapped to mid part of chromosome 3, and because the normolipidemic B6 allele at *Cq3* was associated with increased T-CHO levels, similar to the B6 allele at *D3Mit25*, I performed additional genotyping of *D3Mit60* and *D3Mit254* to determine whether the Chr3 locus and *Cq3* are allelic. As seen in Fig. 5, the Chr3 locus did not share the 95% CI with *Cq3* (the 95% CI for *Cq3* lay from 47 cM to 62 cM)); therefore, it is unlikely that the Chr3 locus and *Cq3* are allelic.

For body weight, on a single-point statistical basis, loci on chromosomes 3 (*D3Mit25, D3Mit230, D3Mit102*, and *D3Mit254*), 7 (*D7Mit228*), 8 (*D8Mit211*), and 17 (*D17Mit139*) showed evidence for linkage (i.e., $P<0.05$) (Fig. 4). Therefore, remaining 104 F_2 mice were genotyped for these loci (several F_2 mice had been already genotyped for T-CHO analysis). As a result, nominal P values at loci on chromosomes 7, 8, and 17 did not attain the threshold for suggestive linkage (data not shown). Thus, only one significant body weight QTL was identified on chromosome 3, near the microsatellite marker *D3Mit102* with a peak LOD score 5.2 (Fig. 5). I named this locus *Bwq7* (body weight QTL 7), because *Bwq1* through *Bwq6* had already been assigned [1, 16, 23]. *Bwq7* explained 14.4% of the F_2 phenotypic variance, with an additive effect of –1.9, and a dominance effect of –0.6 g body weight per R allele [mean ± S.D. body weight (g) for genotypes at *D3Mit102* in F_2 mice were as follows: R/R: 25.3 ± 2.0 (n=32); R/B: 26.5 ± 2.8 (n=81); B/B: 28.7 ± 3.8 (n=37)]. The 95% CI for *Bwq7* lay from 38 cM to 63 cM (Fig. 5); therefore, *Bwq7* shared the 95% CI with Chr3 locus and with *Cq3*. Because the R allele was associated with decreased trait values at these loci, either Chr3 locus or *Cq3* may be allelic with *Bwq7*. Although *D4Mit225* was the closest marker for a suggestive QTL for body weight in B6 × RR F_2 mice [22], this locus had no significant effect on body weight in B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice.

As described in the MATERIALS AND METHODS section, totally 80 F_2 mice were already genotyped for all microsatellite loci at the initial stage; therefore, potential interaction between marker loci was evaluated pairwise using Map Manager QTX b20 software. However, no significant interactions were identified for both traits.

DISUCUSSION

In the present study, QTL analysis on T-CHO levels and body weight was performed in B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice by controlling the effects of the *Apoa2* allele, to identify QTLs in addition to those identified in the previously studied B6 × RR F_2 mice [22]. For T-CHO levels, although no significant QTLs were identified, two suggestive QTLs were identified on chromosomes 2 and 3 in place of the

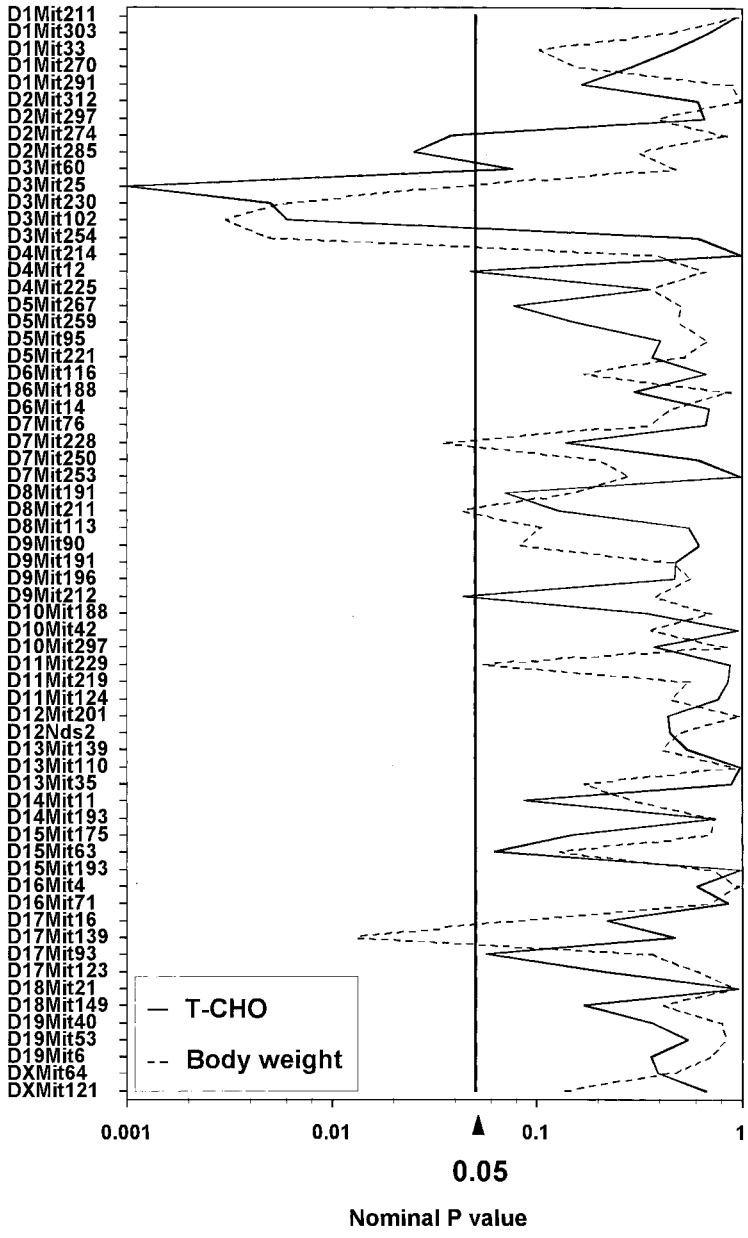

Fig. 4. Result of initial linkage analyses in selected F_2 mice. The x-axis represents log-transformed nominal P values, and the y-axis represents the microsatellite loci genotyped.

effects of the *Apoa2* allele. Because B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice exhibited large variations in T-CHO levels, as much as did B6 × RR F_2 mice, the observed variation should be attributed primarily to loci on chromosomes 2 and 3. The phenotypic effects of these loci are apparently weaker than those of *Cq6*. Nevertheless, I consider that the cumulative and/or combinational action of these loci may make it possible to produce QTL effect that is equal to that of *Cq6* on the basis of following reasons. First, the fact that loci on chromosomes 2 and 3 exert opposite direction with each other might contribute to the variations in T-CHO levels. Indeed, the R allele was associated with increased T-CHO at locus on chromosome 2, whereas the R allele was associated with decreased T-CHO at locus on chromosome 3. Thus, a combination of loci may produce a large variation in T-CHO in F_2 mice. Second, the variation may be dependent on interaction between loci. Indeed, I previously showed that the effect of *Cq3* on T-CHO was further enhanced in combination with the specific *Apoa2* allele from the KK strain [19]; thus, specific combinations between loci produce the QTL

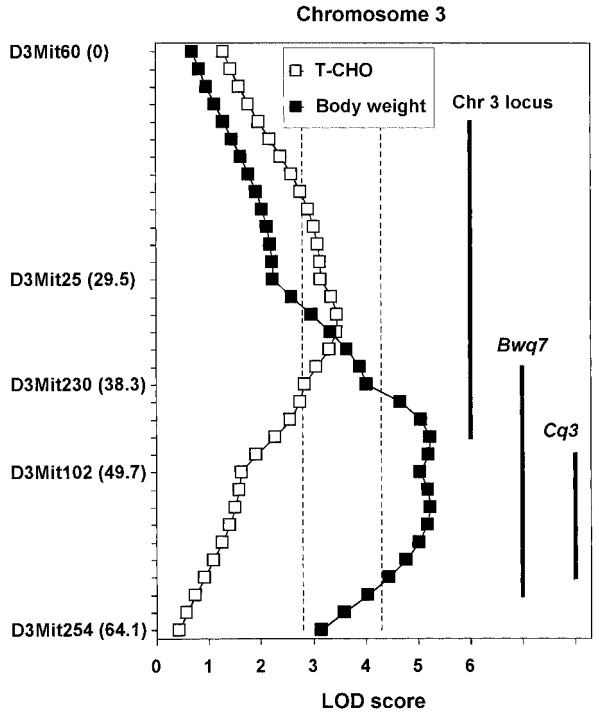

Fig. 5. LOD score plots for T-CHO (□) and body weight (■) on chromosome 3. Broken lines indicate thresholds for suggestive (LOD score=2.8) and significant (LOD score=4.3) linkage. Short thick lines indicate the 95% CI for the Chr 3 locus, *Bwq7*, and *Cq3* respectively. Y-axis: Microsatellite markers genotyped with their chromosomal positions in parentheses. Chromosomal positions are expressed in centi-Morgans (cM) from the centromere based on information from MGI.

effects that cannot be explained by any single locus effect. This possibility was partly tested in the present study; however, no significant epistatic interactions were identified by testing all pair of loci. More than two loci may interact with one another to produce large phenotypic effect; however, the number of F_2 mice (n=152) is too few to test this possibility.

For body weight, significant body weight QTL was identified on chromosome 3 (*Bwq7*) with the peak LOD score 5.2. The effect of *Bwq7* was not significant at the genome-wide linkage level, but was significant on a single point statistical basis (nominal P value < 0.05) in previously analyzed B6 × RR F_2 mice [mean ± S.D. body weight (g) for genotypes at *D3Mit102* in F_2 mice were as follows: R/R: 25.0 ± 3.1 (n=44); R/B: 25.3 ± 3.5 (n=95); B/B: 26.6 ± 3.6 (n=48)] [22]. Furthermore, the R allele at *D3Mit102* was associated with decreased body weight in both F_2 mice. Therefore, I consider that the effect of *Bwq7* would rather be enhanced than be newly produced in the absence of the effect of the *Apoa2* allele. With regard to a suggestive body weight QTL identified in B6 × RR F_2 mice on chromosome 4 (LOD score 3.8), I cannot explain the reason why the effect of this locus was not detected in B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice.

In a similar way as with T-CHO levels, B6.KK-*Apoa2*^{b} N(8) × RR F_2 mice exhibited large variations in body weight, as much as did B6 × RR F_2 mice. The observed variation should be primarily attributed to *Bwq7*. Thus, it was noteworthy that the genetic control of body weight was also altered significantly by controlling of the effects of the *Apoa2* allele.

Murine chromosome 3 contains multiple QTLs and genes for T-CHO and body weight. For T-CHO control, several studies have identified CHO QTLs on chromosome 3. Machleder *et al.* [12] identified an HDL-CHO QTL near *D3Mit120* (28 cM) in B6 × C3H/HeJ F_2 mice, and the C3H allele was associated with increased HDL-CHO levels. Mehrabian *et al.* [14] identified T- and HDL-CHO QTLs near *D3Mit12* (49.2cM) in B6 × CAST/Ei F_2 mice, and the B allele was associated with increased CHO levels. Shike *et al.* [17] identified a T-CHO QTL (*Tcho-1*) near *D3Mit12* (49.2 cM) in a backcross between KK × (BALB/c × KK) F_1, and the K allele was associated with increased T-CHO levels. Anunciado *et al.* [2] identified T-CHO QTL near *D3Mit46* (13.8 cM) in SM/J × A/J F_2 mice. Colinayo *et al.* [6] identified T- and HDL-CHO QTL near *D3Mit241* (33 cM) in B6 × DBA/2J F_2 mice, and the B allele was associated with increased CHO levels. Korstanje *et al.* [8] identified an HDL-CHO QTL (*Hdlq21*) near *D3Mit11* (49 cM) in SM/J × NZB/BlNJ F_2 mice. Several of these loci on chromosome 3 may be the same as the suggestive QTL identified in this study. In addition, the thioredoxin interacting protein (*Txnip*) gene, whose mutation is known to cause hyperlipidemia, is also located on the mid-part of chromosome 3 [3, 5].

For body weight control, the mid-part of chromosome 3 also contains several body weight- and/or obesity-related QTLs and genes. Brockmann *et al.* [4] analyzed the Du6 mouse strain, which had been selected for high body weight, and identified an abdominal fat weight QTL (*Afw1*) and an abdominal fat percent QTL (*Afpq1*). Moody *et al.* [15] identified a body weight QTL at 10 weeks (*Wt10q2*) and a brown fat QTL (*Batq2*). Koza *et al.* [9] identified an obesity-related QTL (*Iba2*) in backcross progeny between B6 and A/J. There are also two body weight/ obesity genes on chromosome 3. One is fatty acid binding protein 2, intestinal (*Fabp2*, 55 cM) [25]. In particular, male *Fabp2*-deficient mice exhibit increased body weight as well as hyperinsulinemia [25]. Because I also identified a suggestive QTL for fasting insulin on the mid-part of chromosome 3 in previously analyzed B6 × KK-*A*^{y} F_2 mice [20], *Fabp2* is plausible candidate gene. The other body weight/ obesity gene is potassium voltage-gated channel, shaker-related subfamily, member 3 (*Kcna3*, 52.3 cM). Xu *et al.* [29] generated *Kcna3*-deficient mice and showed that mutants were significantly lighter than wild-type littermates. They speculated that *Kcna3* has a role in regulating the basal metabolic rate. Because the 95% CI for *Bwq7* extends from 38 cM to 63 cM, both *Fabp2* and *Kcna3* are contained within the CI, and therefore these are plausible candidate genes for *Bwq7*.

Finally, Welch *et al.* [28] reported the results of a genetic

analysis similar to the present study. They produced B6.C-H25c × BALB/cJ F_2 mice, and they analyzed genetic control of T-CHO levels by controlling of the effects of the *Apoa2* allele. Four significant QTLs were identified on chromosomes 6, 13, 15, and 19; of these, the locus on chromosome 6 was female-specific, and the locus on chromosome 13 was male-specific. Thus, none of the QTLs were colocalized with the present loci; suggesting that the procedure used by Welch *et al.* [28] as well as the present study, will be beneficial for searching for additional QTLs. However, as Welch *et al.* [28] did not perform an analysis on B6 × BALB/cJ F_2 mice, it was not certain whether these 4 loci were detected in place of the effects of the *Apoa2* allele. The QTL mapping strategy by controlling of the effects of a major QTL facilitated the identification of additional QTLs.

REFERENCES

1. Anunciado, R.V., Nishimura, M., Mori, M., Ishikawa, A., Tanaka, S., Horio, F., Ohno, T. and Namikawa, T. 2001. Quantitative trait loci for body weight in the intercross between SM/J and A/J mice. *Exp. Anim. (Tokyo)* **50**: 319–324.
2. Anunciado, R.V., Ohno, T., Mori, M., Ishikawa, A., Tanaka, S., Horio, F., Nishimura, M. and Namikawa, T. 2000. Distribution of body weight, blood insulin and lipid levels in the SMXA recombinant inbred strains and the QTL analysis. *Exp. Anim. (Tokyo)* **49**: 217–224.
3. Bodnar, J.S., Chatterjee, A., Castellani, L.W., Ross, D.A., Ohmen, J., Cavalcoli, J., Wu, C., Dains, K.M., Catanese, J., Chu, M., Sheth, S.S., Charugundla, K., Demant, P., West, D.B., de Jong, P. and Lusis, A.J. 2002. Positional cloning of the combined hyperlipidemia gene Hyplip1. *Nat. Genet.* **30**: 110–116.
4. Brockmann, G.A., Haley, C.S., Renne, U., Knott, S.A. and Schwerin, M. 1998. Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth. *Genetics* **150**: 369–381.
5. Castellani, L.W., Weinreb, A., Bodnar, J., Goto, A.M., Doolittle, M., Mehrabian, M., Demant, P. and Lusis, A.J. 1998. Mapping a gene for combined hyperlipidaemia in a mutant mouse strain. *Nat. Genet.* **18**: 374–377.
6. Colinayo, V.V., Qiao, J.H., Wang, X., Krass, K.L., Schadt, E., Lusis, A.J. and Drake, T.A. 2003. Genetic loci for diet-induced atherosclerotic lesions and plasma lipids in mice. *Mamm. Genome* **14**: 464–471.
7. Higuchi, K., Kitagawa, K., Naiki, H., Hanada, K., Hosokawa, M. and Takeda, T. 1991. Polymorphism of apolipoprotein A-II (apoA-II) among inbred strains of mice. *Biochem. J.* **279**: 427–433.
8. Korstanje, R., Li, R., Howard, T., Kelmenson, P., Marshall, J., Paigen, B. and Churchill, G. 2004. Influence of sex and diet on quantitative trait loci for HDL-cholesterol levels in a SM/J by NZB/B1NJ intercross population. *J. Lipid Res.* **45**: 881–888.
9. Koza, R.A., Hohmann, S.M., Guerra, C., Rossmeisl, M. and Kozak, L.P. 2000. Synergistic gene interactions control the induction of the mitochondrial uncoupling protein (Ucp1) gene in white fat tissue. *J. Biol. Chem.* **275**: 34486–34492.
10. Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. and Newburg, L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**: 174–181.
11. Lander, E. and Kruglyak, L. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* **11**: 241–247.
12. Machleder, D., Ivandic, B., Welch, C., Castellani, L., Reue, K., and Lusis, A.J. 1997. Complex genetic control of HDL levels in mice in response to an atherogenic diet. *J. Clin. Invest.* **99**: 1406–1419.
13. Manly, K.F., Cudmore, R.H. Jr. and Meer, J.M. 2001. Map Manager QTX, cross-platform software for genetic mapping. *Mamm. Genome* **12**: 930–932.
14. Mehrabian, M., Castellani, L.W., Wen, P.-Z., Wong, J., Rithaporn, T., Hama, S.Y., Hough, G.P., Johnson, D., Albers, J.J., Mottino, G.A., Frank, J.S., Navab, M., Fogelman, A.M. and Lusis, A.J. 2000. Genetic control of HDL levels and composition in an interspecific mouse cross (CAST/Ei × C57BL/6J). *J. Lipid. Res.* **41**: 1936–1946.
15. Moody, D.E., Pomp, D., Nielsen, M.K. and Van Cleck, L.D. 1999. Identification of quantitative trait loci influencing traits related to energy balance in selection and inbred lines of mice. *Genetics* **152**: 699–711.
16. Reed, D.R., Li, X., McDaniel, A.H., Lu, K., Li, S., Tordoff, M.G., Price, R.A. and Bachmanov, A.A. 2003. Loci on chromosomes 2, 4, 9, and 16 for body weight, body length, and adiposity identified in a genome scan of an F_2 intercross between the 129P3/J and C57BL/6ByJ mouse strains. *Mamm. Genome* **14**: 302–313.
17. Shike, T., Hirose, S., Kobayashi, M., Funabiki, K., Shirai, T. and Tomino, Y. 2001. Susceptibility and negative epistatic loci contributing to type 2 diabetes and related phenotypes in a KK/Ta mouse model. *Diabetes* **50**: 1943–1948.
18. Suto, J. 2005. Apolipoprotein gene polymorphisms as cause of cholesterol QTLs in mice. *J. Vet. Med. Sci.* **67**: 583–589.
19. Suto, J. 2006. Characterization of *Cq3*, a quantitative trait locus that controls plasma cholesterol and phospholipid levels in mice. *J. Vet. Med. Sci.* **68**: 303–309.
20. Suto, J. and Sekikawa, K. 2002. A quantitative trait locus that accounts for glucose intolerance maps to chromosome 8 in hereditary obese KK-A^y mice. *Int. J. Obes.* **26**: 1517–1519.
21. Suto, J. and Sekikawa, K. 2003. Quantitative trait locus analysis of plasma cholesterol and triglyceride levels in KK × RR F_2 mice. *Biochem. Genet.* **41**: 325–341.
22. Suto, J., Takahashi, Y. and Sekikawa, K. 2004. Quantitative trait locus analysis of plasma cholesterol and triglyceride levels in C57BL/6J × RR F_2 mice. *Biochem. Genet.* **42**: 347–363.
23. Suto, J., Matsuura, S., Yamanaka, H. and Sekikawa, K. 1998. Genetics of obesity in KK mouse and effects of Ay allele on quantitative regulation. *Mamm. Genome* **9**: 506–510.
24. Suto, J., Matsuura, S., Yamanaka, H. and Sekikawa, K. 1999. Quantitative trait loci that regulate plasma lipid concentration in hereditary obese KK and KK-A^y mice. *Biochim. Biophys. Acta* **1453**: 385–395.
25. Vassileva, G., Huwyler, L., Poirier, K., Agellon, L.B. and Toth, M.J. 2000. The intestinal fatty acid binding protein is not essential for dietary fat absorption in mice. *FASEB J.* **14**: 2040–2046.
26. Wang, X. and Paigen, B. 2002. Quantitative trait loci and candidate genes regulating HDL cholesterol: a murine chromosome Map. *Arterioscler. Thromb. Vasc. Biol.* **22**: 1390–1401.
27. Wang, X., Korstanje, R., Higgins, D. and Paigen, B. 2004. Haplotype analysis in multiple crosses to identify a QTL gene. *Genome Res.* **14**: 1767–1772.
28. Welch, C.L., Bretschger, S., Wen, P.Z., Mehrabian, M., Latib,

N., Fruchart-Najib, J., Fruchart, J.C., Myrick, C. and Lusis, A.J. 2004. Novel QTLs for HDL levels identified in mice by controlling for Apoa2 allelic effects: confirmation of a chromosome 6 locus in a congenic strain. *Physiol. Genomics* **17**: 48–59.

29. Xu, J., Koni, P.A., Wang, P., Li, G., Kaczmarek, L., Wu, Y., Li, Y., Flavell, R.A. and Desir, G.V. 2003. The voltage-gated potassium channel Kv1.3 regulates energy homeostasis and body weight. *Hum. Mol. Genet.* **12**: 551–559.