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Genetic analysis for diabetes in a new rat model of nonobese type 2 diabetes, Spontaneously Diabetic Torii rat

Taku Masuyama,[a,b,*,1] Masanori Fuse,[c,d] Norihide Yokoi,[e] Masami Shinohara,[b] Hirotada Tsujii,[d] Masao Kanazawa,[f] Yasunori Kanazawa,[g] Kajuro Komeda,[c,2] and Kazuyuki Taniguchi[a,2]

[a] *Laboratory of Veterinary Anatomy, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan*
[b] *Research Laboratories, Torii Pharmaceutical Co. Ltd., 3-4-1 Nihonbashi-honcho, Chuo-ku, Tokyo 103-8439, Japan*
[c] *Division of Laboratory Animal Science, Animal Research Center, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan*
[d] *Department of Developmental Biotechnology in Mammals, Faculty of Agriculture, Shinshu University, 8304 Minamiminowa-Mura, Nagano 399-4598, Japan*
[e] *Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan*
[f] *Third Department of Internal Medicine, Tokyo Medical University, 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan*
[g] *Jichi Medical School, 3311-1 Yakushiji, Minami-kawachi, Tochigi 329-0498, Japan*

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Abstract

The Spontaneously Diabetic Torii (SDT) rat has recently been established as a new rat model of nonobese type 2 diabetes. In this study, we characterized diabetic features in SDT rats, and performed quantitative trait locus (QTL) analysis for glucose intolerance using 319 male (BN × SDT) × SDT backcrosses. Male SDT rats exhibited glucose intolerance at 20 weeks, and spontaneously developed diabetes with the incidence of 100% at 38 weeks, and glucose intolerance is well associated with the development of diabetes. The QTL analysis identified three highly significant QTLs (*Gisdt1*, *Gisdt2*, and *Gisdt3*) for glucose intolerance on rat chromosomes 1, 2, and X, respectively. The SDT allele for these QTLs significantly exacerbated glucose intolerance. Furthermore, synergistic interactions among these QTLs were detected. These findings indicate that diabetic features in SDT rats are inherited as polygenic traits and that SDT rats would provide insights into genetics of human type 2 diabetes.

Keywords: Animal model; Comparative gene map; Diabetes; Gisdt1; Gisdt2; Gisdt3; Glucose tolerance; Nonobese; SDT rat; QTL

Type 2 diabetes mellitus is a multiple complex disease with chronic hyperglycemia caused by the disruption of the balance between pancreatic islet function and peripheral insulin sensitivity, and controlled for its development by the interactions of multiple genetic and environmental factors [1,2]. Its genetic basis is not well-understood yet, because detailed investigations such as the genetic dissection have been restricted in humans due to practical and ethical reasons. The rat–mouse–human comparative genetic information [3–6] has revealed that the genetic dissection of an appropriate animal model is a useful approach to understand the pathogenesis of human diseases [7]. Thus, animal models of type 2 diabetes mellitus would provide important insights into the human disease [8]. A large number of the models of diabetes with obesity have been known such as Zucker–Diabetic–Fatty (ZDF) rat [9], Otsuka–Long–Evans–Tokushima–Fatty (OLETF) rat [10], KK – A^y mouse [11], $Lepr^{db}/Lepr^{db}$ mouse [12], NSY mouse [13], Castaneus-B6 diabetic (CBD) mouse [14], Tsumura–Suzuki obese diabetic (TSOD) mouse [15], New Zealand obese (NZO) mouse [16], and TallyHo (TH) mouse [17]. However, the animal model of type 2

* Corresponding author. Fax: +81-463-82-3444.
E-mail address: t.masuyama@tba.t-com.ne.jp (T. Masuyama).
[1] Present address: Toxicology Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., 23 Naganuki, Hadano-shi, Kanagawa 257-0024, Japan.
[2] These two authors are equally responsible to this work.

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diabetes without obesity is hardly reported except for Goto–Kakizaki (GK) rat [18]. The GK rat is widely used as a good animal model to understand pathogenesis of nonobese type 2 diabetes [18]. Although this disease is thought to involve predominantly impairment of insulin secretion by pancreatic β cells [18,19], pathology of nonobese type 2 diabetes remains controversial which defect is primarily important in either GK rat or human because it cannot be explained by single etiology. It is important to establish and investigate new animal models having features of the disease.

We have recently established a new inbred rat strain with spontaneous diabetes without obesity from an outbred colony of Sprague–Dawley rats and named it Spontaneously Diabetic Torii (SDT) rat [20]. SDT rats spontaneously develop hyperglycemia and glucosuria after 20 weeks of age with the incidence of 100% until 40 weeks of age in males. Since they exhibit no sign of obesity throughout their lives and are affected with histological changes (hemorrhage and fibrosis, but no evidence of autoimmunity) in pancreatic islets prior to the onset of diabetes, SDT rats would serve as a new model of nonobese type 2 diabetes mellitus which has a predominant insulin defect rather than peripheral insulin resistance [20]. Moreover, SDT rats exhibit severe ocular complication such as proliferative retinal degeneration and detachment of retina resembling human diabetic retinopathy [20]. Therefore, genetic characterization of diabetes in SDT rats is important for the understanding of pathogenesis of human nonobese diabetes and its complication. In the present study, we performed QTL analysis for glucose intolerance in the SDT rat, and demonstrate that the SDT rat is a good model of human nonobese diabetes and that diabetes in the SDT rat is polygenetically controlled by at least three genes.

Materials and methods

Animals. SDT rats were maintained at the Research Laboratories of Torii Pharmaceutical Co. Ltd., Brown Norway (BN/Sea) rats were purchased from SEAC Yoshitomi (Fukuoka, Japan) and were used as a non-diabetic control strain for crossing. For the genetic analysis, the BN and the SDT rats were crossed to obtain the F1 progeny [SDT × BN] F1 and [BN × SDT] F1, and [BN × SDT] F1 rats were further crossed with SDT rats to obtain N2 progeny [(BN × SDT)F1 × SDT]. All of the animals were maintained at 23 ± 2 °C and 55 ± 10% relative humidity with a 12-h light-dark cycle, and were provided with water and commercially available pellets, CE-2 (CREA Japan, Tokyo, Japan) ad libitum.

Phenotypic analysis. Glucose tolerance was estimated by the oral glucose tolerance test (OGTT) using a glucose dose of 2 g/kg body weight in male rats at 20 weeks of age following 18 h of fasting, and the blood glucose concentration was measured with a portable glucose meter (ANTSENSE II, Bayer Medical, Tokyo, Japan) immediately before and 60 and 120 min after the administration. The area under the curve of blood glucose concentration (glucose AUC) obtained from results of OGTT was calculated on the basis of blood glucose levels at 0, 60, and 120 min after the glucose administration. The criteria used to diagnose diabetes were the presence of glucosuria (indicated as "positive" determined by the use of Multi-Sticks, Bayer-Sankyo, Tokyo) and a high blood glucose concentration (exceeding 300 mg/dl under nonfasting condition). Body mass index (BMI) was determined by dividing the body weight with the square of the body length (from anus to nose) at 25 weeks of age.

Genetic analysis. The genomic DNA extraction, polymerase chain reaction (PCR), and electrophoresis techniques were performed as described previously [21]. Simple sequence length polymorphism (SSLP) markers (a total of 135), which were described elsewhere [22,23] or purchased from Research Genetics (Huntsville, AL, US), with a polymorphism between the SDT and the BN rats were used for the first whole genome scanning (Table 1). The first whole genome scan was performed by the use of 26 N2 rats having marked glucose intolerance and 30 N2 rats having normal glucose tolerance by the detection of the significant difference between the observed and the expected segregation ratios at each marker according to the approach of selective genotyping [24]. Linkage of markers to glucose AUC was evaluated by a χ^2 test in 2 × 2 table using Stat View software (SAS Institute, NC, US). A criterion of $P < 0.01$ was used as an initial significance level of suggestive linkage. For chromosomes (Chrs. 1, 2, 6, 7, 8, 11, 14, 18, and X) with suggestive linkage to glucose intolerance in the first screening, QTL analysis was performed on the entire population of N2 progeny ($n = 319$) by the use of the computer program Map Manager QTX [25] (http://mapmgr.roswellpark.org/mmQTX.html). The empirical suggestive ($P < 0.63$), significant ($P < 0.05$), and highly significant ($P < 0.001$) threshold levels for detection of QTL were determined by permutation tests in 1-cM steps for 5000 permutations [26,27] using the Map Manager QTX, and evaluated by the likelihood ratio statistics (LRS) scores. The LRS score can be approximately converted to the conventional base − 10 LOD score by dividing it with 4.61 (twice the natural logarithm of 10). To construct a rat–mouse–human comparative map for QTL regions, the gene-based or -flanking SSLP markers (a total of 22) were further added, and mapping information was obtained from available web sites as follows; Welcome Trust Center (http://www.well.ox.ac.uk/rat_mapping_resources/), the OLETF Project (http://ratmap.ims.u-tokyo.ac.jp), the Mouse Genome Database (http://www.informatics.jax.org), and Online Mendelian Inheritance in Man (OMIM) Gene Map (http://www3.ncbi.nlm.nih.gov/Omim/searchmap.html).

Results

Characteristics of SDT rats

To characterize the diabetic traits of SDT rats for genetic study, we first investigated the cumulative incidence of diabetes in male SDT rats. The SDT rats spontaneously developed diabetes after 22 weeks of age, and showed the cumulative incidence of 100% until 38 weeks of age (Fig. 1A) in consistence with previous observations [20]. BN rats never showed any signs of diabetes at least until 50 weeks of age. Next, we performed the OGTT to examine glucose tolerance of the SDT rats at 20 weeks of age before the onset of diabetes. SDT rats showed hyperglycemia after glucose load, while the age-matched BN rats did not (Fig. 1B). The glucose AUC was significantly higher in SDT rats (mean ± SE: 633.3 ± 26.4 mg h/dl) than that in BN rats (294.1 ± 8.5 mg h/dl), suggesting that the glucose AUC is a useful index of glucose intolerance in SDT rats

Table 1
SSLP markers used in this study

Chr.	No. of markers	Marker name
1	13 (**7**)	D1Mgh16, D1Mit9, D1Mit10, D1Rat35(Igf1r), D1Rat32, D1Rat38, D1Rat42(Plin), D1Mit3, D1Mit4, D1Mgh21, D1Rat76, D1Mgh13, D1Mgh14, **D1Wox11(Prkcg)**, **D1Wox80(Nphn)**, **D1Wox78(Grm5)**, **D1Wox52(Cgn2)**, **D1Wox35(Pth)**, **D1Wox49(Sa)**, **D1Arb24(Igf1)**
2	10 (**9**)	D2Rat11, D2Mgh14, D2Mit4, D2Mit6, D2Mit11, D2Mit14, D2Rat118(Col11a), D2Mit15, D2Mgh13(Uri), D2Rat297, **D2Wox15(Cpb1)**, **D2Wox17 (Fgg)**, **D2Wox19 (Pklr)**, **D2Wox52(S100a4)**, **D2Wox25(Hsd3b1)**, **D2Wox37(Ampd1)**, **D2Wox64(Pd5ea)**, **D2Got147(Fabp2)**, **D2Rat156**
3	8	D3Mgh16, D3Mgh8, D3Mgh6, D3Mgh13, D3Mit4, D3Mgh2, D3Mgh1, D3Mgh3
4	8	D4Mgh1, D4Mgh14, D4Mgh15, D4Mgh16, D4Mgh4, D4Rat128, D4Rat34, D4Mit17
5	8	D5Wox8, D5Mgh1, D5Mgh4, D5Mit10, D5Mgh6, D5Mit11, D5Mit13, D5Mgh9
6	8	D6Wox10, D6Mgh8, D6Mit4, D6Mit2, D6Mit8, D6Mgh4, D6Mgh9, D6Mgh1
7	6	D7Mgh11, D7Mgh15, D7Rat31, D7Mit5, D7Mit16, D7Mit1
8	6	D8Rat58, D8Mit5, D8Mgh9, D8Mgh7, D8Mgh4, D8Mgh11
9	4	D9Rat45, D9Mgh3, D9Ra11, D9Rat1
10	7	D10Mit6, D10Mgh11, D10Mit8, D10Mgh6, D10Mgh5, D10Mit1, D10Mgh4
11	9	D11Mgh6, D11Rat17, D11Rat71, D11Yok1*, D11Mgh4, D11Rat4, D11Rat2, D11Mgh3, D11Mgh2
12	3	D12Arb12, D12Mit6, D12Wox10
13	5	D13Mgh1, D13Arb7, D13Mit2, D13Mit5, D13Mit4
14	4	D14Mit1, D14Wox5, D14Mit4, D14Mit10
15	5	D15Mit2, D15Mgh7, D15Mgh4, D15Mgh2, D15Mgh6
16	5	D16Mit2, D16Mgh4, D16Mit1, D16Wox7, D16Wox3
17	4	D17Mit7, D17Mit2, D17Mit4, D17Mit5
18	7	D18Mit1, D18Mgh1, D18Mit8, D18Rat11, D18Rat82, D18Rat5, D18Rat1
19	4	D19Mgh4, D19Mit3, D19Mit5, D19Mit7
20	5	D20Rat71, D20Rat48, D20Rat76, D20Rat56, D20Rat29
X	6 (**6**)	DXRat2, DXRat83, DXRat42, DXRat10, DXMit5, DXMgh7, **DXWox11(Rgn)**, **DXWox20(Xk)**, **DXRat7(Pfkfb)**, **DXWox9(Ar)**, **DXWox26(Plp)**, **DXWox29(Ocrl)**
Total	135 (**22**)	

Bold markers were added when the QTL mapping did. **D11Yok1* described by Yokoi et al. [23]. The Gene-defined or -flanking marker is showed the gene symbol in parenthesis.

(Fig. 1C). Moreover, we found a statistically highly significant negative correlation ($r = -0.894$, $P < 0.001$) between the glucose AUC at 20 weeks of age and the onset age of diabetes (Fig. 1D), suggesting that glucose intolerance is directly associated with the development of diabetes in SDT rats. Accordingly, we adopted the glucose AUC as a target phenotype in genetic analysis to detect genes responsible for diabetes in SDT rats. Since SDT rats had heavier body weight (Fig. 1E) and larger body length (Fig. 1F) than BN rats, they showed BMI similar to BN rats (Fig. 1G), implying that SDT rats are larger in body size than BN rats, but not obese.

Phenotypes in F1 and N2 progenies

To investigate the genetic control of diabetes and glucose intolerance in SDT rats, we arranged the mating between the SDT and BN rats. Two types of F1 progeny were obtained by reciprocal crosses: (SDT female × BN male) and (BN female × SDT male). All of the males of the F1 [(SDT × BN) and (BN × SDT)] rats had normal glucose tolerance with postprandial blood glucose levels (glucose 60 and 120 min) and glucose AUC similar to those of BN rats (Fig. 2). Moreover, F1 rats did not develop diabetes until 50 weeks of age. We then constructed the backcross panel [(BN × SDT) F1 female × SDT male] N2. The N2 progeny showed the distribution pattern of individual glucose AUC widely ranged from normal to abnormal levels (minimum: 202 mg h/dl, maximum: 719 mg h/dl). Postprandial glycemia (at glucose 60 and 120 min) showed a similar distribution pattern to that of glucose AUC. The onset of diabetes was observed in only 6 (1.9%) out of 319 N2 rats until 25 weeks of age. These results show that diabetes and glucose intolerance are inherited as polygenic traits in this cross. On the other hand, the distribution pattern of body weight was different from those of blood glucose levels and glucose AUC. Most of the F1 and N2 rats had body weights similar to SDT rats, indicating that the body weight is inherited in a dominant manner and is independent from genes responsible for glucose tolerance.

Selective genotyping

To scan genomic regions responsible for diabetes and glucose intolerance in the cross, we performed the first whole-genome scanning with selected 56 N2 rats including 26 rats with marked glucose intolerance (glucose AUC > 560 mg h/dl) and 30 rats with normal glucose tolerance (glucose AUC < 220 mg h/dl) using a set of 135 SSLP markers (Table 1). A χ^2 test was applied to examine linkage between glucose AUC and SSLP markers and a criterion of $P < 0.01$ was used as an initial significant level of suggestive linkage. As a result,

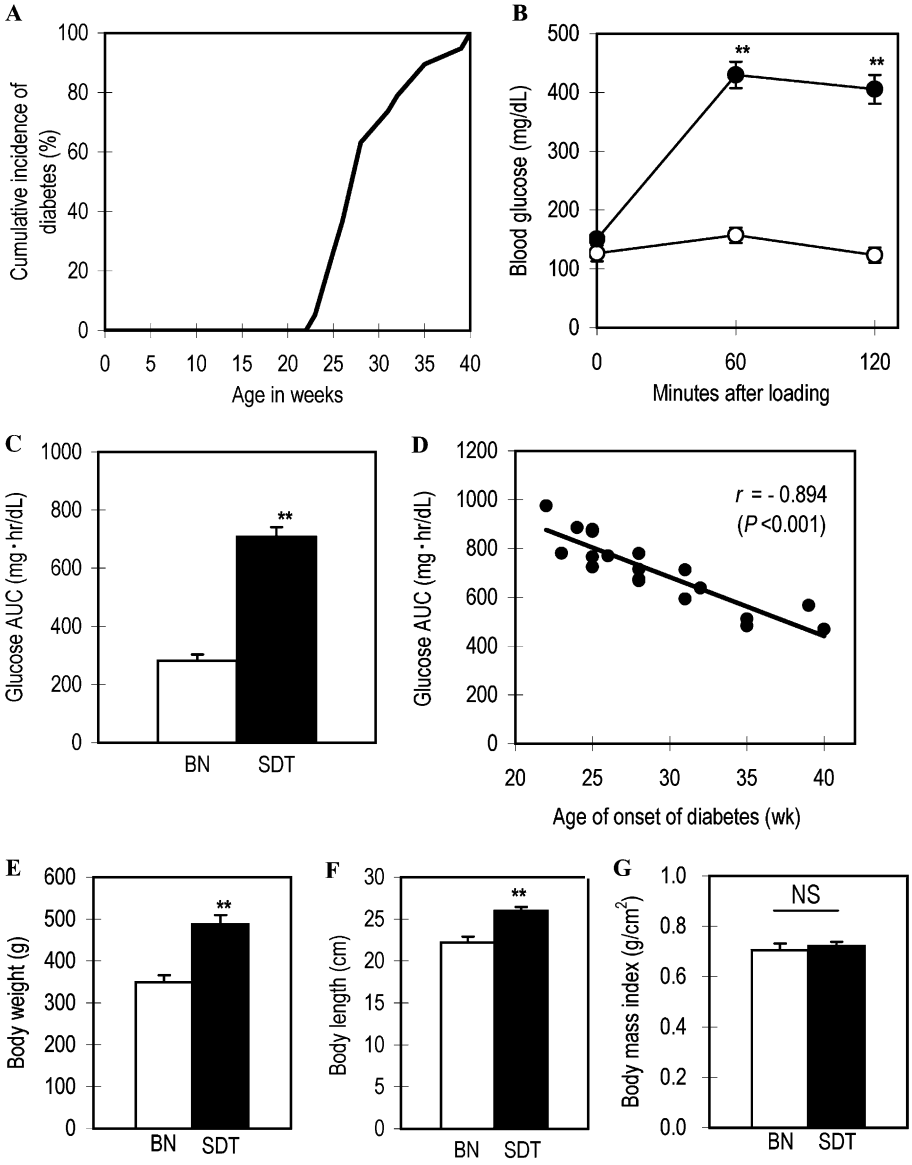

Fig. 1. Diabetic features of male SDT rats. (A) Cumulative incidence of diabetes in male SDT rats used in this study. (B) Blood glucose levels during OGTT with a glucose dose of 2 g/kg body weight in males of SDT and BN rats at 20 weeks of age. (C) Glucose AUC values obtained by the OGTT in males of SDT and BN rats at 20 weeks of age. (D) Correlation analysis between glucose AUC obtained by the OGTT at 20 weeks of age and age of diabetes onset in male SDT rat. (E) Body weights in SDT and BN rats at 20 weeks of age. (F) Body length (from anus to nasal) in SDT and BN rats at 20 weeks of age. (G) Body mass index (body weight (g)/(body length (cm))^2) in SDT and BN rats at 20 weeks of age. All data are presented as means ± SE. Asterisks indicate the significant difference by Student's t test (*, $P < 0.05$; **, $P < 0.01$). NS, not significant. Pearson's product-moment correlation coefficient (r) was used to individually clarify the relationship between glucose AUC and the age of onset of diabetes in the SDT rat.

suggestive linkages were found at SSLP markers on chromosomes 1, 2, 6, 7, 8, 11, 18, and X with prevalence of SDT alleles, and on chromosome 14 with prevalence of BN alleles (Table 2). Accordingly, these chromosomes were further analyzed to detect QTLs for glucose AUC using the entire N2 rats.

QTL mapping

We carried out an interval mapping by the use of the computer program Map Manager QTX, and determined a threshold LRS score by a permutation test on each chromosome where the existence of a putative locus for glucose AUC was suggested by the first screening. In addition to the first screening, gene-based SSLP markers (mainly *Wox* markers) were added to allow direct comparison of the orthologous regions in the mouse and human genomes. We found statistically highly significant evidence for three QTLs ($P < 0.001$) affecting glucose AUC (Fig. 3) and designated these loci as *Gisdt1* (Glucose intolerance in SDT rat 1), *Gisdt2*, and *Gisdt3* on chromosomes 1, 2, and X, respectively.

On chromosome 1 in which a highly significant threshold level was an LRS score of 14.6 for glucose

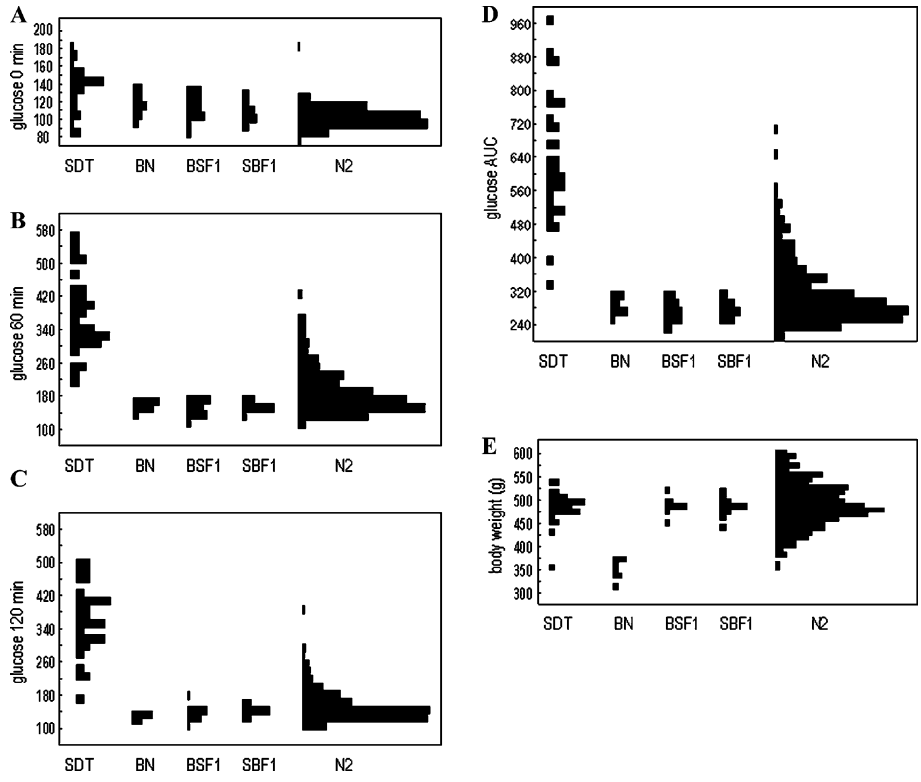

Fig. 2. Distribution patterns of blood glucose concentrations on OGTT and body weights in the parental SDT and BN rats, each F1 progeny of [SDT × BN]F1 (SBF1), [BN × SDT]F1 (BSF1), and [(BN × SDT)F1 × SDT]N2 (N2) progeny at 20 weeks of age. (A) Glucose 0 min; (B) glucose 60 min; (C) glucose 120 min; (D) Glucose AUC; and (E) body weight.

AUC, a maximum LRS of 27.8 was observed at *D1Mit3* (Fig. 3A). We designated the QTL as *Gisdt1*. The 4.61-LRS interval (as equivalent to 1-LOD interval) spanned at 10 cM around the marker, *D1Mit3* (Fig. 3A). This QTL was strongly linked also to glucose 60 min. The LRS peak for glucose 60 min (LRS = 35.6) markedly exceeded a highly significant threshold of an LRS of 16.5 for this trait, and was consistent with the position of QTL (*Gisdt1*) for glucose AUC. This locus accounted for 8%, 10%, and 4% of the phenotypic variances of glucose AUC, 60 and 120 min, respectively, in the N2 progeny (Table 3). Interestingly, we found a highly significant linkage for body weight at *Gisdt1* region on chromosome 1 (Fig. 3A), despite the fact that the body weights were likely controlled by dominant manner. The highly significant LRS for body weight (a peak LRS of 30.7) was found at the position almost the same as that for glucose tolerance, while the LRS curve showed a broad pattern supporting the interval of 19 cM around the *D1Mit3* for the 4.61-LRS (Fig. 3A). This suggests that *Gisdt1* may be one of the genetic loci regulating body weight by recessive manner.

A strongest QTL for glucose AUC, designated as *Gisdt2*, was found on chromosome 2 (Fig. 3B). The QTL peak reached to a highly significant level (LRS = 36.3) at *D2Got147* exceeding a highly significant threshold of an LRS of 14.0 for glucose AUC on this chromosome. This QTL (*Gisdt2*) supported the interval spanning at 7 cM around the *D2Got147* for the 4.61-LRS (Fig. 3B). For glucose 60 min, the highest LRS peak of 40.5 exceeding a highly significant threshold (LRS = 15.2) was also found at *D2Got147* in consistence with QTL for glucose AUC. This locus accounted for 10%, 12%, and 5% of the phenotypic variances of glucose AUC, 60 and 120 min, respectively, in the N2 progeny (Table 3).

A third QTL for glucose AUC, designated as *Gisdt3*, was found between *DXWox20*and *DXRat83* on chromosome X with a highly significant LRS score of 24.7 exceeding a highly significant threshold LRS score of 13.7 on this chromosome (Fig. 3C). The QTL peak was consistent with that for the glucose 60 min (LRS = 26.9). This locus accounted for 7%, 8%, and 4% of overall variances of glucose AUC, 60 and 120 min, respectively, in the N2 progeny.

In addition to the three regions as described above, a total of five regions showed the LRS score exceeding significant threshold level ($P = 0.01$) for at least one of glucose AUC, glucose 60 and 120 min. However, we are reluctant to declare linkage because they may represent either random fluctuations or actual QTLs with small effects. Further analysis using other crossing partners or other phenotypes related to glucose tolerance may be required to suppose them as real QTLs.

Table 2
SSLP markers suggestive of linkage for glucose intolerance by χ^2 test using 56 backcrosses either high or low glucose AUC

Chr.	Locus name	High glucose AUC[a]		Low glucose AUC[b]		χ^2	P value
		SDT/SDT	SDT/BN	SDT/SDT	SDT/BN		
1	D1Rat38	21	5	11	19	11.06	0.0012
	D1Rat42	22	4	13	17	10.13	0.0021
	D1Mit3	22	4	14	16	10.13	0.0023
2	D2Mit11	19	7	10	20	8.81	0.0030
	D2Mit14	20	6	11	19	9.13	0.0025
	D2Rat118	21	5	10	20	12.68	0.00040
	D2Mit15	23	3	12	18	13.96	0.00020
6	D6Mgh1	19	7	10	20	8.81	0.0030
7	D7Mit16	18	8	10	20	7.18	0.0074
8	D8Mgh9	19	7	10	20	8.81	0.0030
	D8Mgh7	21	6	13	17	7.01	0.0081
	D8Mgh4	18	8	9	21	8.59	0.0034
11	D11Yok1	19	7	11	19	7.42	0.0064
	D11Mgh4	20	6	11	19	9.13	0.0025
14	D14Wox5	8	18	20	10	7.18	0.0074
18	D18Rat11	19	7	10	20	8.81	0.0030
	D18Rat82	19	7	11	19	7.42	0.0064
		SDT	BN	SDT	BN		
X	DXRat2	21	5	13	17	8.18	0.0061
	DXRat83	22	4	13	17	10.13	0.0021

First whole-genome scan was performed using 135 SSLP markers as described in Table 1. Linkage of markers to glucose intolerance was evaluated by χ^2 tests in 2×2 tables. Only locus of $P < 0.01$ is shown in this table.

[a] N2 rats having high glucose AUC (>560 mg h/dl) on OGTT.

[b] N2 rats having low glucose AUC (<220 mg h/dl) on OGTT.

Characterization of three QTLs

To investigate the effects of each of the three QTLs on glucose intolerance, N2 rats were allotted by genotypes of their closest SSLP markers, *D1Mit3* for *Gisdt1, D2Got147* for *Gisdt2*, and *DXWox20* for *Gisdt3*. In the N2 progeny, rats having homozygous SDT/SDT genotype at *Gisdt1* showed significantly increased values in glucose AUC, glucose 60 and 120 min, and body weight when compared with rats having heterozygous SDT/BN genotype (Table 3). *Gisdt2* and *Gisdt3* were also shown to have significant effects on postprandial hyperglycemia to exacerbate glucose intolerance when homozygous for SDT allele at *Gisdt2* or having the SDT allele at *Gisdt3* (Table 3). We next examined interaction between these QTLs (Figs. 4A–C). All combination among three QTLs showed synergistic interaction to increase glucose AUC. It was found that the effects of *Gisdt3* on glucose AUC disappear when *Gisdt1* is heterozygous, suggesting that *Gisdt3* is dependent on *Gisdt1*. We next calculated the cumulative effects of three *Gisdt* loci on glucose tolerance in the N2 progeny (Fig. 4B). Furthermore, the accumulation of SDT alleles in three QTLs significantly increased the glucose AUC as compared with that of BN alleles (Figs. 4D and E), indicating that significant interaction (epistasis) is exerted by combination of SDT alleles at three QTLs. This result suggests that glucose intolerance is exacerbated by the synergistic effect of the *Gisdt* loci.

Discussion

In humans, the glucose tolerance impairs prior to maturity-onset of hyperglycemia [28–30] and is widely used as a clinical index to predict the potentiality of developing diabetes [29]. We first found the evidence that glucose intolerance was directly associated with the age of the onset of diabetes in SDT rats (Fig. 1D). This is an important evidence to show that the SDT rat strain is an appropriate animal model of human type 2 diabetes mellitus. In addition, we previously reported that SDT rats spontaneously develop diabetes without obesity and with hypoinsulinemia due to the pathological lesions of pancreatic islets such as fibrosis [20]. Also in this study, SDT rats were confirmed to be nonobese, although they were larger in body size than BN rats of crossing partner. Obesity or increased body mass produces the increase of peripheral insulin requirement and induces insulin resistance and hyperinsulinemia [29]. Thus, it is likely that

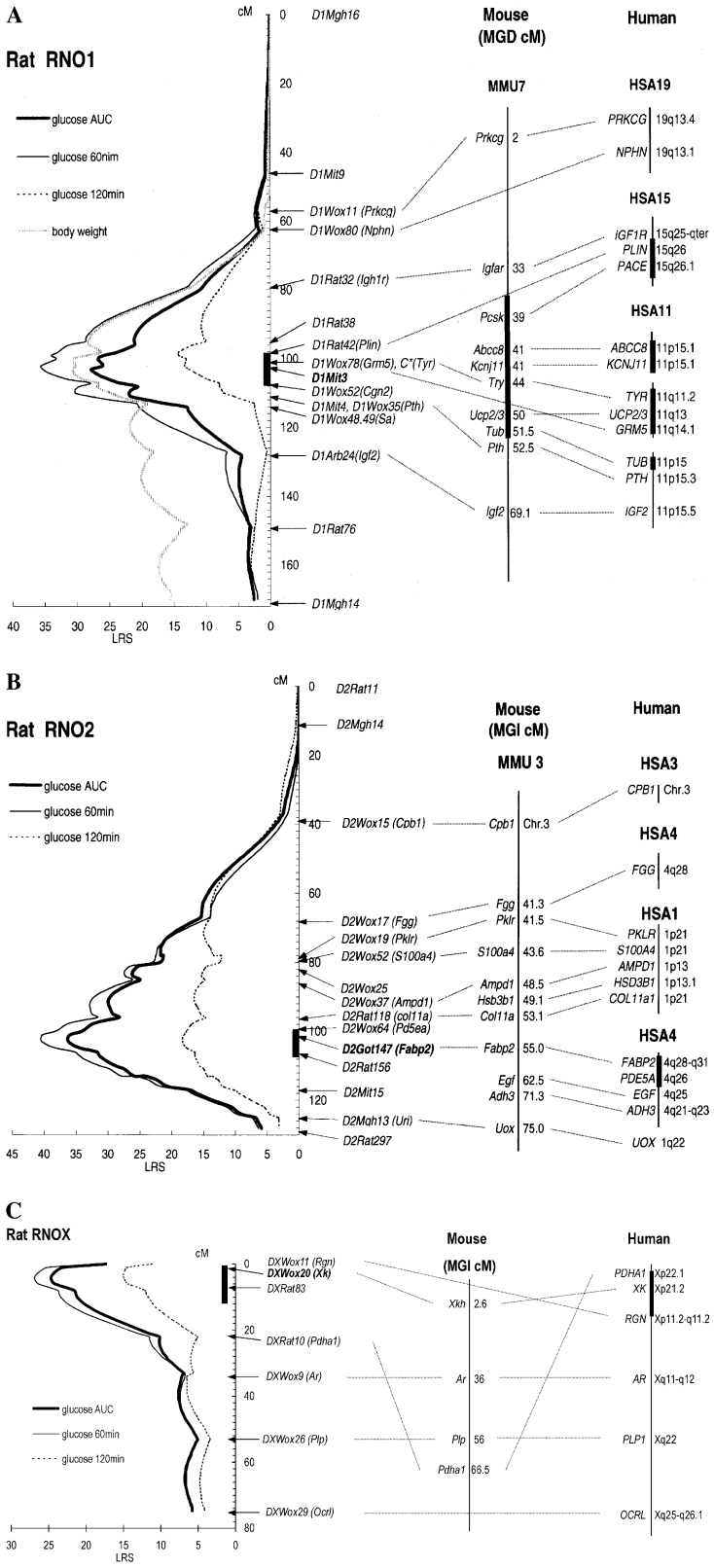

Fig. 3. LRS plots for glucose 60 and 120 min, AUC, and body weight on chromosome 1 (A), chromosome 2 (B), and chromosome X (C) (as shown in the left side of each panel) and the comparative maps among rat–mouse–human homologous to each QTL region (as shown in the right side of each panel). The LRS score and the genetic distance were calculated with the Map Manager QTX program. Highly significant threshold level of LRS score determined by permutation test is shown in Table 3. Supporting interval of 4.61 LRS as equivalent to 1 LOD is represented by bold line on each map. Bold-typed loci represent the closest marker of QTL. Gene-based or -associated SSLP markers indicate with its gene-symbol in parenthesis. To allow the homologous regions for QTLs, the syntenic gene is connected by dotted lines among rat, mouse, and human genome.

Table 3
Likelihood ratio statistics and the effect of each QTL on glucose tolerance in N2 progeny

Phenotypic trait	*Gisdt1*		*P* value[b]	LRS[c]	Threshold[d]	Variance[e] (%)	Add.[f]
	SDT/SDT,[a] $N = 165$	SDT/BN, $N = 154$					
Glucose AUC (mg h/dl)	330.2 ± 7.02	286.3 ± 3.84	1.33E − 07	27.8	14.6	8	43.68
Glucose 60 min (mg/dl)	206.8 ± 5.20	169.8 ± 2.96	3.24E − 09	35.6	16.5	10	37.15
Glucose 120 min (mg/dl)	146.0 ± 3.64	129.6 ± 2.06	1.35E − 04	14.3	14.6	4	16.18
Body weight (g)	512.5 ± 3.79	482.8 ± 3.72	4.37E − 08	30.7	14.8	9	30.82
	Gisdt2		*P* value	LRS	Threshold	Variance (%)	Add.
	SDT/SDT, $N = 158$	SDT/BN, $N = 161$					
Glucose AUC (mg h/dl)	333.9 ± 6.97	284.6 ± 4.11	2.47E − 09	36.3	14.0	10	50.39
Glucose 60 min (mg/dl)	208.8 ± 5.26	169.5 ± 3.03	2.84E − 10	40.5	15.2	12	40.14
Glucose 120 min (mg/dl)	147.2 ± 3.60	129.1 ± 2.25	2.40E − 05	18.3	15.1	5	18.73
	Gisdt3		*P* value	LRS	Threshold	Variance (%)	Add.
	SDT, $N = 157$	BN, $N = 162$					
Glucose AUC (mg h/dl)	329.2 ± 7.04	289.4 ± 4.34	1.83E − 06	24.7	13.7	7	42.67
Glucose 60 min (mg/dl)	204.9 ± 5.26	173.5 ± 3.34	6.33E − 07	26.9	12.9	8	33.65
Glucose 120 min (mg/dl)	146.2 ± 3.68	130.1 ± 2.20	1.72E − 04	15.0	13.4	4	17.11

[a] Gisdt1, Gisdt2, and Gisdt3 were genotyped using the closest markers, *D1Mit3, D2Got147* and *DXWox20*, respectively. Data are given as mean ± SE.
[b] Statistical difference between genotypes in each quantitative trait are shown by *P* value in Student's *t* test
[c] Likelihood ratio statistics (LRS) scores were detected by interval mapping using Map Manager QTX.
[d] Threshold level is indicated as a highly significant LRS ($P < 0.001$) determined by the permutation test in 1-cM steps at 5000 permutations.
[e] Percentage of overall variant of each trait in N2 rats.
[f] Additive regression coefficient in each trait are shown.

diabetes and glucose intolerance in SDT rats were induced by predominance of insulin secretory defect to glucose stimuli rather than insulin resistance.

Based on clinical features of SDT rats we demonstrated, we identified three QTLs responsible for diabetes and glucose intolerance in SDT rats. These QTLs, *Gisdt1-3*, were mapped on rat chromosomes 1, 2, and X, and accounted for 10%, 12%, and 8%, respectively, of the overall variance. This observation reveals that diabetes and glucose intolerance are polygenic traits in SDT rats. It is also shown that each SDT allele of each QTL has a strong effect on the increase of the blood glucose level at 60 min after glucose load. Moreover, of the three QTLs for glucose intolerance, we found that *Gisdt1* on chromosome 1 is involved in the regulation of body weight. Body weights were strongly correlated to the BMIs in N2 rats. The increase of body mass implies the absolute increase of insulin demand [29]. It is likely that *Gisdt1* is a locus responsible for the increased insulin demand in peripheral tissues rather than the decreased insulin secretion. In contrast, the SDT alleles of *Gisdt2* and *Gisdt3* do not link to body weight. Thus, *Gisdt2* and *Gisdt3* may be associated with pancreatic islet function on the regulation of glucose homeostasis.

We showed that glucose intolerance exacerbates when the SDT alleles are accumulated at three loci in the N2 progeny (Fig. 4), demonstrating that glucose intolerance is polygenic in SDT rats, and is induced by the synergistic interaction of three QTLs that we detected. It is well recognized not only in animal models [31,32] but also in humans [2,28] that most of type 2 diabetes mellitus is heterogeneous and polygenic, and that the association of several genetic factors corresponds to the onset of type 2 diabetes. Genetic interaction among genes influences the development of disease. Interestingly, the interaction between *Gisdt1* and *3* was observed in postprandial glycemia, in which the effect of *Gisdt3* on postprandial glycemia disappears when *Gisdt1* is heterozygous. This suggests that *Gisdt3* plays a role as an additive factor of *Gisdt1*.

Many glucose-intolerance-related loci have been identified in rat models with diabetic features such as GK rat [33,34], obese $Lepr^{fa}/Lepr^{fa}$ WKY13M rat [31] or OLETF rat [32,35,36]. Of these loci, several QTLs are localized on chromosomal regions containing *Gisdt* QTLs which we identified. On chromosome 1 containing *Gisdt1*, *Nidd5/of*, and *Nidd6/of* for fasting glucose [35] and *Dmo1* for obesity and glucose intolerance [36] in OLETF rat, *Niddm1* [33] and *Nidd/gk1* [34] for glucose intolerance in GK rat and *Niddm4* for diabetes in obese $Lepr^{fa}/Lepr^{fa}$ WKY13M rats [31] have been mapped previously. The interval of *Gisdt1* we detected is either on centromeric side apart from the intervals in these QTLs or partly overlapping. On chromosome 2

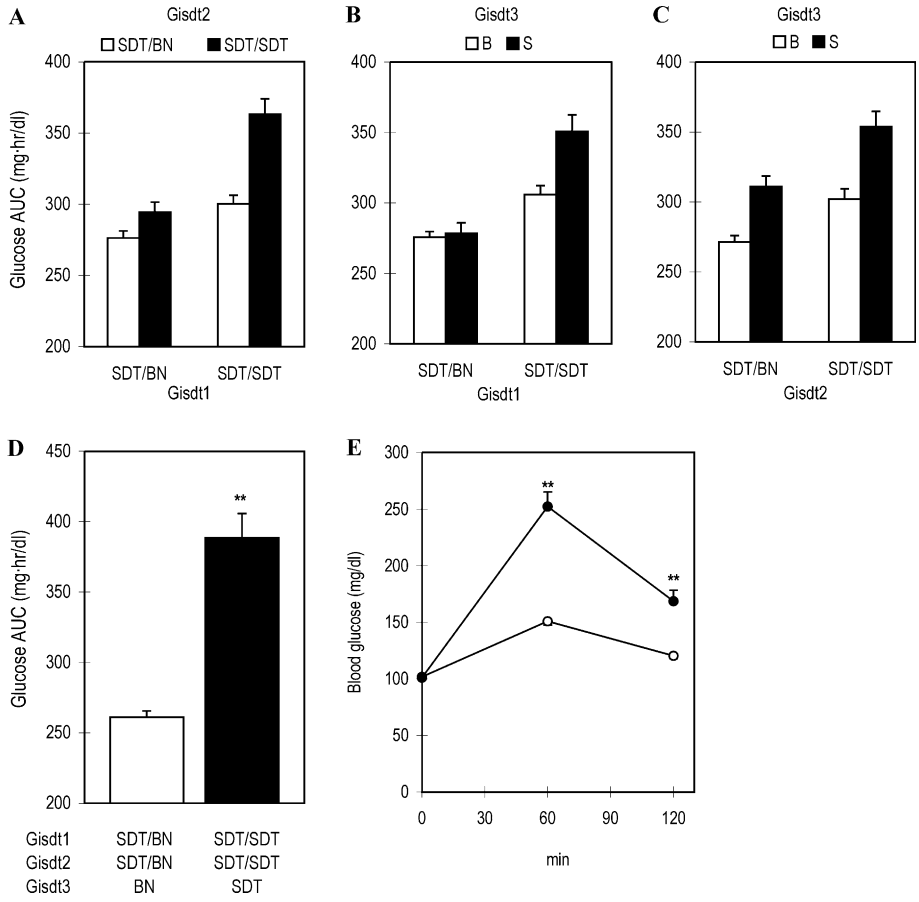

Fig. 4. The interaction between each QTL on glucose AUC in the N2 progeny. Glucose AUC was compared among the four different combinations of genotypes at *Gisdt1* and *Gisdt2* (A), *Gisdt1* and *Gisdt3* (B), and *Gisdt2* and *Gisdt3* (C) for the N2 progeny. Cumulative effects of SDT alleles of three QTLs were evident in glucose AUC (D) and blood glucose levels (E) during OGTT. Genotypes of QTLs were determined by the closest SSLP markers, *D1Mit3* for *Gisdt1*, *D2Got147* for *Gisdt2*, and *DXWox20* for *Gisdt3*. All data are presented as means ± SE. Asterisks indicate the significant difference by Student's *t* test (**, $P < 0.01$) in (D,E).

containing *Gisdt2*, *Niddm2* for glucose intolerance [33] and *Nidd/gk2* for fasting insulin [34] in GK rats have been reported. *Niddm2* mapped by Galli et al. [33] by the use of F2 crosses between GK and F344 rats, is the closest at *D2Mit15* near *Gistd2* on chromosome 2 and is responsible for glucose AUC on glucose tolerance test similar to *Gisdt2*. In contrast, Gauguier et al. [34] used the BN rat as a crossing partner similar to ours to prove QTL for diabetes of the GK rat, and obtained no evidence of linkage for glucose tolerance was obtained, but mapped QTL for fasting insulin at the same region as *Niddm2* or *Gisdt2*. Although this interval may contain common susceptibility gene(s) acting for the glucose intolerance in both GK and SDT rats, further examinations should be required to characterize the nature of *Gisdt1*, because the difference of genetic background may influence phenotypes in SDT rats as well as in GK rats. On chromosome X, *Gisdt3* region was centromeric from *Odb1* for diabetes in OLETF rats reported by Hirashima et al. [35]. This suggests that *Gisdt3* would be a novel QTL for glucose tolerance in rats as distinct from *Odb1*.

The construction of comparative map among human and rodents is one of the common strategies for contribution to human genetic research and for extraction of candidate genes responsible for locus detected in rodents. Based on the comparative map information of genes associated with SSLP markers used in this study, the orthologous regions in mice (MMU) and humans (HSA) for three QTLs are shown in Fig. 3. Genomic regions approximately 40–52.5 cM distant from the centromere on MMU7 and around HSA15q26-qter or 11p15.1 and 11q11.2–14.1 are orthologous to *Gisdt1*, those 55 cM distant from MMU3 centromere and HSA1p13–p21 or 4q22–q31 to *Gisdt2*, and those from the centromere to 36 cM of distance on MMUX and HSAXp11-q11 to *Gisdt3*. In the mouse orthologous region for *Gisdt1*, the causative gene of the tubby mouse, *tub*, is located at 51.5 cM of MMU7 [37]. Although the function of *tub* gene is unclear yet, it is reported that *tub* gene is associated with the late-onset of mild obesity and retinal degeneration [37,38]. Interestingly, we previously reported that diabetic SDT rats develop severe proliferative retinopathy [20], although it is unclear whether retinal

changes in tubby mice and SDT rats are genetically related or not. Moreover, *Gisdt1* is linked to the increased body weights. If *Gisdt1* is *tub*, it is thought that the genetic background of the SDT rat including *Gisdt2* and *Gisdt3* hid the phenotype of mild obesity by *tub*. In humans, three susceptibility loci for common non-insulin dependent diabetes mellitus (NIDDM) have been mapped on HSA2q [39], 12q24.2 [40], and 20q12–q13 [41], but do not correspond to those on rat chromosomes where *Gisdt* loci were mapped in this study. The orthologous regions for *Gisdt* loci should, therefore, be considered as novel candidate regions containing susceptibility genes for glucose intolerance and type 2 diabetes in humans. Physiologically relevant positional candidate genes are perilipin (*PLIN*), sulfonylurea receptor (*ABCC8*), potassium channel, inwardly rectifying, subfamily J, member 11 (*KCNJ11*), and uncoupling proteins 2 (UCP2) and 3 (*UCP3*) for *Gisdt1*. Those for *Gisdt2* and *Gisdt3* are fatty acid-binding protein 2 (*FABP2*) and bifunctional enzyme, fructose 6-P,2-kinase:fructose 2,6-bisphosphatase (*PFKFB1*), respectively. To identify genes involved in the pathogenesis of diabetes in SDT rats, we are now constructing congenic strains for three QTLs. Recently, the human and mouse genome-sequencing is reaching to a final step. Comparative maps and the variable genome-information will help us to apply the positional candidate approach to identify genes responsible for diabetes in SDT rats.

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