Detection of a Quantitative Trait Locus for Intramuscular Fat Accumulation Using the OLETF Rat

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ABSTRACT. The Otsuka Long-Evans Tokushima Fatty (OLETF) rat is an animal model for obese type 2 diabetes. We showed that the OLETF rat exhibits higher levels of intramuscular fat content in *Musculus longissimus* as compared to the Fischer-344 (F344) rat. Our investigation was designed to identify quantitative trait loci (QTLs) contributing to the increased levels of intramuscular fat content by performing a whole-genome search using 108 F_2 intercross obtained by mating the OLETF and the F344 rats. We identified one QTL responsible for intramuscular fat accumulation on rat chromosome 1 with a maximum lod score of 3.4, which accounts for 5% of the total variance. As expected, the OLETF allele corresponds to the increased levels of intramuscular fat content.
KEY WORDS: animal model, intramuscular fat, obesity, OLETF rat, QTL.

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Intramuscular fat, so called marbling is an important factor for evaluation of beef quality, and intramuscular fat accumulation is the main target in beef industries, particularly in Japan. Marbling is more markedly observed in the Japanese Black cattle than other breeds of cattle [2, 34] and heritability for marbling is very high [18], indicating an important genetic component in intramuscular fat accumulation. Recently, Miyake *et al.* [16] have reported presence of a major gene for intramuscular fat accumulation in cattle. However, there is still no more than one report on the mapping of quantitative trait loci (QTLs) representing major genes for intramuscular fat accumulation in cattle [3]. Bovine studies for such QTLs are complicated by genetic heterogeneity, environmental influences, and difficulty in constructing experimental crosses. The use of inbred animal models of intramuscular fat accumulation is an essential component for investigation of such QTLs using the strategy of molecular and statistical genetics.

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat was established by selective breeding based on impaired glucose tolerance [11]. The OLETF rat develops obese type 2 diabetes [11]. Several QTL analyses on obesity and type 2 diabetes in this rat have been reported [9, 17, 20, 25, 29, 30]. Among these analysis, there is our previous study that have identified six QTLs responsible for adiposity indices of fat pads on rat chromosomes 2 (*Obs1* for mesenteric fat), 4 (*Obs2* for retroperitoneal fat), 8 (*Obs3* for mesenteric fat), 9 (*Obs4* for retroperitoneal fat), and 14 (*Obs5* and *Obs6* for retroperitoneal fat) [20]. This study demonstrated that the adiposity indices of individual fat pads were under the control of different genes.

In the present study, we have demonstrated that the OLETF rat possesses higher levels of intramuscular fat content in *Musculus longissimus* than the Fischer-344 (F344) rat. Thus, we set out to search for evidence of QTLs affecting intramuscular fat accumulation by using the interval mapping method in 108 F_2 progenies obtained by mating the OLETF and the F344 rats. We have identified one QTL responsible for intramuscular fat accumulation on chromosome 1.

MATERIALS AND METHODS

Genetic crosses: All of the F_1 rats were generated by female OLETF rats bred with male F344 rats, and F_2 progeny were obtained by intercrossing the F_1 rats. Male rats were used for genetic studies. The difference in degree of adiposity between OLETF and F344 rat strains is more markedly observed in males than in females [11, 20]. Thus, using males was presumed to exclusively maximize the quantitative difference of intramuscular fat content between the two strains for greater statistical power to detect QTLs [12]. All rats were kept under specific pathogen-free conditions. The temperature (21 ± 2°C), humidity (55 ± 5%), and air conditioning were all controlled. Rats had free access to tap water and standard laboratory chow (CRF-1; Oriental Yeast, Tokyo, Japan) and were maintained on a 12-hr light and dark cycle (6 AM/6 PM).

Phenotyping: Rats were killed under deep anesthesia at 35 weeks of age. Immediately after exsanguination, the first to third lumbar region of *Musculus longissimus* was removed from the right side of the carcass and rapidly frozen in dry ice-cooled isopentane. Five serial coronal sections (6 μm thick) were obtained at intervals of 180 μm from frozen *Musculus longissimus*, and the sections stained with Oil Red O for the presence of lipid droplets in adipocyte

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cytoplasm. The section area, occupied by cells positive for Oil Red O staining, were measured in a given area (1 cm²) using MacSCOPE computer program. The fat staining-positive area expressed as an absolute value or as a percentage of the body weight was used as the quantitative phenotype of the levels of intramuscular fat content. The body weight was measured before the sacrifice of rats. The tail was cut and frozen at –80°C for subsequent isolation of high-molecular-weight DNA. This study conformed to the guidelines for the care and use of laboratory animals of Kyoto University.

Genotyping: DNA was isolated from the tail using phenol-chloroform extraction. The (OLETF female × F344 male) F_2 progeny were genotyped by polymerase chain reaction (PCR) amplification of microsatellites with the primers purchased from Research Genetics, Inc. (Huntsville, AL), according to a previous report [17].

Data analysis: Genetic markers were mapped relative to one another with the MAPMAKER/EXP computer package [14]. A candidate for laboratory typing error was identified by an error detection procedure on the MAPMAKER/EXP computer package, which is a systematic method that incorporates the possibility of error into the usual likelihood model for linkage analysis [15]. Potential errors were rechecked against photographs, and, where necessary, the genotyping was repeated. QTLs affecting phenotypes were mapped relative to genetic markers with the MAPMAKER/QTL computer package [22]. We selected a lod score of 2.8 as the genome-wide threshold for establishment of linkage [13]. Phenotypic comparisons for different genotypic groups were performed by using a Student's *t* test or an analysis of variance (ANOVA) with a post hoc test using Scheffe's *F* test (StatView, SAS Institute, Inc., Cary, NC). Multiple QTL analysis was conducted with the 'simultaneous search' function of MAPMAKER/QTL computer package. Briefly, identified QTL is 'fixed', removing that portion of the variance that is explained by that locus from the subsequent analysis. The genome is then rescanned to identify additional QTLs. Multiple QTL analysis was also performed by MQM-mapping on the MapQTL computer program [6, 7]. QTL-likelihood plots were produced by using the marker that flanks closest to the lod score peak of identified QTL as cofactor for QTL mapping on the MapQTL computer program. Identified QTL was confirmed by QTL mapping in which marker closest to the lod score peak of the identified QTL was used as cofactor except for the marker flanking the interval under study. Although, in case of a few cofactors in multiple-QTL models, there may be no clear difference in the mapping efficacy between multiple QTL analyses with MAPMAKER/QTL and with MapQTL, the two analyses were carried out to confirm additional QTLs.

Examination of syntenic conservation among rat, mouse, and human: Syntenic conservation between rat, mouse, and human chromosomes were investigated on the basis of the radiation hybrid map of rat [28], the rat linkage map [1, 5, 31], the human obesity gene map [23], and several Web site databases (http://ratmap.gen.gu.se/; http://www-genome.wi.mit.edu:80/ftp/distribution/; and http://www.otsuka.genome.ad.jp/ratmap).

RESULTS

We stained the coronal sections of *Musculus longissimus*

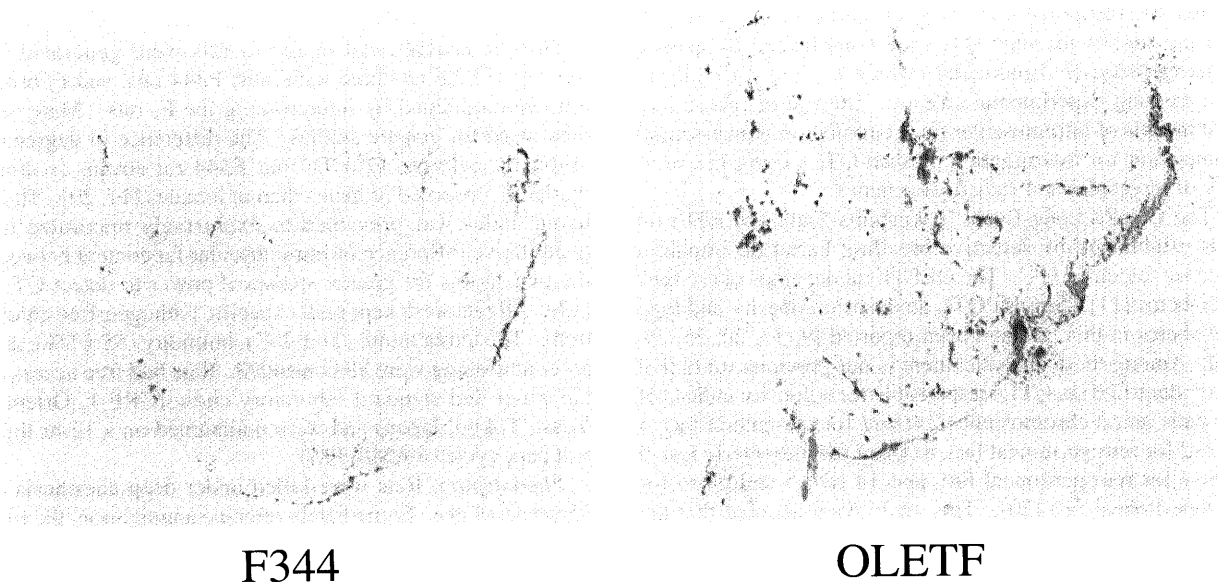F344   OLETF

Fig. 1.   Oil Red O staining of the coronal sections of *Musculus longissimus* from OLETF and F344 rats. The area positive for Oil Red O staining is shown by red color.

Table 1.   Phenotypic characteristics of parental strains, F_1 progeny, and F_2 progeny

Phenotype	OLETF	F344	F_1 progeny	F_2 progeny
Number of animals	7	7	7	108
Fat staining-positive area (mm²)	3.26 ± 0.52[a]	1.34 ± 0.38[b]	2.44 ± 1.03[c]	2.52 ± 1.55
Fat staining-positive area/body weight (mm²/g × 10⁻³)	5.01 ± 2.29[a]	3.62 ± 2.19[b]	4.06 ± 1.71[c]	4.74 ± 2.99
Body weight (g)	650.2 ± 31.2[a]	375.6 ± 20.0[b]	600.2 ± 24.3[c]	531.1 ± 48.4

Phenotypes are given as mean ± SD. Only male rats were phenotyped at 35 weeks of age. Phenotypes in the parental strains and F_1 progeny were compared by a one-way analysis of variance with Scheffe's F analysis as post hoc test. Means labeled with different superscripts are significantly different from one another ($P<0.05$).

from OLETF and F344 rats with Oil Red O. The section area, occupied by cells positive for Oil Red O staining, was visually larger in OLETF than in F344 (Fig. 1), suggesting an increase of intramuscular fat content in *Musculus longissimus* of the OLETF rat.

We crossed female OLETF rats with male F344 rats to generate F_1 progeny which, in turn, were used to generate 108 male F_2 progeny. Only males, 35 weeks of age, from the parental strains, the 7 F_1 progeny, and the 108 F_2 progeny were phenotyped for the levels of intramuscular fat content, that is the fat staining-positive area expressed as an absolute value or as a percentage of the body weight (Table 1). The fat staining-positive area as an absolute value was increased in the OLETF rat as compared with the F344 rat, and the OLETF and F344 rats showed clear statistical difference in this phenotype ($P<0.0001$). The F_1 progeny showed that the trait of this increased positive area is inherited in an additive manner. Compared with the F_1 progeny, the F_2 progeny showed similar means but larger variance in the phenotype. The broad, continuous distributions without evidence of distinct groups in this phenotype among the F_2 progeny suggest that this trait is polygenically regulated. The distribution of the fat staining-positive area as a percentage of the body weight showed a similar trend to that expressed as an absolute value in the four genetic groups of the parental strains and the F_1 and F_2 progenies. These indicate the existence of increased levels of intramuscular fat content in the OLETF rat.

To identify the QTL(s) affecting the increased levels of intramuscular fat content, we carried out a total genome scan on the F_2 progeny, using a set of 222 informative simple sequence length polymorphisms between the OLETF and the F344 rats. The markers were on average 7.6 cM apart, and approximately 94% of the genome (based on a genetic length of 1705 cM calculated in this study) was within 10 cM of an informative marker. No discrepancies were found between maps constructed in this study and the maps reported in Rat Genome Database (http://rgd.mcw.edu/). Phenotypic and genotypic data were analyzed for linkage with MAPMAKER/QTL [22]. According to the criteria set by Lander and Kruglyak [13], we found statistical evidence for one QTL affecting the increased levels of intramuscular fat content on chromosome 1. This locus, between *D1Mit7* and *D1Rat123* showed a maximum

lod score of 3.4 for the fat staining-positive area and a maximum lod score of 3.2 for the fat staining-positive area/body weight, accounting for 5% of the fat staining-positive area variance and 7% of the fat staining-positive area/body weight variance in our cross (Fig. 2). The inheritance pattern at the locus was consistent with the OLETF alleles acting in a recessive mode of inheritance to increase the levels of intramuscular fat content (Table 2). Multiple QTL analysis with MAPMAKER/QTL indicated that no other QTLs were detected that statistically increased the baseline score of the chromosome 1 QTL, nor was any additional variance explained after fixing the chromosome 1 QTL in the 108 F_2 progeny. Additionally, application of MQM-mapping, which is a mapping method based on multiple-QTL models [6, 7], has not led to a revelation of additional QTLs other than the chromosome 1 QTL. Note that no statistical evidence for an X-linked locus affecting the increased levels of intramuscular fat content was found by an analysis of variance.

DISCUSSION

In the present study, we demonstrated that the OLETF rat exhibits intramuscular fat accumulation and identified one QTL influencing this trait on chromosome 1 using the F_2 progeny obtained from the cross of the OLETF and the F344 rats. Our previous study reported QTL analysis on mesenteric, retroperitoneal, and epididymal fat accumulation in the OLETF rat [20]. The maximum lod scores for the adiposity indices of the mesenteric, retroperitoneal, and epididymal fat pads, respectively, in genomic region of the chromosome 1 QTL detected for intramuscular fat accumulation in this study, were 0.01, 2.01, and 0.03, suggesting that this QTL has no impact on mesenteric, retroperitoneal, and epididymal fat accumulation in the OLETF rat. Therefore, the chromosome 1 QTL seems to exert an effect on fat storage only in intramuscular fat tissue. This has been noted by our previous report on obesity loci, that identified two QTLs, *Obs1* (chromosome 2) and *Obs3* (chromosome 8), and four QTLs, *Obs2* (chromosome 4), *Obs4* (chromosome 9), *Obs5* (chromosome 14), and *Obs6* (chromosome 14), as affecting mesenteric and retroperitoneal fat accumulation in the OLETF rat, respectively [20]. Analyses of obesity QTLs in polygenic mouse models have also demonstrated

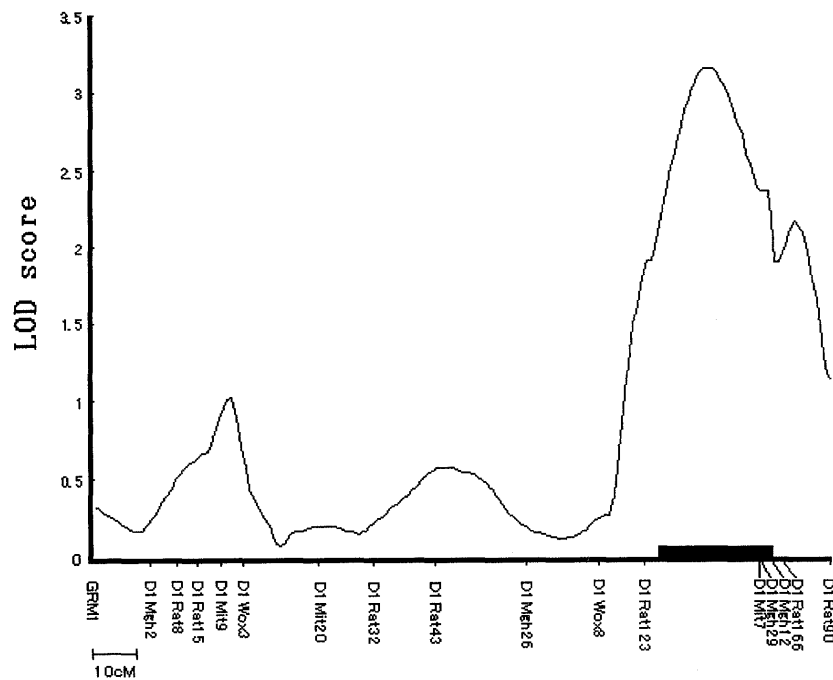48   H. TANOMURA *ET AL.*

Fig. 2.   Scan for lod score for linkage to fat staining-positive area expressed as an absolute value on chromosome 1. The abscissa labels indicate microsatellite markers used for the linkage analysis, and map distances in centimorgans determined by the Kosambi map function are represented. The 1-lod support interval is shown as black bar. The lod scores were calculated by the MAPMAKER/QTL program at 2-cM intervals spanning the distance between each pair of polymorphic markers.

Table 2.   Comparison of means of fat staining-positive area and fat staining-positive area/body weight in F_2 progeny based on genotype at the chromosome 1 QTL

Phenotype	Genotype		
	OLETF/OLETF	OLETF/F344	F344/F344
Number of animals	20	54	21
Fat staining-positive area (mm²)	3.33 ± 2.12[a]	2.26 ± 1.16[b]	2.50 ± 1.61[b]
Fat staining-positive area/body weight (mm²/g × 10⁻³)	6.20 ± 4.05[a]	4.29 ± 2.12[b]	4.89 ± 3.31[b]
Body weight (g)	543.0 ± 46.1[a]	527.9 ± 49.9[a]	527.8 ± 51.6[a]

Animals were grouped according to the genotype of the *D1Mit7* marker closest to the maximum lod scores for fat staining-positive area and fat staining-positive area/body weight within the chromosome 1 QTL region. Phenotypes are given as mean ± SD. Means labeled with different superscripts are significantly different from one another ($P<0.05$) by a one-way analysis of variance with Scheffe's F analysis as post hoc test.

different genetic controls of fat storage in different fat tissues [27, 32].

We estimated the proportion of genetic variance and the effective number of genes for intramuscular fat content in the F_2 progeny. For the fat staining-positive area expressed as an absolute value, the proportion of genetic variance in the F_2 was found to be about 69% by comparison of the variances in F_1 and F_2 progenies, and the number of genes was estimated roughly as 1 by Wright's formula [33]. Roughly 86% of the F_2 variance in the fat staining-positive area as a

percentage of the body weight appeared to be genetic, and the number of genes was estimated to be roughly 1. These were consistent with the results of high heritability [18] and of presence of a major gene [16] for bovine marbling. Thus, detection of a significant QTL with large effect was expected in our experimental design, because it has been reported that there is a reasonable chance of detecting a QTL that explains 10% of phenotypic variance as a significant locus (lod score ≥ 4.3), in an F2 intercross of 100 individuals [26]. However, indeed, only one suggestive QTL (4.3 > lod

score ≥ 2.8) was detected on chromosome 1. Further, this QTL was a small effect QTL that explains 5% and 7% of the total phenotypic variance and 7% and 8% of genetic variance for the fat staining-positive area as an absolute value and as a percentage of the body weight in the F_2 progeny, respectively. The effective number of genes is calculated, assuming that the responsible genes are inherited with additive mode, have additive equal effects on the traits, and are not linked, and a failure of the assumption results in underestimation of the gene number [33]. Our result of detection of only one suggestive QTL with small effect suggests a failure of the assumption and the underestimation. Intra-allelic interaction (heterosis), inter-allelic interaction (epistasis), genes with different effect sizes, and linked genes, respectively, which do not conform to the assumption of the additive mode of inheritance of the responsible genes, of the additive contributions of responsible genes to the traits, of the equal contributions of responsible genes to the traits, and of no linkage between responsible genes, may be involved in intramuscular fat accumulation in the OLETF. This phenotype in the OLETF might have a considerably higher degree of genetic complexity. Therefore, –90% of genetic variance, which remain unexplained for the levels of intramuscular fat content, could be attributable to considerably complex genetic factors.

We have mapped the chromosome 1 QTL to 31 cM 1-lod support interval. The human and mouse genome regions homologous to the interval of the QTL, respectively, would be 9q12 and 10q23-q25.3, and 19 (21–49 cM) [28]. Physiologically relevant candidate gene suggested by the homologous human and mouse regions is adrenergic alpha-2A-receptor (ADRA2A) locus [21]. The ADRA2A locus has been shown to associate with trunk to extremity skinfolds ratio [21]. In addition, Pfat3 locus [24], DAHL3 locus [10], and Dmo1 locus [29] may be defining the same underlying gene as the present QTL. The Pfat3, which has been previously identified as obesity QTL in mouse, affects adiposity in a cross between M16i and CAST/Ei [24]. The rat obesity QTLs, DAHL3 [10] and Dmo1 [29], have been reported to have an effect on body weight.

Recently, suggestive QTLs were identified for marbling on chromosomes 17 and 27 in cattle [3]. The interval of the chromosome 1 QTL detected in this study would correspond to the bovine genome regions, 8q12-q18 and 26q12-q23, but not chromosomes 17 and 27 [19]. Thus, the bovine genome regions of 8q12-q18 and 26q12-q23, in addition to chromosomes 17 and 27, may be an attractive component for beef cattle breeding. In pig, it has been reported that a major gene influences intramuscular fat accumulation [8], and that QTLs for intramuscular fat content are located on porcine chromosomes 2, 4, 6 and 7 [4]. However, comparative map [19] suggests that these QTLs in pig would not be homologous to the chromosome 1 QTL detected in this study. Altogether, the data of the present and previous QTL studies [3, 4] are consistent with genetic heterogeneity of intramuscular fat accumulation.

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