Searching for genetic factors of fatty liver in SMXA-5 mice by quantitative trait loci analysis under a high-fat diet

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Abstract Fatty liver is strongly associated with the metabolic syndrome characterized by obesity, insulin resistance, and type 2 diabetes, but the genetic basis and functional mechanisms linking fatty liver with the metabolic syndrome are largely unknown. The SMXA-5 mouse is one of the SMXA recombinant inbred substrains established from SM/J and A/J strains and is a model for polygenic type 2 diabetes, characterized by moderately impaired glucose tolerance, hyperinsulinemia, and mild obesity. SMXA-5 mice also developed fatty liver, and a high-fat diet markedly worsened this trait, although SM/J and A/J mice are resistant to fatty liver development under a high-fat diet. To dissect loci for fatty liver in the A/J regions of the SMXA-5 genome, we attempted quantitative trait loci (QTLs) analysis in (SM/J× SMXA-5)F2 intercross mice fed a high-fat diet. We mapped a major QTL for relative liver weight and liver lipid content near D12Mit270 on chromosome 12 and designated this QTL *Fl1sa*. The A/J allele at this locus contributes to the increase in these traits. We confirmed the effect of *Fl1sa* on lipid accumulation in liver using the A/J-Chr12^{SM} consomic strain, which showed significantly less accumulation than A/J mice. This suggests that the SM/J and A/J strains, neither of which develops fatty liver, possess loci causing fatty liver and that the coexistence of these loci causes fatty liver in SMXA-5 mice.—Kumazawa, M., M. Kobayashi, F. Io, T. Kawai, M. Nishimura, T. Ohno, and F. Horio. **Searching for genetic factors of fatty liver in SMXA-5 mice by quantitative trait loci analysis under a high-fat diet. *J. Lipid Res.* 2007.** 48: **2039–2046.**

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Fatty liver is increasingly prevalent in Western countries and may lead to steatohepatitis, cirrhosis, and end-stage liver disease (1–3). The majority of patients with fatty liver are those with obesity, insulin resistance, hyperlipidemia, and/or type 2 diabetes in the metabolic syndrome or syndrome X (4, 5). Despite the frequent association between fatty liver and the metabolic syndrome, the genetic basis and functional mechanisms linking fatty liver with other components of this syndrome are largely unknown. The heterogeneity of the syndrome, which is caused by the interaction of genetic and environmental factors, makes it difficult to understand the disease mechanism in humans. Because genetic and environmental factors can be strictly controlled when inbred animal models are used, such models are invaluable for the dissection of complex diseases. Mice with extreme obesity, such as obese ob/ob mice, and those lacking adipose tissue, such as lipoatrophic A-ZIP/F-1 mice and PEPCK-nSREBP-1α mice, have served as extreme models for studies of the pathogenesis of fatty liver (6, 7). However, animal models of more common forms of fatty liver, as observed in the human metabolic syndrome, are limited.

The SMXA-5 mouse is one of 26 SMXA recombinant inbred (RI) substrains that have been established from parental strains SM/J and A/J (8). Each SMXA RI strain has a mosaic genome derived from the SM/J and A/J strains. Although the parental strains were nondiabetic, the SMXA-5 mouse was introduced as a new animal model for polygenic type 2 diabetes that moderately develops impaired glucose tolerance, hyperinsulinemia, and mild obesity (9). In a recent study, we reported that SMXA-5 developed fatty liver and that a short-term high-fat diet markedly worsened this trait (10). On the other hand, we also demonstrated that SM/J and A/J mice are resistant to the development of fatty liver under a high-fat diet (10). These results suggested that the loci for fatty liver, which exist in the nonsteatotic parental SM/J and A/J genomes, interact with each other in the genomes of RI strains, leading to the development of fatty liver.

In this study, using SMXA-5 and SM/J strains that have different degrees of fatty liver, we dissected loci in the A/J regions of the SMXA-5 genome that contribute to fatty liver. We attempted quantitative trait locus (QTL) analysis

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in the (SM/J×SMXA-5)F2 intercross fed a high-fat diet, because such a diet enhances the difference in fatty liver degrees between SM/J and SMXA-5. Moreover, to verify the function of the responsible locus mapped in this study, we chose consomic mapping as a subsequent strategy. Consomic strains eliminate much of the genetic background interference and are efficient tools for dissecting complex diseases.

MATERIALS AND METHODS

Animals and diet composition

Parental SM/J and SMXA-5 strains were obtained from the Institute for Laboratory Animal Research, Nagoya University School of Medicine. SM/J female mice were mated to SMXA-5 males to produce F1 hybrid mice, which were intercrossed to produce (SM/J×SMXA-5)F2 intercross mice. Male F1 and F2 generations (255 mice) were produced and maintained in our facility. A/J male mice were purchased from Japan SLC (Hamamatsu, Japan). The A/J-Chr12^{SM} consomic strain was produced and housed at the Institute for Laboratory Animal Research. All mice were maintained in a room under conventional conditions at a controlled temperature of 23 ± 3°C and 55 ± 5% humidity on a 12 h light/dark cycle. Male SM/J, SMXA-5, F1, and F2 mice were fed the high-fat diet from 6 to 13 weeks of age. Male A/J and A/J-Chr12^{SM} mice were fed the high-fat diet from 6 to 17 weeks of age. The mice were given access ad libitum to drinking water and the high-fat diet, whose composition (g/kg diet) was as follows: casein, 209; carbohydrate (starch sucrose, 1:1), 369; AIN93MX mineral mixture (11), 35; AIN93VX vitamin mixture (11), 10; choline chloride, 2; corn oil, 35; lard, 300; and cellulose powder (AVICEL type FD-101; Asahi Chemical Industry, Osaka, Japan), 40. The content of fat in this high-fat diet is 33.5% (weight percentage). All procedures were performed in accordance with the Animal Experimental Guide of Nagoya University.

In the experiment using SM/J, SMXA-5, F1, and F2 mice, all phenotype determinations and dissection were performed after 7 weeks of the high-fat diet. Serum insulin concentrations and triglyceride concentrations were measured by radioimmunoassay (ShionoRIA; Shionogi, Osaka, Japan) and the Triglyceride-E Kit (Wako, Tokyo, Japan), respectively. In the experiment using A/J and A/J-Chr12^{SM} mice, phenotype determinations and dissection were performed after 11 weeks of the high-fat diet. The intraperitoneal glucose tolerance test was performed using the following protocol. After 14 h of fasting (from 7:00 PM to 9:00 AM), blood samples were collected from the tail vein (fasting blood glucose sample). Then, a 20% glucose solution was injected intraperitoneally (2 g glucose/kg body weight). Blood samples were collected at 30, 60, and 120 min after the injection. Blood glucose concentration was measured by a glucose oxidase method (Glucose-B Test Kit; Wako). The area under the curve was calculated according to the trapezoid rule from the glucose measurements at fasting, 30, 60, and 120 min (mg/min/dl). Body mass index was calculated as body weight (g) divided by the square of the anal-nasal length (cm). Immediately after the mice were dissected in each experiment, the liver was removed, weighed, and stored at −80°C.

Hepatic lipid analysis

Frozen livers were homogenized with chloroform-methanol (2:1), and liver lipids were extracted into organic solvents. A portion of this extract was dried, and the hepatic contents of triglycerides and total cholesterol were measured by the Triglyceride E-Test (Wako) and the Cholesterol E-Test (Wako), respectively. This extract was also used to measure total liver lipids according to the method of Folch, Lees, and Sloane Stanley (12).

Genotyping and linkage analysis

Genomic DNA was prepared from mouse kidney by salt-ethanol precipitation. A total of 73 microsatellite marker loci (Map Pair; Research Genetics, Huntsville, AL), polymorphic between SM/J and SMXA-5, were genotyped in all 255 F2 mice. All microsatellite markers and the positions for linkage analysis are shown in **Fig. 1**. SMXA-5 is one of 26 SMXA RI substrains that possess mosaic genomes derived from the SM/J and A/J strains (9). We selected valuable markers for this study from the SMXA RI strain distribution patterns (13). PCR was performed according to standard methods (14). PCR products were separated by electrophoresis on a 4% NuSieve (FMC, Rockland, ME) agarose gel and visualized with ethidium bromide staining. Linkage analysis was performed using the Map Manager QTXb20 (15, 16) software program. This program is based on interval mapping using the free regression model. The permutation test estimates an experiment-wide probability for given likelihood ratio statistics. Significance was determined by 1 centimorgan steps for 1,000 permutations to provide likelihood ratio statistics that were suggestive, significant, or highly significant (17, 18). 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 likelihood ratio statistics by 4.605 (19). Significant linkage was defined in accordance with the guidelines of Lander and Kruglyak (20) as statistical evidence occurring by chance in the genome scan with $P < 0.05$. The search for QTLs with epistatic interaction effects was performed with Map Manager QTX, as described by Ishikawa et al. (21).

Statistical analysis

One-way ANOVA and subsequent Scheffe's test were used to compare the means among SM/J, SMXA-5, and F1 mice. Student's *t*-test was used to compare means between A/J and A.SM-Chr12 mice. Differences with $P < 0.05$ were regarded as significant. Correlation analyses between liver total lipid content and various parameters, and general statistical analyses, were also performed using StatView version 5.0 software (SAS Institute, Cary, NC).

RESULTS

Steatotic phenotypes in parental strains, F1, and F2 mice

The initial body weight of SMXA-5 mice was higher than that of SM/J mice and similar to that of F1 mice. Final body weight and body mass index of SMXA-5 mice were also significantly higher than the respective values of SM/J mice and F1 mice, but these traits were higher in F1 mice than in SM/J mice (**Table 1**). Relative liver weight, liver triglyceride content, total cholesterol content, total lipid content, and serum triglyceride concentration were measured in parental (SM/J and SMXA-5), (SM/J×SMXA-5)F1, and (SM/J×SMXA-5)F2 intercross mice after 6 weeks of the high-fat diet and are shown in **Table 2**. In relative liver weight, liver total lipid content, liver triglyceride content, and serum triglyceride concentration, the means in the SMXA-5 mice were significantly higher than the respective values in the SM/J mice. Thirty-five percent of the differ-

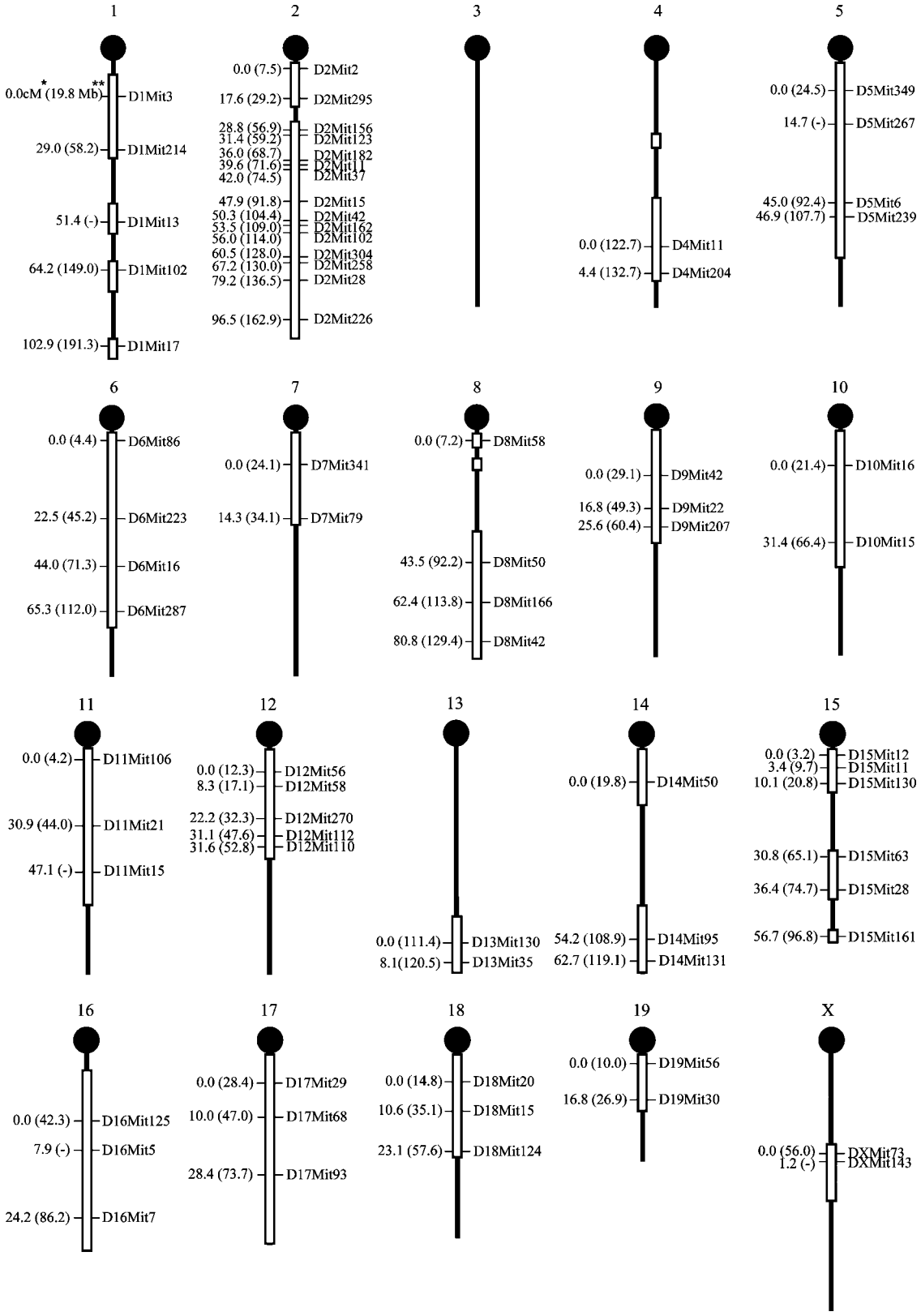

Fig. 1. The microsatellite markers used for linkage analysis in the SM/J and SMXA-5 intercross. Chromosomes are shown as lines; the black lines indicate the SM/J region in the SMXA-5 genome, and the white boxes indicate the A/J region in the SMXA-5 genome. Single asterisks indicate map positions [in centimorgan (cM)] of markers estimated from the F2 intercross using the Kosambi map function (Map Manager QTX). Double asterisks indicate map positions (in Mb) of markers collected from the Ensembl Genome Browser (NCBI Build m36; http://www.ensembl.org/).

TABLE 1. Initial and final body weight, body mass index, relative liver weight, liver total lipid content, liver TG content, liver TC content, and serum TG concentration in parental strains, (SM/J×SMXA-5)F1, and (SM/J×SMXA-5)F2 intercross mice after 7 weeks of feeding a high-fat diet

				P				
Parameter	SM/J (n = 6)	SMXA-5 (n = 6)	F1 (n = 6)	SM versus SMXA-5	F1 versus SM	F1 versus SMXA-5	F2 (n = 255)	F2 Range
Initial body weight (g)	14.0 ± 0.3 a	17.2 ± 0.3 b	16.9 ± 0.3 b	<0.001	<0.001	<0.001	16.4 ± 0.1	12.3–21.3
Final body weight (g)	23.0 ± 0.5 a	30.5 ± 0.7 c	26.0 ± 0.4 b	<0.001	0.013	<0.001	27.5 ± 0.3	18.1–37.1
Body mass index (g/cm^2)	0.251 ± 0.010 a	0.348 ± 0.006 c	0.285 ± 0.004 b	<0.001	0.009	<0.001	0.305 ± 0.002	0.220–0.385
Liver weight (g/100 g body weight)	4.06 ± 0.06 a	5.26 ± 0.21 b	4.27 ± 0.12 a	<0.001	NS	<0.001	4.51 ± 0.03	2.89–5.73
Liver total lipids (mg/100 g body weight)	320 ± 20 a	879 ± 50 b	265 ± 5 a	<0.001	NS	<0.001	421 ± 8	211–909
Liver TG (mg/100 g body weight)	132 ± 13 b	332 ± 24 c	90 ± 9 a	<0.001	NS	<0.001	149 ± 3	57–330
Liver TC (mg/100 g body weight)	11.1 ± 0.5	13.0 ± 0.8	12.9 ± 0.4	NS	NS	NS	11.1 ± 0.1	6.9–17.6
Serum TG	151 ± 12 a	288 ± 21 b	111 ± 8 a	<0.001	NS	<0.001	192 ± 5	16–400

TC, total cholesterol; TG, triglyceride. Values are expressed as means ± SEM. Statistical analysis was done among SM/J, SMXA-5, and F1 hybrid mice. These data were analyzed by one-way ANOVA. When a significant effect was observed by one-way ANOVA, Scheffe's test was used to compare the means of all groups. Means not sharing a common lowercase letter are significantly different by Scheffe's test ($P < 0.05$).

ence in liver total lipid content between SMXA-5 and SM/J mice was attributable to the accumulation of triglycerides. Liver total cholesterol content did not differ between the strains. The liver triglyceride content of F1 mice was significantly lower than those of SMXA-5 and SM/J mice. Relative liver weight, liver total lipid content, and serum triglyceride concentration in F1 mice were similar to those of SM/J mice and significantly lower than those of SMXA-5 mice. In the F2 intercross mice, the means of all parameters lay between those of SM/J and SMXA-5. In all parameters, excluding liver triglyceride content, the individual values in F2 mice showed wide distributions, exceeding the ranges of values in SM/J and SMXA-5 mice.

QTL mapping in the (SM/J×SMXA-5)F2 intercross

On chromosome 2, between D2Mit162 and D2Mit28, we mapped significant QTLs that affect liver total lipid content and relative liver weight. The mean values of these traits among F2 mice with the A/J homozygotes (A/A) between D2Mit162 and D2Mit28 were higher than those with the SM/J homozygotes (SM/SM) (Table 2). This locus was designated *Fl2sa*, for fatty liver 2 in the SMXA RI strains. In contrast, a suggestive QTL for liver total cholesterol content was found in the region near D2Mit156, and the allele originating in SM/J was associated with an increased value of each phenotype based on genotype. A suggestive QTL for liver triglyceride content was also found in the region

TABLE 2. Suggestive, significant, and highly significant QTLs for fatty liver

				Phenotype Based on Genotype[c]		
Phenotype	Nearest Marker	LOD[a]	Percentage[b]	SM/SM	SM/A	A/A
Liver TC (mg/100 g body weight)	D2Mit156	2.5	4	11.8 ± 0.3	11.0 ± 0.2	10.7 ± 0.2
Liver total lipids (mg/100 g body weight)	D2Mit162	4.1*	7	389 ± 12	414 ± 10	486 ± 24
Liver weight (g/100 g body weight)	D2Mit28	3.9*	7	4.37 ± 0.06	4.51 ± 0.04	4.70 ± 0.06
Liver TG (mg/100 g body weight)	D2Mit226	2.1	4	140 ± 4	157 ± 4	140 ± 5
Liver total lipids (mg/100 g body weight)	D6Mit86	2.1	4	455 ± 17	433 ± 13	389 ± 13
Serum TG (mg/dl)	D6Mit86	2.4	5	196 ± 10	203 ± 7	166 ± 8
Serum TG (mg/dl)	D8Mit166	2.0	3	173 ± 8	195 ± 7	208 ± 10
Liver TG (mg/100 g body weight)	D10Mit15	1.9	4	151 ± 6	155 ± 4	139 ± 5
Liver weight (g/100 g body weight)	D11Mit15	2.1	4	4.67 ± 0.08	4.49 ± 0.04	4.42 ± 0.05
Serum TG (mg/dl)	D11Mit15	2.1	4	222 ± 11	183 ± 6	186 ± 9
Liver TG (mg/100 g body weight)	D12Mit58	3.7*	7	138 ± 5	148 ± 4	166 ± 6
Liver weight (g/100 g body weight)	D12Mit270	8.8**	15	4.29 ± 0.05	4.49 ± 0.04	4.74 ± 0.06
Liver total lipids (mg/100 g body weight)	D12Mit270	7.7**	13	358 ± 10	418 ± 10	488 ± 20
Liver TC (mg/100 g body weight)	D17Mit29	3.7*	7	10.9 ± 0.3	10.9 ± 0.1	11.9 ± 0.3
Liver weight (g/100 g body weight)	D17Mit68	2.5	4	4.33 ± 0.06	4.55 ± 0.04	4.55 ± 0.05
Liver total lipids (mg/100 g body weight)	D17Mit68	2.7	5	384 ± 12	448 ± 13	401 ± 12

LOD, logarithm of odds; QTL, quantitative trait locus. Single asterisks indicate significant levels and double asterisks indicate highly significant levels; the other LOD scores without asterisks denote suggestive linkage.

[a] Maximum LOD scores, exceeding suggestive threshold levels. Suggestive, significant, or highly significant thresholds derived from free model permutation for each trait were as follows: for liver weight (1.9, 3.3, and 4.7, respectively); for liver total lipid content (1.8, 3.2, and 5.4); for liver triglyceride content (1.9, 3.3, and 4.6); for liver total cholesterol content (1.8, 3.3, and 5.2); and for serum triglyceride concentration (1.9, 3.2, and 5.3).

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

[c] Phenotype based on genotype at the nearest marker. Data are expressed as means ± SEM for genotypes.

near D2Mit226. The mean values of this trait among F2 mice with the heterozygote were higher than those with the SM/J or A/J homozygote.

On chromosome 12, a QTL for relative liver weight was found in the region near D12Mit270, with the highest LOD score being 8.8 (a highly significant LOD score is ⩾4.7). This allele explains 15% of the phenotypic variance in relative liver weight (Table 2). We also mapped a highly significant QTL for liver total lipid content in the region near D12Mit270 and a significant QTL for liver triglyceride content in the region near D12Mit58. We named this QTL for fatty liver *Fl1sa*. On chromosome 12, the A/J allele at *Fl1sa* increased the value of each phenotype based on genotype in comparison with the SM/J allele. All LOD score curves for these traits showed more than one peak (**Fig. 2**). Concerning liver weight and total lipid content, the chromosomal regions exceeding significant thresholds of linkage were broad.

On chromosome 17, we mapped significant and suggestive QTLs that affected relative liver weight, total lipid content, and total cholesterol content between D17Mit29 and D17Mit68 (Table 2). On significant QTLs for liver total cholesterol content in the region near D17Mit29, the mean value among F2 mice with A/J homozygotes (A/A) at D17Mit29 was higher than that with the SM/J homozygotes (SM/SM).

In this study, significant and highly significant QTLs were detected on three chromosomes (2, 12, and 17). In addition, suggestive QTLs were detected on chromosomes 2, 6, 8, 10, 11, and 17. On chromosomes 6, 10, and 11, a suggestive QTL for liver total lipid content, one for liver triglyceride content, and one for liver weight were detected, respectively (Table 2). Three suggestive QTLs for serum triglyceride concentration were detected on chromosomes 6, 8, and 11. Each locus explains 3–5% of the phenotypic variance in each trait. The allele originating in SM/J on chromosomes 6 (but not on the QTL for serum triglyceride concentration) and 11 was associated with an increased value of each phenotype based on genotype. In the QTL on chromosome 10, the mean values of the traits among F2 mice with the heterozygote were higher than those with the SM/J or A/J homozygote.

On all traits analyzed in this study, there was no significant QTL with an epistatic interaction effect.

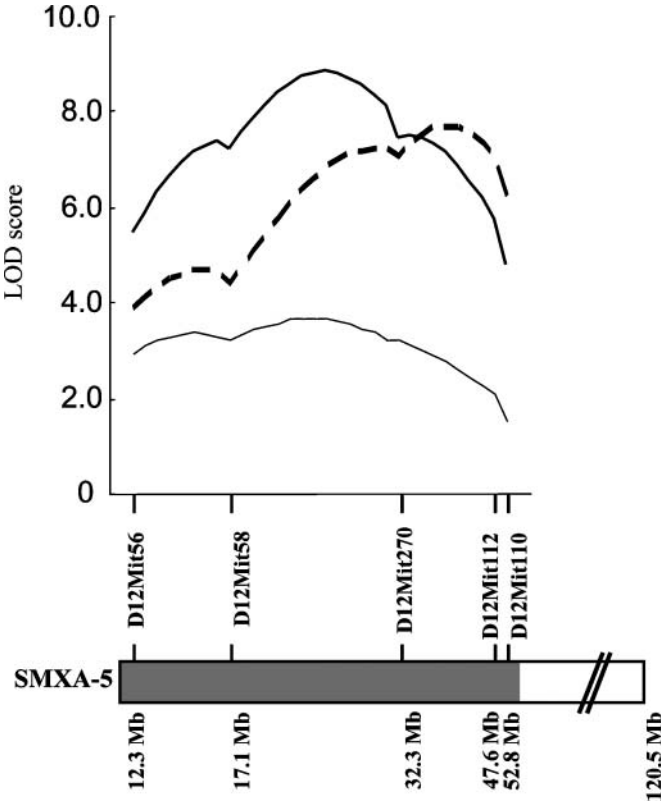

Fig. 2. Logarithm of odds (LOD) score curves for liver weight (thick line), liver total lipid content (broken line), and liver triglyceride content (thin line) for chromosome 12, and the chromosomal construction of the SMXA-5 strain (bottom). The gray bar shows the A/J-derived chromosomal region of the SMXA-5 strain. Map positions (in Mb) on the x axis are collected from the Ensembl Genome Browser (NCBI Build m36; http://www.ensembl.org/). Suggestive, significant, and highly significant thresholds of LOD scores for all traits are as follows: for liver weight (1.9, 3.3, and 4.7, respectively); for liver total lipid content (1.8, 3.2, and 5.4); and for liver triglyceride content (1.9, 3.3, and 4.6).

Correlations of fatty liver with several parameters in the (SM/J×SMXA-5)F2 intercross

There were significant positive correlations between liver total lipid content and relative liver weight, liver triglyceride content, serum triglyceride concentration, body weight, body mass index, relative epididymal fat weight, and mesenteric fat weight (**Table 3**). The correlation between liver total lipid content and mesenteric fat weight was strongest among adipose tissues. In addition, blood glucose concentration, glucose intolerance, and serum insulin concentration were positively correlated with liver

TABLE 3. Correlation of liver total lipid content with fatty liver-related traits, serum lipid concentration, obesity, and diabetes-related traits in (SM/J×SMXA-5)F2 intercross mice

Trait	*r*	*P*
Liver weight	0.704[a]	<0.0001
Liver TG	0.535[a]	<0.0001
Liver TC	−0.176[a]	0.0050
Serum TG	0.136[b]	0.0307
Serum TC	0.047	0.4613
Body weight	0.588[a]	<0.0001
Body mass index	0.560[a]	<0.0001
Epididymal fat	0.220[a]	0.0004
Retroperitoneal fat	−0.049	0.4373
Mesenteric fat	0.542[a]	<0.0001
Nonfasting BG	0.475[a]	<0.0001
Fasting BG	0.439[a]	<0.0001
IPGTT:120	0.530[a]	<0.0001
IPGTT:AUC	0.501[a]	<0.0001
Nonfasting insulin	0.458[a]	<0.0001
Fasting insulin	0.391[a]	<0.0001

BG, blood glucose concentration; IPGTT:120, serum glucose concentration at 120 min in intraperitoneal glucose tolerance test; IPGTT:AUC, area under the curve in intraperitoneal glucose tolerance test.

[a] $P < 0.001$, significant correlation.

[b] $P < 0.05$, significant correlation.

total lipid content. There were no significant correlations between liver total lipid content, serum total cholesterol concentration, and retroperitoneal fat weight.

Phenotype of the consomic strain A.SM-Chr12

To confirm the effect of a highly significant QTL on chromosome 12 on lipid accumulation in liver, we characterized the fatty liver-related traits of A/J-Chr12^{SM}. In the A/J-Chr12^{SM} consomic strain, chromosome 12 of A/J mice was replaced as a whole by chromosome 12 from SM/J mice, leaving the rest of the A/J chromosomes intact. The initial body weight of the A/J-Chr12^{SM} mice was slightly higher than that of the A/J mice, but the final body weight and body mass index were similar between the strains (**Table 4**). Relative epididymal fat weight in A/J-Chr12^{SM} mice was higher than that of A/J mice. The other two white adipose tissue weights did not differ between A/J-Chr12^{SM} and A/J mice. The relative liver weight was significantly lower in A/J-Chr12^{SM} mice than in A/J mice (Table 4), as were both the liver total lipid and liver triglyceride contents. Fasting blood glucose concentration and glucose tolerance were not different between these two strains.

DISCUSSION

In this study, QTL analysis revealed the existence of highly significant or significant QTLs (*Fl1sa* for relative liver weight, liver total lipid content, and liver triglyceride content on chromosome 12), significant QTLs (*Fl2sa* for relative liver weight and liver total lipid content on chromosome 2), and a significant QTL for liver total cholesterol content on chromosome 17. On chromosome 12, because LOD score curves for relative liver weight and liver total triglyceride content were similar, the increase in liver weight could be attributable mainly to the accumulation of triglycerides in liver. We recently reported that QTL analysis using this F2 intercross mouse with a high-fat diet revealed the loci for diabetes-related traits such as glucose tolerance, blood glucose, serum insulin concentration, and body mass index on chromosomes 2 and 12 (22). Moreover, the mean values of these traits were higher among F2 mice with the A/J allele at these loci than in mice with the SM/J allele. It has been reported that the pathogenesis of fatty liver is closely related to the development of obesity and insulin resistance, which are nearly universal in patients with fatty liver (23). The relationship between the accumulation of intracellular fatty acid-derived metabolites and insulin resistance has also been demonstrated in experimental mice (24, 25). Therefore, it is speculated from the present results that the A/J-derived allele for liver lipid content on chromosomes 2 and 12 also affected the development of insulin resistance in SMXA-5 mice. In a previous study (22), we detected significant linkages for diabetes-related traits on these two chromosomes. On chromosome 12, the same region where we mapped QTLs for relative liver weight in the present study, we found significant linkage for body mass index and serum insulin levels (22). The chromosome 2 region, where we mapped QTLs for relative liver weight in the present study, also had major QTLs for glucose tolerance, free-fed blood glucose level, and body mass index. Moreover, correlation analysis in F2 mice showed positive correlations between liver total lipid content and blood glucose level, glucose intolerance, and serum insulin level. These results show that fatty liver has a strong association with obesity and insulin resistance.

The region of chromosome 12 where *Fl1sa* was mapped in the present study was previously reported to be associated with the existence of the fatty liver-related allele *fld* (for fatty liver dystrophy) in mouse (26). Mice carrying mutations in the *fld* allele have features of human lipodystrophy characterized by loss of body fat, fatty liver, hypertriglyceridemia, and insulin resistance (27–30). *Lpin1*, the gene responsible for the *fld* mutant allele, was isolated and found to encode the nuclear protein lipin (16.7 Mb on mouse chromosome 12) (31). Phan and Reue (32)

TABLE 4. Initial and final body weights, body mass index, relative tissue weights, liver total lipid content, liver TG content, liver TC content, fasting blood glucose concentration, IPGTT:120, and IPGTT:AUC in A/J and A/J-Chr12^{SM} consomic mice after 11 weeks of feeding a high-fat diet

Characteristic	A/J (n = 8)	A/J-Chr12^{SM} (n = 8)	*P*
Initial body weight (g)	21.2 ± 0.5	22.7 ± 0.3[a]	0.023
Final body weight (g)	38.1 ± 1.5	37.0 ± 1.3	NS
Body mass index (g/cm^2)	0.328 ± 0.010	0.323 ± 0.008	NS
Tissue weight (g/100 g body weight)			
Epididymal fat	4.26 ± 0.28	5.65 ± 0.12[b]	<0.001
Retroperitoneal fat	1.19 ± 0.10	1.33 ± 0.08	NS
Mesenteric fat	2.19 ± 0.13	2.22 ± 0.15	NS
Liver	4.20 ± 0.10	3.65 ± 0.10[b]	<0.001
Liver total lipid (mg/100 g body weight)	415 ± 51	291 ± 12[a]	0.032
Liver TG (mg/100 g body weight)	124 ± 5	104 ± 4[b]	0.009
Liver TC (mg/100 g body weight)	9.7 ± 0.4	10.1 ± 0.5	NS
Fasting blood glucose (mg/dl)	93 ± 5	91 ± 5	NS
IPGTT:120 (mg/dl)	210 ± 20	180 ± 8	NS
IPGTT:AUC (min/mg/dl)	32,201 ± 3,136	33,273 ± 1,390	NS

IPGTT:120, serum glucose concentration at 120 min in intraperitoneal glucose tolerance test; IPGTT:AUC, area under the curve in intraperitoneal glucose tolerance test. Values are expressed as means ± SEM.

[a] $P < 0.05$, significant difference from the value of A/J by Student's *t*-test.

[b] $P < 0.01$, significant difference from the value of A/J by Student's *t*-test.

demonstrated that lipin caused lipodystrophy in its absence and promoted obesity when its levels were enhanced. Thus, at present, we consider *Lpin1* a plausible candidate gene for *Fl1sa* controlling liver lipid content on chromosome 12 in SMXA-5 mice. The chromosomal region is homologous to human chromosomes 2p25.3-23.3 and 14q12 and to rat chromosome 6 (http://ensembl.org/). It has not been reported that the loci controlling fatty liver development exist in these regions in human and rat.

As our next step in the QTL analysis in this study, we chose consomic mapping as a strategy to dissect *Fl1sa* for liver lipid content on chromosome 12. In A/J-Chr12^{SM} consomic mice, chromosome 12 of A/J mice was replaced as a whole by chromosome 12 from SM/J mice, so we expected that relative liver weight and liver lipid accumulation would be lower in these mice than in A/J mice. Consistent with the result of QTL analysis, A/J-Chr12^{SM} mice showed a significant reduction of relative liver weight, liver total lipid content, and liver triglyceride content compared with A/J mice. These results verify the existence of an allele that strongly induces the development of fatty liver on chromosome 12 of A/J mice. Moreover, the prevention of fatty liver induced by a high-fat diet might improve insulin resistance in A/J-Chr12^{SM} consomic mice compared with A/J mice. However, glucose tolerance was not different between A/J-Chr12^{SM} and A/J mice. We speculate that *Fl1sa* primarily affects lipid accumulation in liver and subsequently causes glucose intolerance and insulin resistance. As these two strains were fed the high-fat diet for 11 weeks in this study, prolongation of feeding the high-fat diet might suppress the development of a diabetic phenotype in A/J-Chr12^{SM} mice.

The present results suggest that the SM/J and A/J strains, neither of which develops remarkable fatty liver, possess genes causing fatty liver and that the coexistence of these genes is necessary to elicit fatty liver in SMXA-5 mice. It is also speculated that the fatty liver of SMXA-5 mice would be the result of gene-gene interactions. Although we have performed a search of QTLs with epistatic interaction effects, any loci with such effects were not detected. QTLs for fatty liver with epistatic interaction effects might exist within the SM/J regions of the SMXA-5 genome, which were excluded from the present analysis of the gene-gene interaction.

In conclusion, we detected 16 QTLs associated with fatty liver as relative liver weight, liver lipid content, or serum triglyceride concentration on chromosomes 2, 6, 8, 10, 11, 12, and 17 by QTL analysis in F2 intercross mice derived from SM/J mice and SMXA-5 mice that developed fatty liver from a high-fat diet. On chromosome 12, we mapped the locus *Fl1sa*, which strongly affected relative liver weight, liver total lipid content, and liver triglyceride content, and we used the A/J-Chr12^{SM} consomic strain to confirm that the A/J allele at *Fl1sa* led to the development of fatty liver. The fatty liver of SMXA-5 mice would be attributable to the interaction of causative genes. Studies using animal models such as SMXA-5 mice will facilitate the genetic dissection and elucidation of the complex mechanisms underlying the metabolic syndrome, including fatty liver, obesity, insulin resistance, and type 2 diabetes.

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