Combinations of Nondiabetic Parental Genomes Elicit Impaired Glucose Tolerance in Mouse SMXA Recombinant Inbred Strains

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Type 2 diabetes in humans is not a single gene disorder but a multifactorial disease caused by the interaction of multiple genes and environmental factors. Recombinant inbred (RI) strains are a powerful tool for analyzing not only single genetic traits but also multifactorial genetic traits. By using the SMXA RI mice, we genetically dissected diabetes-related traits (BMI, nonfasting blood glucose concentration, and blood glucose concentration during intraperitoneal glucose tolerance tests). For minimizing the variation of glucose tolerance in each strain, all mice were fed the high-carbohydrate diet and subjected to phenotypic and genetic analyses. The parental strains, SM/J and A/J, were nondiabetic, and the differences of the mean values of diabetes-related traits were small. In contrast, an impaired glucose tolerance was observed in (SM × A)F1 mice, and marked differences in diabetes-related traits were observed in 19 SMXA RI strains. In particular, several SMXA RI strains showed markedly impaired glucose tolerance and hyperglycemia. Quantitative trait locus (QTL) analysis revealed a locus on chromosome (Chr) 10 contributing significant effect on nonfasting blood glucose concentration, as well as six diabetes-related loci on four chromosomes with suggestive evidence of linkage with diabetes-related phenotypes. The A/J-derived QTLs on Chr 2 and 18 and an SM/J-derived QTL on Chr 10 contributed to the impairment of glucose tolerance and/or the increase of blood glucose concentration. The present study indicates that QTLs derived from parental SM/J and A/J genomes, both of which are nondiabetic, interact in the RI genomes, leading to the development of hyperglycemia and diabetic phenotypes. Genetic dissection of this kind of diabetogenesis will increase our understanding of the complex gene-gene interaction and mode of inheritance in human type 2 diabetes. *Diabetes* 52:180–186, 2003

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Chr, chromosome; IPGTT, intraperitoneal glucose tolerance test; LOD, logarithm of odds; QTL, quantitative trait locus; RI, recombinant inbred.

Type 2 diabetes is the most common form of diabetes and is characterized by two metabolic defects: a deficiency of insulin secreted by pancreatic β-cells and an inability of peripheral tissues to respond to insulin. Type 2 diabetes in humans is not a single gene disorder but rather is caused by the interaction of multiple genetic and environmental factors (1). Some rare monogenic forms of early-onset subtype, maturity-onset diabetes of the young, and rare mutations in the candidate genes included in glucose metabolism have been identified (2). However, these known gene defects account for only <1–2% of all type 2 diabetes cases, and the genes responsible for the common form of late-onset type 2 diabetes remain unidentified (3). Therefore, there has been considerable effort to identify the loci of the genes responsible for type 2 diabetes using genome-wide scans. Genome-wide scans have identified *NIDDM1* on chromosome (Chr) 2q in Mexican-Americans (4) and *NIDDM2* on Chr 12q in the Swedish-speaking population in a small area of western Finland (5). Recently, the calpain 10 gene (*CAPN10*) was first isolated as a diabetes susceptibility gene in the *NIDDM1* region by positional cloning (6). In general, it has been difficult to identify the genes involved in human diabetes because of the heterogeneous cause of this disease.

Inbred strains are genetically homogeneous, and the genetic and environmental components can be strictly controlled in the experimental setting. The genetic analysis of a multifactorial disease in experimental animals such as mice and rats is much more revealing than that of a similar disease in human subjects. Strategies for isolating multiple genes that individually account for small amounts of variance (quantitative trait loci [QTLs]) have been developed to identify chromosomal regions associated with diabetes traits. The underlying genetic factors in several polygenic model animals with type 2 diabetes have been studied by QTL mapping analysis. These studies have used the GK rat (7), the OLETF rat (7), the NSY mouse (8), the NZO mouse (9), the TSOD mouse (10), the KK-A^y mouse (11,12), and the TH mouse (13). QTL mapping analyses in these model animals identified several QTLs that influenced glucose tolerance, blood glucose concentration, serum insulin concentration, and parameters that define glucose homeostasis. At present, isolated diabetogenic genes are insufficient for deriving an explanation of the complex and polygenic nature of this disease in

TABLE 1
Body weight, BMI, nonfasting blood glucose concentration, and blood glucose concentration during IPGTT in SM/J, A/J, and (SM × A)F1 mice at 5 weeks on the high-carbohydrate diet

Traits	SM/J (n = 12)	A/J (n = 15)	F1 (n = 14)
Body weight (g)	18.5 ± 0.3	28.1 ± 0.8*	26.5 ± 0.4*
BMI (g/cm^2)	0.236 ± 0.002	0.274 ± 0.005*	0.276 ± 0.005*
Nonfasting blood glucose concentration (mg/dl)	168 ± 7	164 ± 8	166 ± 8
Blood glucose concentration in IPGTT (mg/dl)			
0 min	67 ± 7	83 ± 5	76 ± 3
30 min	368 ± 17	411 ± 21	438 ± 14*
120 min	116 ± 9	175 ± 14*	263 ± 18*†

Data are means ± SE. *Significantly different from SM/J ($P < 0.05$); †significantly different from A/J by Student's t test.

humans. It remains necessary to further isolate responsible genes to account for human diabetes.

Recombinant inbred (RI) strains are derived by inbreeding different sets of F_2 progeny from a cross between two inbred strains of mice. Each of the RI strains has a different combination of the parental genomes in a homozygous state (14,15). RI strains are a powerful tool for analyzing not only single genetic traits but also multifactorial genetic traits. Nishimura et al. (16) constructed a novel set of 26 SMXA RI mouse strains from SM/J and A/J strains. The parental strains (SM/J and A/J) possess differences in a variety of phenotypic traits (16,17). In particular, genetic analyses for pulmonary adenoma (18) and body weight (19) were performed using the SMXA RI strains, and these QTLs have already been mapped. The SMXA RI strains have not been used for genetic analyses of a number of diabetes-related traits, with the exception of analyses for serum insulin concentration (20). In this study, we used SMXA RI strains to determine the loci that control diabetes-related traits such as BMI, nonfasting blood glucose concentration, and blood glucose concentration during intraperitoneal glucose tolerance tests (IPGTTs).

RESEARCH DESIGN AND METHODS

Animals. Male mice of parental (SM/J, A/J) strains, (SM/J × A/J)F1 mice, and 19 SMXA RI strains were obtained from The Institute for Laboratory Animal Research, Nagoya University School of Medicine (Nagoya, Japan) and The Institute for Experimental Animals, Hamamatsu University School of Medicine (Hamamatsu, Japan). All mice were maintained in a room under conventional conditions at a controlled temperature of 23 ± 3°C and humidity of 55 ± 5% on a 12-h light/dark cycle. In this study, mice were fed a semipurified high-carbohydrate diet containing sucrose and starch. In our preliminary investigations, it was found that feeding mice this diet minimized the variation of glucose tolerance in mice of each strain. From 6 weeks of age, all mice were fed this high-carbohydrate diet ad libitum for 11 weeks. The composition (g/kg diet) of the high-carbohydrate diet was as follows: casein, 120; carbohydrate (starch/sucrose, 1:1), 743; AIN93MX mineral mixture (21), 35; AIN93VX vitamin mixture (21), 10; choline chloride, 2; corn oil, 50; cellulose powder (AVICEL type FD-101; Asahi Chemical Industry, Osaka, Japan), 40. All procedures were performed in accordance with the Animal Experimentation Guide of Nagoya University.

Phenotypic characterization. Body weight and anal-nasal length of the mice were measured at 5 and 10 weeks of feeding on this high-carbohydrate diet. BMI was calculated as body weight (g) divided by the square of the anal-nasal length (cm). Blood samples were obtained from the orbital sinus in nonfasting mice after 5 and 10 weeks of feeding on the high-carbohydrate diet. Serum samples were collected, centrifuged, and stored at −30°C until assay. Serum immunoreactive insulin concentrations were measured by radioimmunoassay (ShionoRIA; Shionogi, Osaka, Japan) with rat insulin as a standard. IPGTTs were performed at 5 and 10 weeks of feeding on the high-carbohydrate diet using the following protocol. After a 14-h fast, blood samples were collected from tail vein (fasting blood sample, 0-min sample in IPGTT). Then, a 20% glucose solution was injected intraperitoneally (2 g of glucose/kg body wt), and, subsequently, blood samples were collected at 30, 60, and 120 min after the injection. Blood glucose concentrations were measured by a glucose oxidase method with a Glucose-B Test Kit (WAKO, Osaka, Japan). Using the IPGTT, impairment of glucose tolerance was defined as a blood glucose concentration of >200 mg/dl at 120 min. Hyperglycemia was defined as a nonfasting blood glucose concentration of >200 mg/dl.

Linkage and statistical analyses. The number of mice from each strain was 7–16, referring to the report by Belknap (22). The phenotypic data collected on the SMXA RI strains (the average of each strain) were subjected to a QTL analysis using Map manager QT b28 (23). The program was based on interval mapping, using the free regression model. The strain distribution pattern of 789 polymorphic markers reported in SMXA RI strains (24) was used in the QTL analysis. The positions of the microsatellite (Mit) markers were collected from the Mouse Genome Database (25) in December 2001. Significance was determined by 1,000 permutations to provide likelihood ratio statistics that were suggestive, significant, or highly significant (26). Logarithm of odds (LOD) scores were obtained by dividing the likelihood ratio statistic by 4.605 (27). Significant linkage was defined in accordance with the guidelines of Lander and Kruglyak (28) as statistical evidence occurring by chance in the genome scan with $P \leq 0.05$.

RESULTS

Diabetes-related traits of parental strains and SMXA RI strains. Measurements of BMI, nonfasting blood glucose concentration, and blood glucose concentration during IPGTT were performed in parental (SM/J and A/J) strains, (SM × A)F1 hybrid, and 19 SMXA RI strains at 5 and 10 weeks of feeding mice the high-carbohydrate diet. The results in SM/J, A/J, and F1 mice at 5 weeks are shown in Table 1. Body weight and BMI of A/J or F1 mice were significantly higher than those of SM/J mice. There were no differences in nonfasting blood glucose concentrations among SM/J, A/J, and F1 mice, and these values were in the range of normoglycemia. The blood glucose concentration of A/J mice at 120 min during IPGTT was significantly higher than that of the SM/J mice. However, the glucose tolerance of the A/J mice was recognized as normal from this result. The blood glucose concentrations of F1 mice at 30 and 120 min during IPGTT were higher than those of SM/J or A/J mice. At 10 weeks of feeding mice the high-carbohydrate diet, the values of the parental strains were similar to those at 5 weeks.

Nonfasting blood glucose concentrations of the parental strains and 19 SMXA RI strains at 5 weeks of feeding are shown in Fig. 1*A*. The concentrations of the parental strains and F1 mice were clustered in the middle of the histogram. Four substrains (SMXA-5, -21, -26, and -30) of mice showed hyperglycemia. Blood glucose concentration at 120 min during IPGTT of the parental strains, F1 mice, and 19 SMXA RI strains is shown in Fig. 1*B*. The concentration observed in SM/J mice was near the lower end of the histogram, whereas that of the A/J mice was in the

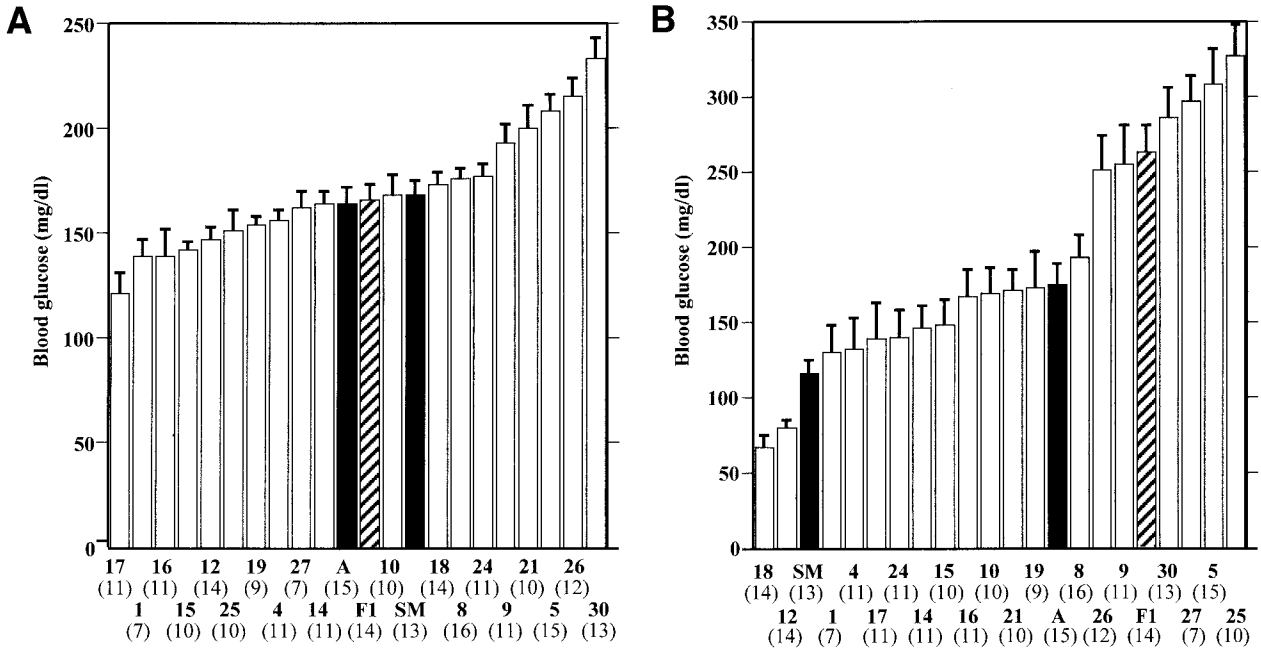

FIG. 1. The nonfasting blood glucose concentration (*A*) and blood glucose concentration at 120 min during IPGTT (*B*) in the SMXA RI strains, SM/J, A/J, and (SM × A)F1 mice at 5 weeks on the high-carbohydrate diet. Data are expressed as means ± SE. Number in parentheses.

middle of the histogram (Fig. 1*B*). Although neither SM/J nor A/J mice showed abnormalities in glucose tolerance, F1 mice and six SMXA RI substrains (SMXA-5, -9, -25, -26, -27, and -30) showed impaired glucose tolerance. It is interesting that nonfasting blood glucose concentration and blood glucose concentration at 120 min during IPGTT of SMXA RI strains showed wide distributions exceeding the values of the parental strains at 5 weeks of feeding. Such wide distributions in these two traits were also observed at 10 weeks of feeding (data not shown).

QTL analysis in the SMXA RI strains. Table 2 shows the LOD scores and 1-LOD support interval from the QTL analyses for BMI, nonfasting blood glucose concentration, fasting blood glucose concentration, and IPGTT at 30 and 120 min at 5 weeks of feeding. By using the data of IPGTT curve, we calculated the area under the curve in each strain. However, neither significant nor suggestive QTLs for the area under the curve were detected at 5 weeks. The genetic markers used in this study were randomly distributed throughout the genome of the RI strains with roughly one genetic marker every 4 cM (23). The following LOD scores were used for detecting suggestive/significant associations for the five traits at 5 weeks: BMI, 2.2/3.7; nonfasting blood glucose concentration, 2.2/3.8; fasting blood glucose concentration, 2.2/3.6; IPGTT at 30 min, 2.0/3.1; IPGTT at 120 min, 2.3/4.2. A significant QTL was found at the region near D10Mit136 on Chr 10, with the highest LOD score of 3.8 for nonfasting blood glucose concentration at 5 weeks of feeding. In addition, suggestive QTLs for BMI (D6Mit17), nonfasting blood glucose concentration (D6Mit287, D18Mit20), fasting blood glucose concentration (D18Mit17), and IPGTT at 30 min (D2Mit6) and 120 min (D10Mit136, D18Mit17) at 5 weeks were detected. As nonfasting blood glucose and blood glucose concentration at 120 min during IPGTT both are important diagnostic criteria for diabetes, the genotypic effect of QTLs on Chr 6, 10, and 18 in regard to these values are shown in Fig. 2.

On Chr 2, we mapped the locus concerning IPGTT at 30 min with a LOD score of 2.0 near D2Mit6. The mean value of IPGTT at 30 min among the SMXA RI strains (15 substrains) with the A/J allele at D2Mit6 was 352.8 ± 51.0 mg/dl, and the mean value of the other strains (4 sub-

TABLE 2
LOD scores of QTLs for BMI, nonfasting blood glucose concentration, and blood glucose concentration during IPGTT

	Chr 2	Chr 6		Chr 10	Chr 18	
	D2Mit6 12.5	D6Mit17 30.3	D6Mit287 49.0	D10Mit136 62.0	D18Mit20 5.0	D18Mit17 20.0
BMI		3.2 (6.0 cM)				
Nonfasting blood glucose			2.3 (25.2 cM)	3.8* (10.0 cM)	2.5 (8.0 cM)	
IPGTT						
Fasting blood glucose						2.4 (21.0 cM)
30 min	2.0 (8.5 cM)					
120 min				2.6 (10.0 cM)		2.9 (21.0 cM)

Highest LOD scores (exceeding suggestive threshold levels) calculated by Map manager QT are shown. *Significant linkage; other scores denote suggestive linkage. Chromosomal regions on the genetic map with values falling within one LOD score of the maximum value in parentheses.

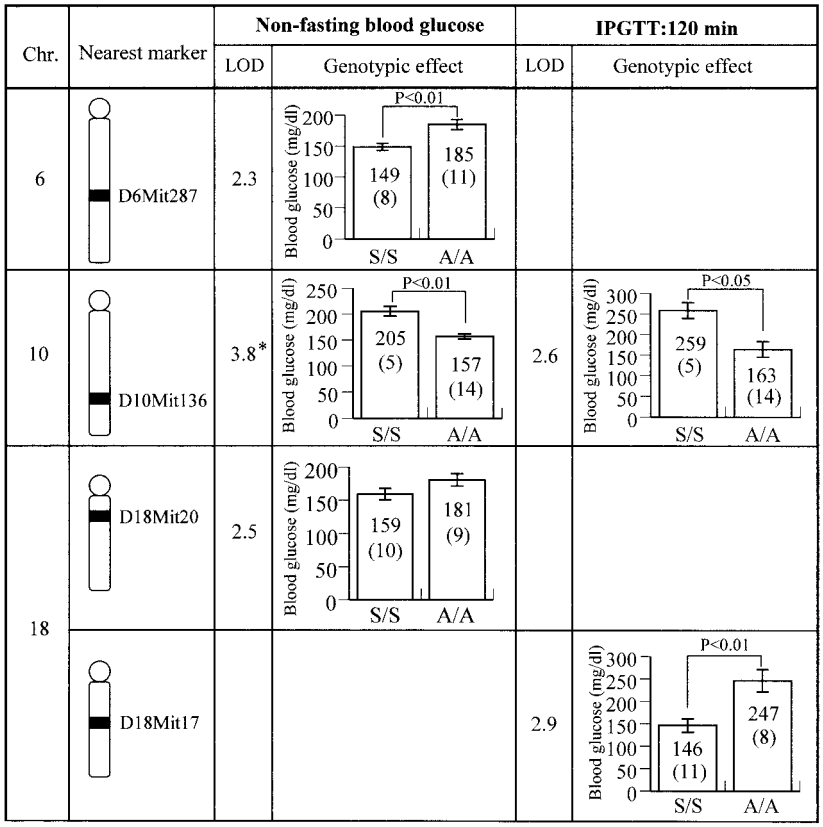

FIG. 2. Chromosomal locations of loci (D6Mit287, D10Mit136, D18Mit20, D18Mit17) identified in the SMXA RI strains, controlling the nonfasting blood glucose concentration and blood glucose concentration at 120 min during IPGTT. Idiograms of four chromosomes are shown together with the locations of markers linked to individual QTL. Histogram bars indicate the distributions of the SM/J (S) and A/J (A) alleles in the high- and low-blood glucose concentration RI strains. *Significant linkage; the other scores denote suggestive linkage. Number in parentheses. The differences of the means between the two groups were statistically analyzed by Student's *t* test. The statistical significance is shown as $P < 0.01$ or $P < 0.05$.

strains) with the SM/J allele was 301.7 ± 13.2 mg/dl. The A/J allele at this locus was associated with increased blood glucose concentration during IPGTT. On Chr 6, we mapped two loci that control BMI (LOD score = 3.2, near D6Mit17) and nonfasting blood glucose concentration (LOD = 2.3, near D6Mit287; Table 2).

As shown in Fig. 2, the mean value for nonfasting blood glucose concentration among the SMXA RI strains (11 substrains) with the A/J allele at D6Mit287 was higher than that of those strains with SM/J allele (8 substrains). The A/J allele at D6Mit287 contributed to an increase in nonfasting blood glucose concentration. A significant linkage (LOD score = 3.8) with nonfasting blood glucose concentration was observed near D10Mit136 on Chr 10 (Table 2). A suggestive linkage (LOD score = 2.6) with IPGTT at 120 min was detected near the same locus. The mean value of nonfasting blood glucose concentration among 5 substrains (SMXA-5, -8, -9, -26, and -30) with the SM/J allele at D10Mit136 was 205.0 ± 9.7 mg/dl, and the mean value of the others (10 substrains) with the A/J allele was 156.6 ± 5.2 mg/dl (Fig. 2). This genotypic effect at the same locus was also observed in the case of the value of IPGTT at 120 min. Thus, the SM/J allele at D10Mit136 on Chr 10 contributed to the elevation of nonfasting blood glucose concentration and to the impairment of glucose tolerance.

On Chr 18, two loci related to nonfasting blood glucose concentration (LOD score = 2.5, near D18Mit20) and fasting blood glucose concentration and IPGTT at 120 min (LOD score = 2.4 and 2.9, respectively, near D18Mit17) were found to be located on the centromeric portion of Chr 18 (Table 2). The A/J alleles at these loci, D18Mit20 and D18Mit17, were also associated with increased nonfasting blood glucose concentration and increased blood glucose concentration during IPGTT, respectively (Fig. 2).

At 10 weeks of feeding mice the high-carbohydrate diet, the results of QTL analyses for three traits (BMI, nonfasting blood glucose concentration, and blood glucose concentrations in IPGTT) were similar to those at 5 weeks (data not shown), but the significant QTL was not found. Compared with the results at 5 weeks, the mean value of nonfasting blood glucose among the SMXA RI strains (5 substrains) with the SM/J allele at D10Mit136 did not change at 10 weeks, whereas the mean value of the other strains (14 substrains) with the A/J allele increased at 10 weeks. However, QTL analyses at 10 weeks revealed a new locus near D2Mit11 (41.8 cM) regarding IPGTT results at 60 min and 120 min.

DISCUSSION

RI strains have proved to be powerful tools in studying the genetic basis of biological properties that differ between parental strains. Thus, parental strains, which show great differences in the mean values of their phenotypes, have been used in most genetic studies of complex traits in RI strains (29–31). In contrast, genetic analyses of traits associated with parental strains that show only small differences in the mean values are few in number (32). In the present study, SMXA RI strains were used to attempt a genetic dissection of BMI, nonfasting blood glucose concentration, and blood glucose concentration during IPGTT. Regarding these traits, the mean values of two parental strains, SM/J and A/J, were only slightly different, and these strains are nondiabetic. However, among all traits tested, we observed notable differences in the mean

TABLE 3
Summary of microsatellite-based chromosomal maps of susceptibility QTLs in the present study and previous studies

Chr.	Present study	Previous study			
	Locus (cM)	Locus (cM)	Traits	Crosses of mice	Ref.
2	D2Mit6 (12.5)	D2Mit6 (12.5)	Body weight	(SM × A)F2	(19)
	D2Mit11 (41.8)	*Nidd5* (32.0)	Body weight, insulin	(TSOD × BALB/cA)F2	(10)
		t2dm3 (42.7)	Insulin	(BTBR × C57BL/6J)F2	(35)
6	D6Mit17 (30.3)	D6Mit17 (30.3)	Body weight	SMXA RI	(20)
		D6Mit209/D6Mit178 (32.5/38.5)	Fat weight	(NSY × C3H/He)F2	(8)
	D6Mit287 (49.0)	D6Mit287 (49.0)	Body weight	(SM × A)F2	(19)
		Nidd3nsy (61.4)	Impaired glucose tolerance	(NSY × C3H/He)F2	(8)
10	D10Mit136 (62.0)	D10Mit70 (59.0)	Insulin	SMXA RI	(20)
18	D18Mit20 (5.0)	D18Mit15 (16.0)	Body weight	(SM × A)F2	(19)
	D18Mit17 (20.0)	*Nidd2* (16.0)	Nonfasting blood glucose	(NZO × NON)F2	(9)

values of the SMXA RI strains. The wide and continuous distribution patterns of all traits in the RI strains suggested that more than one locus influenced each phenotype in these strains. At 5 weeks of feeding with high-carbohydrate diet, six SMXA RI strains (SMXA-5, -9, -25, -26, -27, and -30) showed impaired glucose tolerance, suggesting that diabetogenic genes exist in the genomes of both parental strains and that some specific combinations of these genes lead to the development of hyperglycemia and diabetic phenotypes. As expected, several diabetogenic QTLs were identified from the genomes of both parental strains. Our analysis identified a minimum of six loci (on Chr 2, 6, 10, and 18) with a complex relationship to diabetes-related traits. A previous QTL mapping study using the SMXA RI strains (20) and the intercross offspring from parental strains (SM/J and A/J) (19) identified QTLs for body weight and serum insulin concentration. In the present study, QTLs on Chr 2, 6, 10, and 18 were detected for nonfasting blood glucose concentration and/or blood glucose concentration during IPGTT. The A/J alleles at Chr 2, 6, and 18 loci were associated with hyperglycemia and/or the impairment of glucose tolerance. In contrast, the SM/J allele at Chr 10 locus was associated with hyperglycemia and the impairment of glucose tolerance. Development of hyperglycemia in SMXA RI strains derived from the combination of normoglycemic parental strains suggests that specific combinations of these loci from SM/J and A/J genomes transfer some of the RI strains across a diabetogenic threshold.

It is interesting that an impaired glucose tolerance was observed in (SM × A)F1 mice but not in either parental strain. There are many instances in which the combinations of the separate genomes in F1 hybrids unmask latent diabetes susceptibility. Leiter et al. (9,33) clearly demonstrated such instance observed in outcross of NZO mice with NON mice. By 24 weeks of age, 60% of NZO male mice develop type 2 diabetes, and NON male mice do not at all. However, the incidence of type 2 diabetes of (NZO × NON) F1 mice was 97%, which is definitely higher than those of the parental strains. As another instance, it was reported by Attie et al. (34) that (C57BL/6J × BTBR)F1 mice showed severe insulin resistance, although C57BL/6J and BTBR mice have normal insulin responsiveness. It is important to analyze the phenotype of F1 hybrids for confirming the existence of latent diabetogenic genes in the parental strains. Thus, in this study, an interaction between silent SM/J and A/J alleles seems to elicit the impaired glucose tolerance in (SM × A)F1 mice but not changes in BMI and nonfasting blood glucose concentration. This result of IPGTT observed in (SM × A)F1 mice might support the possibility that the interaction of genes from the parental strains develops impaired glucose tolerance in SMXA RI substrains.

On Chr 2, we mapped two loci near D2Mit6 and D2Mit11 that were related to IPGTT at 30 min at 5 weeks and IPGTT at 60 min and 120 min at 10 weeks. Table 3 shows the previously mapped loci for diabetic traits around this region. The region containing D2Mit6 includes the gene *Rxra*, encoding retinoid X receptor α. The region containing D2Mit11 includes glycerol-3-phosphate dehydrogenase (*Gdm1*) and glucagon (*Gcg*) genes, which are involved in producing proteins necessary for glucose metabolism. In addition, two QTLs, *Nidd5* (10) and *t2dm3*, (35) are included in this region. The *Nidd5* locus (32.0 cM) that affects body weight and serum insulin concentration was mapped in crosses between BALB/cA and TSOD mice that are characterized as a diabetic model with obesity, hyperglycemia, hyperinsulinemia, and increased β-cell mass (36). The *t2dm3* locus (42.7 cM) concerning fasting plasma insulin concentration was mapped in crosses between BTBR-+/+ and C57BL/6J (B6)-ob/+ mice (35). Although we do not have data regarding serum insulin concentration during IPGTT, these two loci near D2Mit6 and D2Mit11, concerning the IPGTT at 30 min, 60 min, and 120 min, may play a role in the control of insulin secretion. At present, it remains unclear whether these QTLs possess the same diabetogenic gene.

On Chr 6, we mapped two loci concerning BMI and nonfasting blood glucose concentration near D6Mit287 and D6Mit17, respectively. The region containing D6Mit287 includes two genes, namely, a fatty acid binding protein in the liver (*Fabp1*), and hexokinase 2 (*Hk2*), as well as a QTL, *Nidd3nsy* (8). The *Nidd3nsy* locus (61.4 cM) that affects glucose tolerance was mapped in crosses between C3H/He and NSY mice characterized by type 2 diabetes with mild obesity (Table 3) (37,38). The region containing D6Mit17 includes the genes peroxisome proliferator–activated receptor γ (*Pparg*) and glyceraldehyde-3-phosphate dehydrogenase (*Gapd*); in addition, the region containing D6Mit17 is near two previously mapped QTLs. One of these QTLs is the locus concerning body weight in the SMXA RI strains (19) and in crosses between SM/J and A/J mice (20). The other locus concerns epididymal fat weight and serum insulin concentration, shown in a study

using NSY mice (8) (Table 3). In these studies using different mouse strains, QTLs that affect fat weight or serum insulin concentration have been reported in the limited region of Chr 6. We speculate that genes concerning type 2 diabetes with adiposity exist on Chr 6.

Although SM/J mice showed lower blood glucose concentrations at all time points (fasting blood glucose, 30 and 120 min) during IPGTT than did the A/J mice, the present study revealed a SM/J-derived diabetogenic locus that was related to nonfasting blood glucose concentration and blood glucose concentration during IPGTT; this locus was near D10Mit136 on Chr 10. In particular, significant linkage was observed for nonfasting blood glucose concentration at 5 weeks of feeding. This locus was designated as *t2dm1sa* and is relevant for *NIDDM1* in the SMXA RI strains. Anunciado et al. (20) also reported that an SM/J allele at this region (59.0 cM) used SMXA RI strains to reveal a suggestive linkage to the increase in nonfasting serum insulin concentration (Table 3). The possibility exists that this region contains the SM/J-derived gene that controls diabetic traits. As diabetogenic QTLs have not been reported elsewhere on this chromosomal region around *t2dm1sa*, we had the task of identifying this SM-derived diabetogenic gene on Chr 10. The region includes the gene *Kcnc2*, encoding a potassium voltage-gated channel, shaw-related subfamily 2. Activation of the K^+ channel can suppress insulin secretion coupling by damping oscillations in membrane potential and $[Ca2^+]_i$. The K^+ channel regulates membrane repolarization, $[Ca2^+]_i$, and insulin secretion (39). We have been unable to identify additional candidate genes that affect glucose metabolism in this region of mouse Chr 10 as well as in the syntenic regions of human Chr 6, 12, and 19 (40).

On Chr 18, we mapped two loci that were related to nonfasting blood glucose concentration, fasting blood glucose concentration, and IPGTT at 120 min; these loci were near D18Mit20 and D18Mit17. The region containing D18Mit20 and D18Mit17 includes early growth response 1 (*Egr1*) and the glucocorticoid receptor 1 (*Grl1*) genes, as well as a QTL, *Nidd2* (9). However, no information concerning the sequence and expression of these genes in SM/J and A/J mice was obtained. The diabetogenic locus *Nidd2* (16.0 cM) controlling nonfasting blood glucose concentration in crosses between NON and NZO mice has already been mapped near D18Mit17 (Table 3). In separate studies using different mouse strains, the QTLs that control the same trait, nonfasting blood glucose concentration, were mapped near D18Mit20 and D18Mit17 (9; current study). This suggests that these two loci included an identical gene and a ubiquitous locus controlling the development of hyperglycemia on Chr 18. Additional studies are needed to determine the identity of these loci mapped in the respective studies. In addition to *Nidd2*, several QTLs for obesity and diabetes were identified in crosses between NON and NZO mice (9). More recently, it was demonstrated that the different combinations of these QTLs, including *Nidd2*, differentially expressed obesity and diabetes in interval-directed recombinant congenic strains (33).

In conclusion, this study demonstrates that QTLs derived from nondiabetic genomes contribute to gene-gene interaction, leading to the development of hyperglycemia and diabetic phenotypes. Genetic analysis of such gene-gene interaction may contribute to elucidate the complex mode of inheritance in human type 2 diabetes. The chromosomal regions where diabetes-related QTLs have been mapped in this study are wide. Using the standard method for dissecting mapped loci that control polygenic traits, we are establishing four congenic strains (SM.A-D2Mit6-D2Mit28, SM.A-D6Mit312-D6Mit367, A.SM-*t2dm1sa*, and SM.A-D18Mit20-D18Mit139). Additional work using these congenic and parental (SM/J and A/J) strains will elucidate the function of each responsible locus.

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