Genetics of the Spontaneously Diabetic Torii Rat

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Abstract: The Spontaneously Diabetic Torii (SDT) rat has recently been established as a novel model of nonobese type 2 diabetes. SDT rats exhibit inflammation and fibrosis in and around the islets during development of the disease. To clarify the genetic basis of the disease, we previously performed quantitative trait locus (QTL) analysis of glucose tolerance at 20 weeks of age using backcrossed progeny produced from the (BN×SDT)F1×SDT cross. The analysis identified three major QTLs (*Gisdt1*, *Gisdt2*, and *Gisdt3*) on rat chromosomes 1, 2, and X, respectively. To examine genetic factors for diabetes, glucose tolerance, islet inflammation, and fibrosis in the SDT rat, we also performed genetic analysis of these traits at 60 weeks of age using intercrossed progeny produced from the (F344×SDT)F2 cross. Genetic analysis of diabetes identified a major locus, *Dmsdt1,* on chromosome 3. QTL analysis of blood glucose levels revealed, in addition to *Dmsdt1*, three other loci (*Dmsdt2*, *Dmsdt3*, and *Dmsdt4*) on chromosome 8, 13, and 14, respectively. Analysis of a congenic strain for *Dmsdt1* (F344.SDT-*Dmsdt1*) indicates that the dominantly acting SDT allele induces islet inflammation and fibrosis. These data clearly demonstrate that development of diabetes in the SDT rat is controlled by the combination of several QTLs with considerable effects. Identification of the genes responsible would provide greater understanding of the pathogenesis and pathophysiology of diabetes.

Keywords: Congenic strain, fibrosis, genetic analysis, glucose tolerance, inflammation, QTL.

INTRODUCTION

Type 2 diabetes mellitus is a multifactorial disease involving interaction of multiple genetic and environmental factors. Since the genetic basis of type 2 diabetes has not been fully clarified yet, analysis using spontaneous animal models would provide useful information on genetic factors involved in the development of the disease. There have been many obese models of type 2 diabetes such as the Otsuka Long-Evans Tokushima fatty (OLETF) rat [1], Wistar fatty rat [2], Zucker diabetic fatty (ZDF) rat [3], db/db mouse [4], KK-A^y mouse [5], Nagoya Shibata Yasuda (NSY) mouse [6], New Zealand obese (NZO) mouse [7], TallyHo (TH) mouse [8], and the Tsumura Suzuki obese diabetic (TSOD) mouse [9], but there is no known model of type 2 diabetes without obesity except for the Goto-Kakizaki (GK) rat [10]. The GK rat has, therefore, been exclusively utilized as a nonobese model of the disease.

The Spontaneously Diabetic Torii (SDT) rat was recently established as a novel nonobese model of type 2 diabetes [11]. Male SDT rats show 100% incidence of diabetes by 40 weeks of age. Before the onset of diabetes, pathological changes such as inflammation and fibrosis in and around the islets continue for several months, and are accompanied by a decrease in the number of pancreatic β-cells [12].

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To clarify the genetic basis of diabetes, glucose intolerance, islet inflammation, and fibrosis in SDT rats, we performed genetic analysis of these traits using two different crosses: the (BN×SDT) F1×SDT backcross [13] and the (F344×SDT) F2 intercross [14]. Analysis of the former cross identified three QTLs (*Gisdt1*, *Gisdt2*, and *Gisdt3*) for glucose intolerance on rat chromosomes 1, 2, and X, respectively. Analysis of the latter cross revealed four QTLs (*Dmsdt1*, *Dmsdt2*, *Dmsdt3*, and *Dmsdt4*) for blood glucose levels on rat chromosome 3, 8, 13, and 14, respectively. Further congenic analysis clarified *Dmsdt1* as a major locus for islet inflammation and fibrosis.

GENETIC ANALYSIS OF GLUCOSE INTOLERANCE USING (BN×SDT) F1×SDT BACKCROSSED PROGENY

To investigate genetic control of diabetes and glucose intolerance in SDT rats, we produced (BN×SDT)F1×SDT backcrossed (N2) progeny, performed oral glucose tolerance test (OGTT) at 20 weeks of age, and phenotyped for the onset of diabetes up to 25 weeks of age [13]. The cumulative incidence of diabetes in N2 rats was only 1.9% (6/319) at 25 weeks of age.

By selective genotyping on N2 rats with marked glucose intolerance (n = 26) and normal glucose tolerance (n = 30), followed by chi-square test, we found significant differences ($P < 0.01$) in the genotype frequencies at several markers on chromosomes 1, 2, 6, 7, 8, 11, 14, 18, and X. These chromosomes were then subjected to QTL analysis using all of the N2 rats. We found three major QTLs affecting postprandial blood glucose levels on chromosomes 1, 2, and X, designated *Gisdt1* (glucose intolerance in SDT rat 1),

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Gisdt2, and *Gisdt3*, respectively. These loci have been registered as *Gluco13*, *Gluco14*, and *Gluco15*, respectively (Rat Genome Database, RGD). We also found a major QTL for body weight at the *Gisdt1* region. The recessively acting SDT allele at *Gisdt1* (*Gluco13*) on chromosome 1 was involved in the higher blood glucose levels and higher body weight in N2 rats. The recessively acting SDT allele at *Gisdt2* (*Gluco14*) on chromosome 2 and the single SDT allele at *Gisdt3* (*Gluco15*) on X chromosome were involved in the higher blood glucose levels in N2 rats. The genetic analysis using N2 rats revealed that there are at least three major QTLs affecting postprandial blood glucose levels in SDT rats.

GENETIC ANALYSIS OF DIABETES AND GLUCOSE INTOLERANCE USING (F344×SDT)F2 INTERCROSSED PROGENY

To perform genetic analysis of diabetes and glucose intolerance in SDT rats, we produced (F344×SDT) F2 rats, phenotyped for the onset of diabetes up to 60 weeks of age, and performed OGTT at the same age [14]. F2 rats developed diabetes at 25 weeks of age or later, and the cumulative incidence of diabetes reached 19% (31/167) at 60 weeks of age. Interestingly, postprandial blood glucose levels of (F344×SDT) F1 rats were significantly higher than those of control F344 rats, indicating that dominantly acting SDT alleles are involved. Although none of the F1 rats developed diabetes till 75 weeks of age, F1 rats did show pathological inflammatory changes in the pancreas, such as inflammation and fibrosis in and around the pancreatic islets.

By selective genotyping on diabetic F2 rats (n = 31) and non-diabetic F2 rats with the area under the blood glucose response curve value of the lowest 20% (n = 33), followed by chi-square test, we found highly significant differences ($P < 0.001$) in the genotype frequencies at several markers on chromosomes 3, suggesting the presence of a major locus responsible for diabetes. The locus was designated *Dmsdt1* (diabetes mellitus locus in the SDT rat No.1), and has been registered as *Gluco35* (RGD). A dominantly acting SDT allele at *Dmsdt1* is involved in the development of diabetes in F2 rats.

By QTL analysis using all of the F2 rats, in addition to *Dmsdt1* we identified three major QTLs affecting blood glucose levels on chromosome 8, 13, and 14, designated *Dmsdt2*, *Dmsdt3*, and *Dmsdt4*, respectively, and one major QTL affecting body weight on chromosome 3, designated *Bwsdt1*. These loci have been registered as *Gluco36*, *Gluco37*, *Gluco38*, and *Bw82*, respectively (RGD). The dominantly acting SDT allele at *Dmsdt1* (*Gluco35*) on chromosome 3 was involved in the higher postprandial blood glucose levels in F2 rats. The recessively acting SDT allele at *Dmsdt2* (*Gluco36*) on chromosome 8 was involved in the higher fasting and postprandial blood glucose levels in F2 rats. The recessively acting SDT allele at *Dmsdt3* (*Gluco37*) on chromosome 13 was involved in the higher fasting blood glucose levels in F2 rats. The additively acting SDT allele at *Dmsdt4* (*Gluco38*) on chromosome 14 was involved in the higher postprandial blood glucose levels in F2 rats. The dominantly acting SDT allele at *Bwsdt1* (*Bw82*) on chromosome 3 was involved in the higher body weight in F2 rats.

By QTL analysis on insulin levels at OGTT, we identified three significant QTLs on chromosome 3, 13, and 14 (Fig. **1**). The LOD score plot suggested that the loci on chromosome 3 and 14 correspond to *Dmsdt1* (*Gluco35*) and *Dmsdt4* (*Gluco38*), respectively, while the locus on chromosome 13 is different from *Dmsdt3* (*Gluco37*).

To clarify the role of the dominantly acting SDT allele at *Dmsdt1* (*Gluco35*) on the development of diabetes, we produced a congenic strain carrying an SDT allele on the control F344 genetic background (F344.SDT-*Dmsdt1*) [14]. At 35 weeks of age, inflammation in and around the pancreatic islets and hemosiderin deposition and fibrosis in the islets were frequently found in congenic rats, indicating that a dominantly acting SDT allele at *Dmsdt1* (*Gluco35*) induces the pathological inflammatory changes in the pancreas.

Genetic analysis using F2 rats clarified that at least four major QTLs are involved in impaired regulation of blood glucose levels in SDT rats. These loci are completely different from those identified by the analysis using N2 rats, except for *Dmsdt2* (*Gluco36*), the recessively acting locus on chromosome 8. These data indicates *Dmsdt2* (*Gluco36*) to be a common genetic factor to the two different crosses, but does not indicate the locus is the most definite one. The inconsistency between the two studies is likely due to differences in crossing, control strains, and age of phenotyping. It is much more straightforward and efficient to analyze complex genetic traits through a backcross (N2) rather than an F2 intercross, since each backcrossed (N2) animal have one of two genotypes at each locus, while each F2 animal can have one of three genotypes at each locus. Although the backcross (N2) analysis is concentrated on detecting recessively-acting genetic factors, the analysis using F2 cross makes it possible to detect both recessively- and dominantly-acting genetic factors.

COMPARISON WITH REPORTED QTLS IN RATS, MICE, AND HUMANS

There have been reported hundreds of QTLs affecting blood glucose levels or body weights in rats, mice, and humans. QTLs mapped to the rat genomic regions harboring SDT QTLs, or those mapped to the orthologous regions in mice and humans are listed (Table **1**). Several QTLs, such as *Niddm40* and *Niddm45* affecting postprandial glucose levels in OLETF rats [15, 16] have been mapped to the *Gisdt1* (*Gluco13*) region on rat chromosome 1. In the *Gisdt2* (*Gluco14*) region on chromosome 2, no QTL has been reported in rats and mice. On chromosome X, *Niddm16* affecting glucose levels in OLETF rats has been reported [15, 17] on the genomic region overlapped with *Gisdt3* (*Gluco15*). *Dmsdt1* (*Gluco35*) induces inflammation and fibrosis in the pancreatic islets that are the main pathogenic features of SDT rats, while uncommon in human type 2 diabetes. Although *Gluco39* affecting glucose levels in Wistar Ottawa Karlsburg W (WOKW) rats has been reported [18] in the *Dmsdt1* (*Gluco35*) region on chromosome 3, no such loci has been reported in the homologous region in humans. *Dmsdt2* (*Gluco33*) on chromosome 3 is involved in both fasting and postprandial glucose levels in SDT rats. Interestingly, *Gluco43* affecting fasting glucose levels in Wistar Kyoto (WKY) rats [19] and *Niddm21* affecting

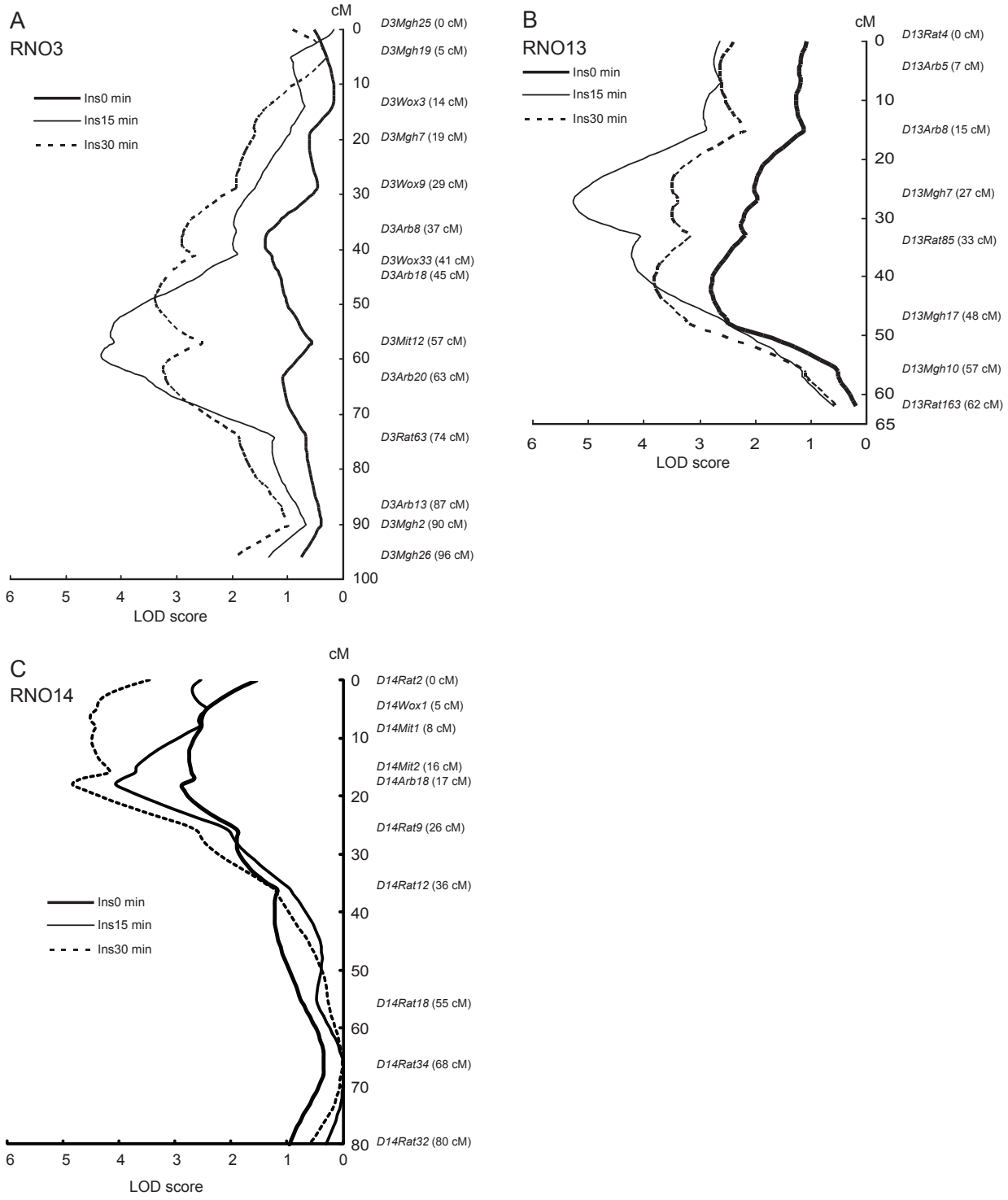

Fig. (1). LOD score plots for quantitative traits on chromosome 3 (**A**), 13 (**B**), and 14 (**C**). Ins 0, 15, and 30 min indicate insulin levels at 0 (fasting), 15, and 30 min during OGTT, respectively. On y-axis, cM shows the map position based on our linkage map. X-axis shows LOD score.

postprandial glucose levels in OLETF rats [20] have been mapped to this region. No QTL has been reported in rats and mice mapped to the *Dmsdt3* (*Gluco37*) region on chromosome 13. In the *Dmsdt4* (*Gluco38*) region on chromosome 14, *Niddm20* affecting postprandial glucose levels in OLETF rats has been reported [21].

In the *Bwsdt1* (*Bw82*) region on chromosome 3, several QTLs affecting body weights have been reported, such as *Bw24* in Dahl Salt Sensitive (SS) rats [22], *Bw31* and *Bw36* in Lyon Hypertensive (LH) rats [23], and *Bw81* in Genetically Hypertensive (GH) rats [24]. There are several QTLs thought to be common for SDT and OLETF rats,

Table 1. Comparizon of SDT QTLs with Reported QTLs in Rats, Mice and Humans

SDT QTLs	Rat		Mouse		Human	
	Chr.	QTLs	Chr.	QTLs	Chr.	QTLs
Gisdt1 (Gluco13)	1 (147Mb)	Gluco41, Niddm23, -40, -44, -45, Bw18, -47, -55	7 (98Mb)	(-)	11 (85Mb)	GLUCO256_H
Gisdt2 (Gluco14)	2 (221Mb)	(-)	3 (124Mb)	(-)	4 (118Mb)	GLUCO243_H
Gisdt3 (Gluco15)	X (13Mb)	Gluco34, Niddm16	X (20Mb)	(-)	X (47Mb)	GLUCO289_H
Dmsdt1 (Gluco35)	3 (115Mb)	Gluco39, Niddm39	2 (127Mb)	Bglu1, T2dm2sa	2 (97Mb)	(-)
Dmsdt2 (Gluco36)	8 (43Mb)	Gluco43, Niddm21	9 (41Mb)	(-)	11 (124Mb)	GLUCO29, -83, -84, -129, -130, -138_H
Dmsdt3 (Gluco37)	13 (39Mb)	(-)	1 (129Mb)	(-)	2 (134Mb)	GLUCO165_H
Dmsdt4 (Gluco38)	14 (22Mb)	Niddm20, -37	5 (88Mb)	(-)	4 (71Mb)	GLUCO77, -202_H
Bwsdt1 (Bw82)	3 (48Mb)	Bw24, -31, -36, -81	2 (66Mb)	Mob5, -7, Mobe1	2 (167Mb)	BW124, -133_H

Mapping information on rat and human QTLs is derived from the Rat Genome Database (RGD) and that on mouse QTLs from the Mouse Genome Informatics (MGI).
Rat QTLs: Gluco, Glucose level QTL; Niddm, Non-insulin dependent diabetes mellitus QTL; Bw, Body weight QTL.
Mouse QTLs: Bglu, blood glucose level QTL; T2dm, type 2 diabetes mellitus QTL; Mob, multigenic obesity QTL; Mobe, modifier of obesity QTL.
Human QTLs: GLUCO, Glucose level QTL; BW, Body weight QTL.

while there seems to be no such QTL for SDT and GK rats [25, 26]. To our surprise, the loss of function polymorphism in *Prlhr* (prolactin releasing hormone receptor, also known as *Gpr10*) affecting hyperphagia, dyslipidaemia, and obesity in OLETF rats [27] has been found in SDT and GK rats (Yokoi N, unpublished observation). In fact, there was a significant association with body weight around the *Gpr10* region on chromosome 1 [13]. Although SDT rat is a non-obese model of type 2 diabetes, SDT alleles at several other loci have also been involved in the higher body weight in the N2 or F2 rats. Since SDT rats showed larger body size as compared to BN or F344 rats which were used as control strains in the genetic studies [13, 14], the SDT alleles affecting body weight may well be involved in large body size rather than obesity. These data indicate that large body size may have some role on the phenotype of SDT rats.

CONCLUSION

So far, we have identified seven QTLs (*Gisdt1*, *Gisdt2*, *Gisdt3*, *Dmsdt1*, *Dmsdt2*, *Dmsdt3*, and *Dmsdt4*) affecting blood glucose levels on chromosome 1, 2, X, 3, 8, 13, and 14, respectively, and one QTL *Bwsdt1* affecting body weight on chromosome 3 in SDT rats. Among them, *Dmsdt1* has been found to be involved in islet inflammation and fibrosis, the major pathogenic features of this model. Since retinal inflammation is among the early pathological changes linked causally to the development of retinopathy, *Dmsdt1* might also be involved in the pathogenesis of retinopathy, the major diabetic complication of SDT rats. At present, there is no data which suggests the relation of other QTLs to retinopathy. Further characterization of *Dmsdt1* and other QTLs are needed to identify the genes responsible and to clarify the mechanism of pathogenesis of diabetes in SDT rats.

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