Genetic analysis of abdominal fat distribution in SM/J and A/J mice[S]

Misato Kobayashi,* Tamio Ohno,† Natsuko Hada,* Masato Fujiyoshi,* Masako Kuga,† Masahiko Nishimura,† Atsushi Murai,* and Fumihiko Horio[1,*]

Department of Applied Molecular Bioscience,* Graduate School of Bioagricultural Sciences; and Division of Experimental Animals,† Center for Promotion of Medical Research and Education, Graduate School of Medicine, Nagoya University, Nagoya, Japan

Abstract **Each abdominal fat depot, such as mesenteric or epididymal, differently contributes to the development of insulin resistance. The aim of this study was to identify the genetic regions that contribute to fat accumulation in epididymal/mesenteric fat and to examine whether or not the genetic regions that affect glucose metabolism and body fat distribution are coincident. We previously mapped a major quantitative trait locus (QTL) (*T2dm2sa*) for impaired glucose tolerance on chromosome 2 and revealed that SM.A-*T2dm2sa* congenic mice showed not only glucose tolerance but also fat accumulation. In the present study, to identify the loci/genes that control the accumulation of abdominal fat, we perfomed QTL analyses of epididymal/mesenteric fat weight by using (A/J×SM.A-*T2dm2sa*)F2 mice in which the effect of *T2dm2sa* was excluded. As a result, two highly significant QTLs for mesenteric fat, as well as three significant QTLs for epididymal/mesenteric fat, were mapped on the different chromosomal regions. This suggests that the fat accumulations in individual fat depots are controlled by distinct genomic regions. Our comparison of these QTLs for abdominal fat distribution with those for glucose metabolism revealed that the major genetic factors affecting body fat distribution do not coincide with genetic factors affecting glucose metabolism in (A/J×SM.A-*T2dm2sa*)F2.—**Kobayashi, M., T. Ohno, N. Hada, M. Fujiyoshi, M. Kuga, M. Nishimura, A. Murai, and F. Horio. **Genetic analysis of abdominal fat distribution in SM/J and A/J mice. *J. Lipid Res.* 2010.** 51: **3463–3469.**

Supplementary key words mesenteric fat • epididymal fat • glucose tolerance •genetic factor

In humans, obesity is a major risk factor for insulin resistance, type 2 diabetes, dyslipidemia, cardiovascular disease, fatty liver, and stroke (1, 2). The accumulation of abdominal fat but not of subcutaneous fat might be linked to the development of these metabolic complications (3–5). Moreover, each abdominal fat depot, such as mesenteric or epididymal, is thought to contribute differently to the development of insulin resistance (6). Metabolic complications such as obesity, insulin resistance, and type 2 diabetes are complex traits controlled by multiple genes and environmental factors. To search for obesity genes, numerous quantitative trait locus (QTL) analyses have been performed using mice, starting in 1993 by Warden et al. (7–9). In 2002, Brockmann et al. (8) reviewed 166 QTLs for obesity, that is body weight and body fat weight, from 24 studies. Wuschke et al. (9) performed a meta-analysis of 279 QTLs for body weight and body fat weight using 34 published mouse experiments. Recently, some groups reported the analysis of relation between QTLs for body fat distribution and QTLs for traits of obesity-related diseases using mouse models (10–14). Although the association of diabetes with obesity is suggested in various animal models of type 2 diabetes, a few reports have focused on the simultaneous analyses of QTLs for type 2 diabetes and fat distribution. The genetic basis and mechanisms linking fat distribution to these metabolic complications remain poorly understood.

SXMA recombinant-inbred (RI) strains are established from parental strains SM/J and A/J mice (15), and the genome of each SMXA RI strain consists of a mixture of the SM/J and A/J mouse genomes. The SMXA-5 mouse, which belongs to the 26 SMXA RI strains, has been shown to develop impaired glucose tolerance, fatty liver, and moderate obesity (16). The development of these metabolic complications in SMXA-5 mice are quite accelerated

This work was supported by a Grant-in-Aid for Scientific Research (B) (No. 21380079) and a Grant-in-Aid for Young Scientists (B) (No. 20780093) from the Japan Society for the Promotion of Sciences, and by a grant from the Kao Research Council for the Study of Healthcare Sciences, Japan.

Manuscript received 2 July 2010 and in revised form 27 August 2010.

Published, JLR Papers in Press, August 27, 2010
DOI 10.1194/jlr.M009563

Abbreviations: Chr., chromosome; cM, centimorgan; LOD, logarithm of odds; LRS, likelihood ratio statistics; QTL, quantitative trait locus; RI, recombinant-inbred.

[1] To whom correspondence should be addressed.
e-mail: horiof@agr.nagoya-u.ac.jp

[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one table.

by a high-fat diet (17, 18). In order to determine the genetic factors involved in the impaired glucose tolerance of SMXA-5 mice, we previously performed a QTL analysis using (SM/J × SMXA-5)-F2 intercross and mapped a major QTL (*T2dm2sa*) for impaired glucose tolerance on chromosome (Chr.) 2 (19). The chromosomal region near *T2dm2sa* had QTLs for serum insulin level and body mass index. The A/J allele at *T2dm2sa* contributed to the impairment of glucose tolerance, hyperinsulinemia, and obesity. We then constructed a congenic strain of mice, SM.A-*T2dm2sa*, in which the A/J-derived *T2dm2sa* region was introgressed into the SM/J mouse genome. Compared with SM/J mice, the SM.A-*T2dm2sa* congenic mice, when both strains were fed a high-fat diet, showed not only impaired glucose metabolism and hyperinsulinemia but also an increase of mesenteric fat weight (19). SM.A-*T2dm2sa* congenic mice appearing in the region (D2Mit295-D2Mit281) near *T2dm2sa* on Chr. 2 possessed gene(s) affecting glucose tolerance, serum insulin level, and the accumulation of fat in abdominal cavity (19 and this study). To reveal unknown diabetogenic loci excluding *T2dm2sa*, we previously performed QTL analyses of impaired glucose tolerance using (A/J × SM.A-*T2dm2sa*)F2 intercross mice (20). As all of these mice possess the A/J allele of *T2dm2sa*, the effect of *T2dm2sa* on glucose tolerance is excluded in the QTL analysis using these intercross mice. By our analysis of (A/J × SM.A-*T2dm2sa*) F2 mice, several significant QTLs for glucose tolerance have been detected (20). This cross was also useful for examining the coincidence of genetic factors controlling fat distribution and glucose metabolism. In this study, to identify unknown loci/genes, excluding *T2dm2sa*, controlling the accumulation of fat in the abdominal cavity, we performed QTL analyses of epididymal fat weight and mesenteric fat weight using (A/J × SM.A-*T2dm2sa*)F2 intercross mice fed a high-fat diet. This analysis would enable the comparision of QTLs for glucose metabolism with those for body fat composition.

The aim of this study was to identify the genetic regions that contribute to fat accumulation in the abdominal cavity, and to examine whether or not the genetic regions controlling glucose metabolism coincide with those controlling body fat weights.

MATERIALS AND METHODS

Experimental animals

SM/J and A/J strains were obtained from the Division of Experimental Animals, Center for Promotion of Medical Research and Education, Graduate School of Medicine, Nagoya University (Nagoya, Japan). The SM.A-*T2dm2sa* congenic strain was produced as previously described (19). Male SM/J mice were mated to A/J female mice to produce F1 mice. Male F1 mice were backcrossed to SM/J females to produce the N2 generation. Male heterozygous carriers of Chr. 2 intervals between D2Mit295 and D2Mit281 were continuously bred to SM/J mice to produce the next generation. The marker-assisted speed congenic procedure resulted in >99% replacement of the SM/J background genome by N5. Then, N6 congenic animals were intercrossed to produce F1 animals (N6:F1) homozygous for the introgressed D2Mit295-D2Mit281 region from A/J mice. SM.A-*T2dm2sa* congenic mice possessed the A/J homozygous alleles between D2Mit295 (proximal, 29.3 Mb) and D2Mit281 (distal, 148.4 Mb) (**Fig. 1**).

Parental strains, F1 hybrid, and F2 intercross mice

A/J female mice were mated to SM.A-*T2dm2sa* male mice to produce F1 hybrid mice and F1 mice were intercrossed to produce (A/J × SM.A-*T2dm2sa*) F2 intercross mice. F1 and F2 generations (255 mice) were produced and maintained at our animal facility. All mice were maintained in a room under conventional conditions at a controlled temperature of 23 ± 3°C and humidity of 55 ± 5% with a 12 h light/dark cycle. Starting at 6 weeks of age, F2 male mice were fed a high-fat diet. Mice were given ad libitum access to drinking water and the high-fat diet. The composition (g/kg diet) of the high-fat diet was as follows: casein, 209; carbohydrate (starch sucrose, 1:1), 369; AIN93MX mineral mixture (21), 35; AIN93VX vitamin mixture (21), 10; choline chloride, 2; corn oil, 35; lard, 300; and cellulose powder (AVICEL type FD-101, Asahi Chemical Industry, Osaka, Japan), 40. All procedures were performed in accordance with the Regulations for Animal Experiments at Nagoya University.

Experimental measurements and linkage analysis

All mice were euthanized after 11 weeks of having been fed a high-fat diet (at 17 weeks of age). Individual body weights were measured before dissection. Epididymal fat and mesenteric fat tissues were dissected and weighed. The relative tissue weights (tissue g/100 g body weight) were calculated individually as weight divided by body weight. Genomic DNA was prepared from the mouse kidney by salt/ethanol precipitation. A total of 125 microsatellite marker loci, polymorphic between SM/J and A/J, were genotyped in all F2 mice, and these loci have all been described previously (20). Polymerase chain reactions (PCRs) were performed according to standard methods (22). The PCR products were separated by electrophoresis on 4% NuSieve (FMC, Rockland, ME) agarose gel and were visualized by ethidium bromide staining. Linkage analysis was performed using the

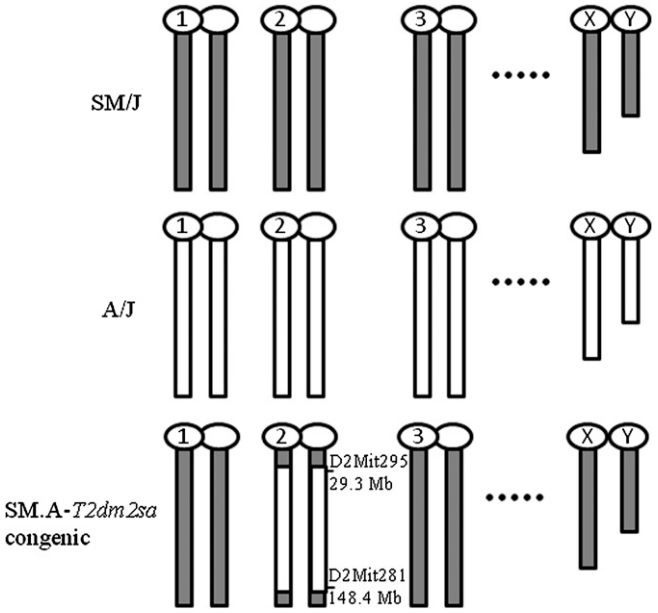

Fig. 1. The chromosomal construction of SM.A-*T2dm2sa* congenic mice. The gray bar shows the SM/J-derived chromosomal region. The white bar shows the A/J-derived chromosomal region.

MapManager QTXb20 (23, 24) software program, which is based on interval mapping using the free-regression model. The permutation test estimates the experiment-wide probability for given likelihood ratio statistics (LRS). Significance was determined by 1-centimorgan (cM) steps for 1,000 permutations in order to provide LRS that were suggestive, significant, and highly significant (25, 26). Suggestive, significant, and highly significant correspond to the 37th, 95th, and 99.9th percentiles, respectively. The logarithm of odds (LOD) score was obtained by dividing the LRS by 4.605 (27). Significant linkage was defined in accordance with the guidelines of Lander and Kruglyak (28), i.e., as statistical evidence occurring by chance in the genome scan with a *P*-value of less than 0.05. The positions for all microsatellite markers were based on the NCBI m37 mouse assembly.

Statistical analysis

All results are expressed as the mean ± SEM except for Table 2, in which the results are expressed as the mean ± SD. One-way ANOVA and subsequent Tukey-Kramer test were used to compare the means of A/J, SM.A-*T2dm2sa*, F1, and SM/J mice. Differences of $P<0.05$ were regarded as significant. All statistical analyses were performed using StatView version 5.0 software (SAS Institute, Cary, NC).

RESULTS

Fat weights in the abdominal cavity

In the phenotypic analyses after 11 weeks of feeding a high-fat diet, we first compared SM.A-*T2dm2sa* congenic mice with SM/J, which was a background strain of SM.A-*T2dm2sa* (**Table 1**). Epididymal fat weight tended to be higher in SM.A-*T2dm2sa* mice than in SM/J mice. Mesenteric fat weight was significantly higher in SM.A-*T2dm2sa* mice than in SM/J mice. We confirmed that genes in the region (D2Mit295-D2Mit281) near *T2dm2sa* on Chr. 2 had an effect on the accumulation of fat in the abdominal cavity.

Second, we compared epididymal fat weight and mesenteric fat weight between A/J and SM.A-*T2dm2sa* mice. Epididymal fat weight was significantly higher in SM.A-*T2dm2sa* mice than in A/J mice but there was no significant difference in the mesenteric fat. Third, we compared the fat weights among (A/J × SM.A-*T2dm2sa*)F1 mice and the parental strains. Epididymal fat weight in F1 mice was similar to that in A/J mice and lower than in SM.A-*T2dm2sa* mice. Mesenteric fat weight was significantly higher in F1 mice than in the parental strains. Across these parameters, the individual values in F2 intercross mice showed a wide range and a normal distribution (Table 1).

TABLE 1. Fat tissue weights in parental strains, (A×SM.A-*T2dm2sa*) F1, (A/J×SM.A-*T2dm2sa*)F2, and SM/J strains

	Epididymal Fat (g/100g body weight)	Mesenteric Fat (g/100g body weight)
A/J (n = 8)	4.09 ± 0.19^{a}	1.45 ± 0.05^{ab}
SM.A-*T2dm2sa* (n = 18)	4.96 ± 0.15^{b}	1.64 ± 0.08^{b}
F1 (n = 17)	4.31 ± 0.22^{a}	2.28 ± 0.09^{c}
F2 (n = 255)	3.89 ± 0.08	2.22 ± 0.03
F2 range	1.22-8.83	0.36-3.96
SM/J (n = 12)	4.42 ± 0.18^{ab}	1.10 ± 0.07^{a}

Data are expressed as means ± SEM. Values of these mice in the same row not sharing a common superscript letter are significantly different at $p<0.05$.

[abc] This statistical analysis was done among A/J, SM.A-*T2dm2sa*, F1, and SM/J mice.

QTL mapping in the (A/J × SM.A-*T2dm2sa*)F2 intercross

Regarding epididymal fat weight, we mapped two significant QTLs (on Chrs. 10 and 12) and six suggestive QTLs (on Chrs. 1, 8, 9, 11, 15, and 18) (**Table 2** and **Fig. 2**). Significant QTLs for epididymal fat weight were found in the region near D10Mit12 and D12Mit58, with LOD scores of 3.8 and 4.5, respectively. For a significant QTL (*Efatq1sa*, Epididymal fat weight qtl 1 in SMXA) at D12Mit58, the mean value of this trait was higher among F2 mice with SM/J homozygous than among those with A/J homozygous. In contrast, in the case of another significant QTL (*Efatq2sa*) at D10Mit12, the mean value was higher among F2 mice with A/J homozygous (A/A) than among those with SM/J homozygous (SM/SM). These results indicated that the A/J allele on Chr. 10 and the opposite allele (SM/J) on Chr. 12 contributed to increased epididymal fat weight.

As regards mesenteric fat weight, two highly significant QTLs (on Chrs. 7 and 16), one significant QTL (on Chr. 5), and three suggestive QTLs (on Chrs. 1, 10, and 13) were detected (Table 2 and Fig. 2). A highly significant QTL (*Mfatq1sa*, Mesenteric fat weight qtl 1 in SMXA) for mesenteric fat weight was found in the region near D7Mit155 with an LOD score of 7.7. This locus explained 13% of the observed phenotypic variance in mesenteric fat weight. The mean values of these traits were higher among F2 mice with A/J homozygous (2.44 g/100 g body weight) at D7Mit155 than among those with SM/J homozygous (1.96 g/100 g body weight). Another highly significant QTL (*Mfatq2sa*) was found in the region near D16Mit211, with an LOD score of 5.0. For two highly significant QTLs at D7Mit155 and D16Mit211, the A/J allele at each QTL contributed to increased mesenteric fat weight. As regards the significant QTL (*Mfatq3sa*) found at D5Mit6, the mean value of this trait was higher among F2 mice with SM/J homozygous than in those with A/J homozygous.

We also performed the analysis of the epistatic interaction of loci controlling epididymal fat weight or mesenteric fat weight. There were no significant interactions between each QTL detected for epididymal or mesenteric fat weight. Moreover, any other loci, which have significant interaction with QTLs shown in Table 2, were not detected by epistatic analysis.

We showed that epididymal fat weight and mesenteric fat weight are positively correlated with fatty liver (liver total lipid contents) in (SM × SMXA-5)F2 mice (18). Therefore, in the present study, we performed the QTL analysis for liver weight and mapped two highly significant QTLs (*Livq3sa* and *Livq4sa*) for liver weight on Chrs. 11 and 15, and two suggestive QTLs on Chrs. 13 and 17 (supplementary Table I). As regards the highly significant QTLs, the SM/J allele on Chr. 11 and the opposite allele (A/J) on Chr. 15 contributed to increased liver weight.

TABLE 2. QTLs for epididymal and mesenteric fat weights in (A/J×SM.A-*T2dm2sa*)F2 mice after 11 weeks feeding of a high-fat diet

Phenotype	Nearest Marker	Position (Mb)	LOD[a]	QTL	%[b]	Phenotype Based on Genotype[c] SM/SM	SM/A	A/A
Epididymal fat (g/100g bw)	D1Mit102	149.1	2.2		4	**4.05 ± 1.09 (53)**	4.06 ± 1.43 (130)	3.52 ± 1.10 (72)
	D8Mit166	113.4	2.3		4	3.77 ± 1.10 (54)	3.76 ± 1.30 (129)	**4.29 ± 1.36 (71)**
	D9Mit10	89.9	2.2		4	**4.16 ± 1.53 (72)**	3.92 ± 1.16 (135)	3.50 ± 1.17 (48)
	D10Mit12	**98.9**	**3.8***	***Efatq2sa***	**7**	3.55 ± 1.18 (63)	3.83 ± 1.23 (129)	**4.43 ± 1.38 (63)**
	D11Mit338	115.3	2.2		4	**4.25 ± 1.41 (65)**	3.93 ± 1.32 (120)	3.55 ± 1.04 (70)
	D12Mit58	**17.0**	**4.5***	***Efatq1sa***	**8**	**4.50 ± 1.12 (57)**	3.87 ± 1.32 (129)	3.48 ± 1.21 (69)
	D15Mit52	22.4	3.2		6	**4.22 ± 1.26 (72)**	4.01 ± 1.30 (112)	3.44 ± 1.20 (71)
	D18Mit17	39.6	2.0		4	3.64 ± 1.20 (64)	3.84 ± 1.20 (128)	**4.31 ± 1.47 (63)**
Mesenteric fat (g/100g bw)	D1Mit8	83.0	2.5		4	2.12 ± 0.44 (69)	2.16 ± 0.43 (113)	**2.36 ± 0.55 (73)**
	D5Mit6	**91.8**	**3.5***	***Mfatq3sa***	**6**	**2.36 ± 0.53 (69)**	2.21 ± 0.46 (128)	2.02 ± 0.39 (58)
	D7Mit155	**32.0**	**7.7****	***Mfatq1sa***	**13**	1.96 ± 0.35 (63)	2.22 ± 0.51 (132)	**2.44 ± 0.41 (60)**
	D10Mit14	118.1	2.6		5	2.30 ± 0.51 (63)	2.10 ± 0.42 (126)	**2.31 ± 0.52 (66)**
	D13Mit290	103.6	2.7		5	**2.38 ± 0.52 (55)**	2.20 ± 0.45 (129)	2.09 ± 0.48 (71)
	D16Mit211	**35.6**	**5.0****	***Mfatq2sa***	**9**	2.03 ± 0.40 (70)	2.20 ± 0.44 (118)	**2.41 ± 0.56 (67)**

* Significant level; ** highly significant level; the other LOD scores without asterisks denote suggestive level linkage.

[a] The maximum LOD scores, exceeding suggestive threshold level. Suggestive, significant, or highly significant thresholds derived from free model permutation for each trait were as follows: for epididymal fat weight (2.0, 3.4, 4.7); for mesenteric fat weight (2.0, 3.4, 5.0).

[b] Percentage of phenotypic variance explained by the QTL.

[c] Phenotype based on genotype at the nearest marker. Data are expressed means ± SD for genotypes. The number of mice of each genotype is indicated in parentheses. The higher mean of SM/SM or A/A are in boldface type.

DISCUSSION

In this study, to identify the genetic regions that contribute to fat accumulation in each abdominal fat pad, we performed the QTL analyses for mesenteric fat weight and epididymal fat weight. Using (A/J × SM.A-*T2dm2sa*) F2 mice, we detected two highly significant QTLs for mesenteric fat weight (*Mfatq1sa, Mfatq2sa*). We also detected three significant QTLs for epididymal fat weight (*Efatq1sa, Efat2sa*) and mesenteric fat weight (*Mfatq3sa*).

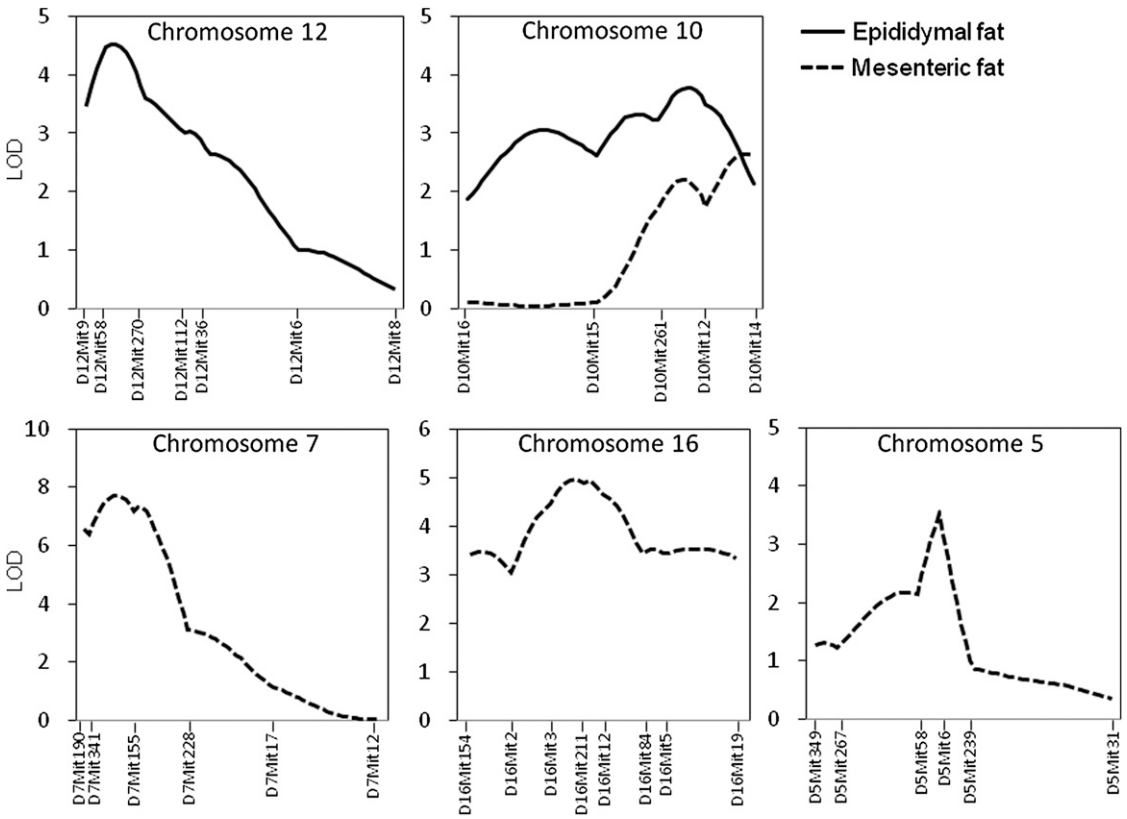

Fig. 2. Logarithm of odds (LOD) score curves of highly significant or significant quantitative trait loci (QTLs) for abdominal fat weights. QTLs were detected for epididymal fat weight on chromosome (Chr.) 12 and 10, and for mesenteric fat weight on Chr. 7, 16, and 5. On Chr. 10, suggestive QTL for mesenteric fat was also detected. A bold line indicates an LOD score curve of epididymal fat. A dashed line indicates an LOD score curve of mesenteric fat.

On Chr. 7, we detected a highly significant QTL (*Mfatq1sa*) for mesenteric fat weight. The results obtained by another group using C57BL/6J-Chr. $7^{129S1/SvImJ}$ consomic mice confirmed that genes affecting fat weight are present on Chr. 7 (29, 30). In the distal region of Chr. 7, it was reported that the locus controlling body fat percent coincided with those controlling obesity-related traits, such as serum total cholesterol concentration (7). However, in this study, *Mfatq1sa* is mapped on the centromeric region of Chr. 7. In the centromeric region of Chr. 7, a number of QTLs have been mapped for body weight (four QTLs) and body fat weight (six QTLs) by other groups (9). In a meta-analysis using mice, Wuschke and coworkers (9) detected the QTL (LOD 14.8) for body fat weight in the region (26.6-53.2 Mb) containing our *Mfatq1sa*. At present, it remains unknown whether or not the genes that affect fat weight in the present study are the same as those in previous studies.

On *Mfatq3sa* of Chr. 5, the SM/J allele contributed to the accumulation of mesenteric fat, although the mesenteric fat weight of SM/J mice tended to be lower than that of A/J mice (Tables 1, 2). Similarly, on *Efatq2sa* of Chr. 10, the A/J allele contributed to the accumulation of epididymal fat, although the epididymal fat weight of A/J mice tended to be lower than that of SM/J. Therefore, mesenteric fat weight and epididymal fat weight are controlled by the combination of multiple genetic factors derived from both parental strains.

Both epididymal fat and mesenteric fat were present in the abdominal cavity. However, the present results indicate that highly significant or significant QTLs for epididymal fat weight and mesenteric fat weight were detected in different chromosomal regions (Table 2, **Fig. 3**). The genomic regions affecting epididymal fat weight did not coincide with those for mesenteric fat weight in (A/J × SM.A-*T2dm2sa*)F2 mice. We therefore speculate that fat accumulation in each fat depot is controlled by distinct genetic factors. Reed et al. (30) performed QTL analysis for gonadal fat and retroperitoneal fat in mice of either sex and reported that 12 chromosomes affected fat distribution. However, the chromosomal regions affecting both fat weights were detected on only three chromosomes. The same group, using QTL analysis, also showed that *Adip5* on Chr. 9 affected epididymal fat weight but not retroperitoneal fat weight (31). Stylianou et al. (32) reported in QTL analysis using mice that there was no coincident chromosomal region (QTLs) that controlled both gonadal and mesenteric fat weight. On the other hand, in the same study, a portion of QTLs for gonadal fat weight or retroperitoneal fat weight were mapped on the same chromosomal regions. Subsequently, Reed et al. (29) reported by using consomic mice that the chromosomal region controlling fat tissue weight between subcutaneous and visceral fat (gonadal fat and retroperitoneal fat) was the same. However, the chromosomal region controlling mesenteric fat weight was different from that controlling the

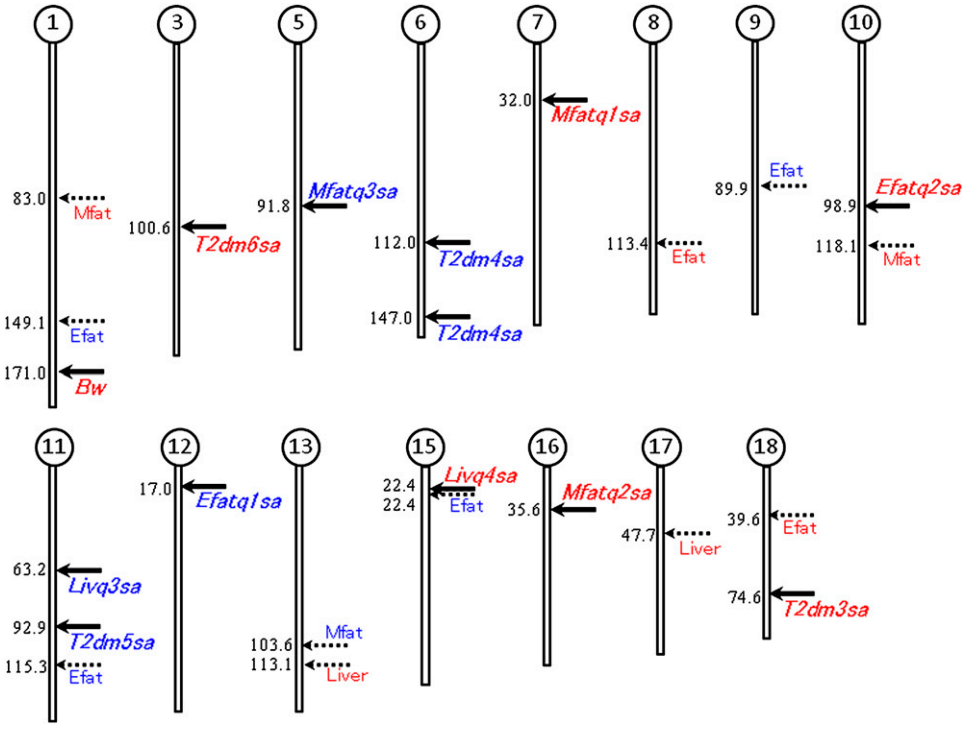

Fig. 3. Summary of quantitative trait loci (QTLs) for fat weights and diabetes-related traits in (A/J × SM.A-*T2dm2sa*)F2 mice. Solid arrows indicate highly significant or significant QTLs for epididymal fat weight and mesenteric fat weight that were mapped in this study, and significant QTLs for diabetes-related traits that were mapped in the previous study (20). Dashed arrows indicate suggestive QTLs. Blue QTLs, higher allele is SM/J; red QTLs, higher allele is A/J. Mfat, suggestive QTL for mesenteric fat; Efat, suggestive QTL for epididymal fat.

weight of all other kinds of fat. The present results and the previous studies suggested that the weight of each kind of fat in the abdominal cavity were controlled by distinct genetic factors.

In addition, we performed QTL analysis for liver weight to search the relation between abdominal fat weight and liver weight (supplementary Table I, Table 2, Fig. 3). The QTLs for epididymal fat weight and liver weight (*Livq3sa*) were detected on the same chromosome (Chr. 11) but not the same/near region. In contrast, on Chr.15, the QTLs for epididymal fat weight (Table 2) and liver weight (*Livq4sa*) were detected at the same chromosomal region that is around 22.4 Mb. Suggestive QTL for mesenteric fat weight on Chr. 13 was also detected close to suggestive QTL for liver weight. Interestingly, the A/J allele on Chrs. 15 (D15Mit52) and 13 (D13Mit53-D13Mit290) contributed to increased liver weight; oppositely, the A/J allele on same regions contributed to decreased epididymal/mesenteric fat (supplementary Table I, Table 2, Fig. 3). The gene(s) in this region might control the fat distribution between liver and abdominal fat tissues.

To examine whether or not the genetic regions controlling the fat weights in the abdominal cavity coincide with those controlling glucose metabolism, we compared the chromosomal regions of the present QTLs for abdominal fat weight with those for type 2 diabetes-related traits detected in the same F2 intercross mice (Fig. 3). We previously mapped the significant QTLs (*T2dm6sa*, *T2dm4sa*, *T2dm5sa*, and *T2dm3sa*) for diabetes-related traits on Chrs. 3, 6, 11, and 18, respectively (20) (Fig. 3). As regards the QTLs for body weight and body mass index, we also detected one significant QTL for body weight on Chr. 1 (20) (Fig. 3). Consequently, the genomic regions, in which significant QTLs for fat tissue weight (*Efatq1sa*, *Efatq2sa*, *Mfatq1sa*, *Mfatq2sa*, and *Mfatq3sa*) and QTL for body weight were detected, did not coincide with those for diabetes-related traits (Fig. 3). In contrast, we previously mapped the highly significant QTL (LOD 7.9) for body mass index on the same region of diabetic QTL: *T2dm2sa* on Chr. 2 (19). Moreover, in the previous (19) and present studies, SM.A-*T2dm2sa* congenic mice showed impaired glucose tolerance and the accumulation of abdominal fat weights. These findings suggest that the genetic factors for impaired glucose tolerance, body mass index, and abdominal fat weights on Chr. 2 exist within the introgressed region (D2Mit295-D2Mit281, 119 Mb) of SM.A-*T2dm2sa* congenic mice. By using novel congenic strains, we investigated whether or not the same genomic region on Chr. 2 has a gene or genes that affects impaired glucose tolerance and the accumulation of abdominal fat. Based on the present comparison of QTLs for abdominal fat weights and those for glucose metabolism, we unexpectedly revealed that the major genetic factors affecting the accumulation of epididymal/mesenteric fat did not coincide with those affecting glucose metabolism in (A/J × SM.A-*T2dm2sa*)F2 mice.

In the present study, epididymal fat weight and mesenteric fat weight tended to be higher in SM.A-*T2dm2sa* congenic mice than the weights in recipient SM/J and donor A/J (Table 1). These observations suggest that the combination of *T2dm2sa* with the A/J allele and unknown loci with the SM/J allele causes abdominal fat accumulation in SM.A-*T2dm2sa* congenic mice. Although we surveyed the loci that control the accumulation of abdominal fat in (A/J × SM.A-*T2dm2sa*)F2 mice, all F2 progeny had the A/J homozygotes allele at the introgressed Chr. 2 region (D2Mit295-D2Mit281). Therefore, we could not analyze the epistatic interactions between *T2dm2sa* and the detected loci. On the other hand, there were no significant QTLs that have epistatic interaction with QTLs shown in Table 2. Taken together with the result of epistatic interaction analysis, it is speculated that *1*) the interaction between *T2dm2sa* and the other loci on abdominal fat weights exists, and *2*) the combinations of *T2dm2sa* with the QTLs detected in this study have additive effect.

In summary, we successfully detected five significant QTLs for epididymal fat weight and mesenteric fat weight by QTL analysis of F2 intercross mice derived from A/J and SM.A-*T2dm2sa* mice. In addition, we revealed that *1*) genetic factors affecting each fat depot were distinct, and *2*) genetic factors affecting the accumulation of epididymal fat and mesenteric fat do not coincide with those affecting glucose metabolism. This genetic analysis may contribute to our understanding of body fat distribution and of the association between the accumulation of body fat and glucose metabolism.

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