

**High-resolution QTL Analysis Reveals Multiple Diabetes Susceptibility Loci
Mapped to Intervals less than 800-kb in the Species Conserved *Niddm1i* of the
GK Rat**

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Running head: Fine-mapping of type 2 diabetes locus

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Abbreviations: IPGTT, intraperitoneal glucose tolerance test; LOD, logarithm of odds ratio; LR, likelihood ratio; Mb, mega base pairs; NIDDM, non-insulin dependent diabetes mellitus; QTL, quantitative trait locus

ABSTRACT

Niddm1i, a 16 Mb locus within the major diabetes QTL in the diabetic GK rat, causes impaired glucose tolerance in the congenic NIDDM1I strain. *Niddm1i* is homologous to both human and mouse regions linked with type 2 diabetes-susceptibility. We employed multiple QTL analyses of congenic F2-progeny selected for one recombination event within *Niddm1i* combined with characterization of subcongenic strains. Fine-mapping located one hyperglycemia locus within 700 kb (*Niddm1i4*, $P=5 \times 10^{-6}$). Two adjacent loci were also detected, the GK allele at *Niddm1i2* (500 kb) showed a glucose-raising effect, whereas it had a glucose-decreasing effect at *Niddm1i3* (400 kb). Most proximal, *Niddm1i1* (800 kb) affecting body weight was identified. Experimental data from subcongenics supported the four loci. Within *Niddm1i3* *Sorcs1* resides, one of the two known diabetes susceptibility genes in the region, while *Tcf7l2* maps outside all four loci. Multiple-marker QTL analysis incorporating the effect of cosegregating QTLs as cofactors together with genetically selected progeny can remarkably enhance resolution of QTLs. The data demonstrate that the species conserved *Niddm1i* is a composite of at least four QTLs affecting type 2 diabetes susceptibility, and that two adjacent QTLs (*Niddm1i2*^{GK} and *Niddm1i3*^{GK}) act in opposite directions.

INTRODUCTION

Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM) is defined by chronic elevation of plasma glucose, but the underlying pathophysiology is complex and profoundly influenced by both polygenic background and environmental factors (such as dietary habits, smoking, and physical activity). Consequently, the phenotypes associated with type 2 diabetes susceptibility usually show a quantitative variation (Permutt *et al.* 2005).

The risk to type 2 diabetes in humans has a measurable genetic component as indicated by familial clustering and higher concordance rates in monozygotic twins compared with dizygotic twins (Medici *et al.* 1999; Poulsen *et al.* 1999), and by the high heritability of insulin secretion and insulin action (Iselius *et al.* 1985; Lehtovirta *et al.* 2000; Poulsen *et al.* 2005). Genetic studies of inbred animals raised in standardized environments facilitates the identification of disease mechanisms via identification of naturally occurring alleles capable of influencing the progression from health to diabetes (Aitman *et al.* 1999; Fakhrai-Rad *et al.* 2000). The GK rat was developed by selective breeding of the most hyperglycemic offspring of outbred Wistar rats during nine generations, followed by inbreeding to generate a strain with stably inherited and spontaneously developing diabetes without concurrent excessive obesity (Goto Y 1975). Progeny from F2-intercrosses arranged between GK and normoglycemic strains have been subjected to genome-wide linkage analyses, and several significant quantitative trait loci (QTL) for diabetes-associated phenotypes have been identified (Galli *et al.* 1996; Gauguier *et al.* 1996). The *Niddm1i* locus, on the telomeric end of rat chromosome 1q, is a locus within the major glucose-controlling QTL (*Niddm1*) in F2-intercrosses between GK and the normoglycemic F344 rat. Studies of the congenic strain NIDDM1I demonstrated that *Niddm1i*^{GK} encoded hyperglycemia and insulin secretion defects in pancreatic islets (Fakhrai-Rad *et al.* 2000; Galli *et al.* 1999; Lin *et al.* 2001).

Genome-wide linkage analyses in human (Duggirala *et al.* 1999; Reynisdottir *et al.* 2003), mice (Kim *et al.* 2001; Stoehr *et al.* 2000), and the OLETF rat model (Watanabe *et al.* 1999) have also located QTLs for diabetes to chromosome regions homologous to *Niddm1i*, human chromosome 10q24.3-q26.11 and mouse chromosome 19. Recently, two genes residing within *Niddm1i* have been associated with diabetes in humans (*Tcf7l2*, (Grant *et al.* 2006)), and fasting insulin levels in an obesity induced mouse model for diabetes (*Sorcs1*, (Clee *et al.* 2006)). The strong support for contributions to diabetes-associated phenotypes within the *Niddm1i* locus, prompted us to undertake a high-resolution genetic study of glucose and body weight regulation. We used a combination of two genotypically different sets of rats; F2-progeny from normoglycemic F344 and congenic NIDDM1I selected for a single recombination event within *Niddm1i*, and subcongenic strains with homozygous GK genotype in different intervals of *Niddm1i*. QTL analyses of the F2-progeny were used to narrow down the confidence intervals for diabetes susceptibility genes, and five subcongenic strains substantiated the presence of four sub-loci within *Niddm1i*.

MATERIALS AND METHODS

Animals: Inbred normoglycemic F344 /DuCr12Swe were originally purchased from Charles River Laboratories (Wilmington, MA). Diabetic GK/Swe was originally from Kyoto University. Generation of the congenic strain F344.GK-*Niddm1i*, NIDDM1I for short, has been described (Galli *et al.* 1999). It is homozygous for GK from 252 Mb to the end of chromosome 1 at 268 Mb (16 Mb, with the last marker *D1Rat90* at 257.1 Mb) on a homozygous F344 genetic background with mitochondrial DNA and chromosomes X plus Y from F344. The congenic F2-intercross between F344 and NIDDM1I was initiated from female F344 and male NIDDM1I. Markers *D1Rat83* and *D1Rat90* were genotyped in 1594 male progeny to select rats with a single recombination event within *Niddm1i* for further testing. This protocol was designed to generate a homogeneous set of animals with recombination events spread throughout the locus. In each marker locus, the expected Mendelian segregation ratio of 1:2:1 in our F2-population was not distorted by the genotypic selection procedure. At birth approx. 10 males from different litters were pooled, and at 30 days of age they were weaned and five progeny from different litters were housed per cage. In the end 210 male progeny had complete genotypic and phenotypic information. The subcongenic strains N1IREC6, N1I12, N1I3, N1IREC1, and N1IREC11 were generated from F2-progeny and carried homozygous GK genome in different segments of *Niddm1i* on a homozygous F344 background (Table 1). All strains were maintained by sister-brother breeding. Litter sizes and number of rats per cage were matched in all experiments with congenic strains. Rats were maintained at constant temperature and humidity in a 12-h cycle of light and dark with free access to standard laboratory chow and water. The local Ethics Committees approved all experiments.

Phenotypic characterization: To avoid effects of the estrus cycle and other minor gender specific influences, only males were included in this study. No major sex-specific

effect for *Niddm1* was seen in the original genome-wide scan between GK and F344 (Galli *et al.* 1996). Weight measurements and an intraperitoneal glucose tolerance test (IPGTT) with 2.0 g glucose/ kg body weight were performed after 6 h fasting without anesthesia at 95 days of age (Galli *et al.* 1999).

Genotype analysis: Genomic DNA was extracted from both ear and tail biopsies. Biopsies were incubated at 55°C overnight in 500 µl lysis buffer (100 mM Tris-HCl, pH 8.0, 5 mM EDTA, 0.2 % SDS, 200 mM NaCl) and 0.1 mg/ml proteinase K. The supernatant was cleared by centrifugation at 12 000 x g for 10 min; an equal volume of isopropanol was added and DNA was collected by centrifugation, 12 000 x g for 30 min, dried and dissolved in 75 µl 10 mM Tris-HCl, pH 7.6, 0.1 mM EDTA. Genetic markers were selected from public databases and in-house information and were mapped within the *Niddm1i*-region in 45 (GKxF344)F2 rats (Galli *et al.* 1996). Eight new microsatellite markers (*DISwe1-8*, available at RatMap at <http://ratmap.gen.gu.se/>) were added in regions lacking informative markers. The PCR profile consisted of: 94°C for 4 min, followed by 35 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 90 s, and a final 7-min incubation at 72°C (markers *DISwe7-8* annealed at 50°C). PCR amplification was performed with one primer in each pair labeled with [γ -³³P]ATP or fluorescence (hex or fam) (DNA technology A/S, Aarhus, Denmark). The locations of markers were taken from Ensembl Rattus Norvegicus version 40.34j based on RGSC 3.4 (<http://www.ensembl.org>) (see Table 1).

QTL analyses: Single-marker QTL analysis was performed based on linear regression using Minitab (Minitab Inc., State College, PA). Conditional probabilities of the QTL genotypes given the observed marker data were estimated using the R/qtl package (Broman *et al.* 2003). These probabilities were used to calculate coefficients of additive and dominance components for putative QTLs at each marker (Lynch and Walsh 1998). Phenotypic values were regressed onto the additive and dominance coefficients at each marker to compute likelihood ratios (LR) using the following equation

$$LR = n \log_e(SSE_{\text{reduced}}/SSE_{\text{full}}),$$

where SSE_{full} is the error sums of squares for the full regression model with a QTL at the marker locus, and SSE_{reduced} is total sums of squares for the null model without a QTL. n denotes the number of observations. The genomic position with the highest LR was taken as the most likely position of a QTL. Multiple-marker QTL analysis was also performed to dissociate multiple linked gene effects during the identification of individual QTLs. This was achieved by multiple regression analyses testing the hypothesis of existence of a QTL at a particular marker conditioned on selected markers as cofactors (Jansen 1993; Zeng 1993). The cofactors were chosen by a forward selection-backward elimination stepwise regression. The LR test statistic for the multiple-marker QTL analysis was calculated using the same equation as above, where SSE_{full} is the error sums of squares is for the full model and SSE_{reduced} is error sums of squares for the reduced model incorporating all the cofactors except the QTL effect at the marker locus. Residuals of each trait were evaluated for normality by examining the normal probability plot of the residuals. Both the multiple regressions and the cofactor selection were conducted using the Minitab program. The numbers of cofactors were: 15-min glucose 3, 30-min glucose 3, and body weight 1.

Multiple testing issues in both the single- and the multiple-marker QTL analyses were addressed by calculation of experiment-wise empirical thresholds using a numerical method (Piepho 2001). Experiment-wise thresholds for significant linkage ($\alpha=0.05$), and highly significant linkage ($\alpha=0.001$) were employed. Experiment-wise 20% significance levels were used as threshold for suggestive linkage. Thus, we applied a more conservative threshold for suggestive QTLs compared with the suggestive threshold used in genome-wide scans (Lander and Kruglyak 1995). Unless otherwise stated the P -values are nominal. We applied the 1.5-LOD drop method to estimate support intervals for QTLs (Sen and Churchill 2001).

Evidence for two-QTL interactions were investigated by two-dimensional scans for all marker pairs within *Niddm1i* using the *scantwo* function of the R/*qtl* package (Broman *et al.* 2003; Sen and Churchill 2001). Experiment-wise significance ($\alpha=0.05$) of a joint LOD was established by 1,000 permutations of data (Churchill and Doerge 1994). The level of significance for an interaction LOD was set at $P < 0.05$.

RESULTS

The F2-progeny were established from a cross between normoglycemic F344 and congenic NIDDM11 with a 13.9 cM (16 Mb) genome segment on the telomeric part of chromosome 1 derived from the diabetic GK strain on a F344 genetic background. Only progeny with one recombination event within *Niddm1i* were included in the QTL analyses. Subsequently, subcongenic strains were established and genotyped (Table 1).

Single-marker QTL analysis: Single-marker QTL analyses were performed to determine the presence and mode of inheritance of QTLs by testing for additive and dominant models against the null model (no QTL) at each marker position within *Niddm1i*. An experiment-wise highly significant QTL for postprandial glucose at 15 minutes (G15) during the intraperitoneal glucose tolerance test (IPGTT) was localized to the distal half of *Niddm1i* (Figure 1A, LR=21.7, $P=3 \times 10^{-6}$). This QTL co-localized with a highly significant QTL for postprandial glucose at 30 minutes (G30, Figure 1B, LR=17.0, $P=4 \times 10^{-4}$). Maximum LR for both traits were obtained at marker *DISmu2*. The two QTLs were additive and explained 9.9% and 7.9% of the residual phenotypic variance. The GK allele was associated with higher postprandial glucose levels.

Most proximal in *Niddm1i*, one significant QTL for body weight was mapped with a negative additive effect of the GK allele (Figure 1C, LR=10.9, $P=1 \times 10^{-3}$). The body weight of F2-progeny homozygous for the GK allele at this QTL was approximately 5% lower than those homozygous for F344 at this locus. The residual variance was reduced by 5.1% by including the QTL in the model. Interestingly, all marker loci showed an additive inheritance since no dominant effect was observed; and all the identified QTLs were best fitted by additive models (data not shown). Therefore we assumed strictly additive alleles in the further QTL analyses. Single-marker QTL analysis of postprandial insulin concentrations was unable to resolve conclusive evidence for distinct insulin-loci within *Niddm1i* (data not shown).

Improved mapping resolution by multiple-marker QTL analysis: Incorporation of markers as cofactors was used to enhance the ability to detect and locate closely linked QTLs and estimate their effects (Jansen 1993; Zeng 1993). The maximum LR test statistics and QTL map positions obtained with multiple-marker QTL analysis were very similar with those obtained with single-marker regression (Table 2). The explained residual variances were also of similar magnitude (data not shown). However, the 1.5-LOD support intervals (SIs) for the QTLs identified by multiple-marker regression were considerably narrower than those for the single-marker regression. Whereas SIs of the major loci at *DISmu2* for G15 and G30 based on single-marker QTL analysis were more than 6.0 Mb, they were reduced to 0.7 Mb by multiple-marker QTL analysis (Figures 1A and 1B, Table 2). The location of the QTL for body weight was narrowed down to a 0.8 Mb interval between *DIRat83* and *DIRat175* (Table 1, Figure 1C). The QTL affecting body weight was designated *Niddm1i1* and the major hyperglycemia QTL linked with *DISmu2* was denoted *Niddm1i4*.

Applying multiple-marker QTL analysis revealed two additional QTLs for G15 at markers *DISwe2* and *DIGot244*, separate from the major hyperglycemia locus at *DISmu2* (Figure 1A). Surprisingly, while the GK allele at *DISwe2* was associated with higher glucose concentration, the opposite effect was seen for the GK allele at *DIGot244* (Table 2, Figure 2). The effect size of the two opposing loci were in a similar magnitude (+2.4 and -2.6 mmol/l), and comparable to that of the locus at *DISmu2* (Table 2, Figure 2). The hyperglycemia locus at *DISwe2* was denoted *Niddm1i2*, and the adjacent QTL with an opposite effect of the GK-allele was denoted *Niddm1i3*. We found no evidence of interaction between any two loci by examinations of all pair-wise combinations of marker loci for associations with the traits.

Analysis of subcongenic strains: To substantiate the presence of multiple loci regulating diabetes-associated phenotypes within *Niddm1i*, a set of five subcongenic strains containing homozygous GK-derived segments covering parts of the locus were phenotypically investigated (Table 1). Glucose homeostasis during IPGTT in congenic

NIDDM1I was, as previously described (Galli *et al.* 1999), impaired compared with F344 (Table 3). N1IREC6, with the entire *Niddm1i*-locus except for 0.8 Mb in the most proximal end, was also hyperglycemic compared with F344. In addition, N1I12, harboring GK-derived genome in the 9-Mb distal end of *Niddm1i*, displayed 13% increase in G15 and 14% increase in G30. Three subcongenic strains did not show increased glucose levels compared with F344. These were N1IREC1, with 4 Mb GK genotype most proximal in *Niddm1i*, N1IREC11 with 3 Mb GK genotype within the proximal half of *Niddm1i*, and N1I3 with 4 Mb GK genotype in the most distal end of *Niddm1i* (Table 1 and Table 3). This supported the presence of the major hyperglycemic locus *Niddm1i4* (*DISmu2*) in the genomic interval differing between N1I12 and N1I3. In fact, N1IREC11 with GK genotype in the proximal half of *Niddm1i* showed an improved glucose homeostasis compared with F344, which substantiated the location of *Niddm1i3* (where the GK-haplotype resulted in lower postprandial glucose concentration). The lower glucose levels of N1IREC11 was not simply an effect of the significantly lower body weight of N1IREC11, since it remained after adjustment for the effect of body weight on glucose concentration in plasma (data not shown). The N1IREC1 congenic strain covers both *Niddm1i2* and *Niddm1i3* (Tables 1 and 2); therefore the glucose-lowering effect of *Niddm1i3* would be expected to counteract the glucose-elevating effect of *Niddm1i2*. This was also seen, since N1IREC1 did not display an improved glucose homeostasis compared with F344. There is no congenic strain with GK genome solely in the *Niddm1i2* locus; as a result we could only obtain indirect evidence for the presence of *Niddm1i2* by comparing N1IREC1 with N1IREC11 ($P=0.02$ for G15, $P=0.01$ for G30).

There was no difference in body weight between NIDDM1I compared with F344 (Table 3). However, the presence of a body-weight reducing QTL encoded by the GK-haplotype was confirmed most convincingly by the difference in body weight between NIDDM1I and N1IREC6. NIDDM1I, differing from N1IREC6 only in the 0.8 Mb most proximal part of *Niddm1i* (Table 1), had 7% lower body weight compared with N1IREC6

(Table 3, $P=3 \times 10^{-6}$). This confirmed the localization of *Niddm1l* to the small GK-derived segment most proximal in *Niddm1i*. In the congenics, the *Niddm1l*^{GK} effect is penetrant only in the two congeneric strains (N1IREC6 and N1I12) that also harbor GK alleles in the region differing between N1I12 and N1I3 (*DIGot250* to *DISmu1*), since N1IREC1 with GK genotype at *Niddm1l* did not display lower body weight than F344. Hence, an explanation of this observation would be the presence of additional loci for body weight between *DIGot250* and *DISmu1* (*Niddm1i4*). Indeed, in the F2-progeny there was an indication of three linked loci with opposite effects on body weight in this region. These three indicated body weight QTLs mapped within a genetic distance considerably shorter than for postprandial glucose at *DISwe2* and *DIGot244* (*Niddm1i2* and *Niddm1i3*). As a result, there were too few recombinants to significantly resolve and map these potential loci (data not shown). Furthermore, the N1IREC11 congeneric strain was significantly lighter than F344, indicating the presence of an additional body weight locus between *DISwe4* and *DIRat85* that was not observed in the F2-progeny.

NIDDM1I and N1IREC6 were the only strains that displayed significantly lower postprandial insulin levels compared with F344 (Table 3). N1IREC11, containing GK genome at the glucose-lowering locus *Niddm1i3*, displayed higher insulin levels compared with F344, which supported its improved glucose control. The phenotype displayed by N1IREC11 demonstrated that the GK-haplotype between *DISwe4* and *DIRat85* encode a complex pattern of diabetes-associated phenotypes (low glucose, high insulin, and low body weight).

Figure 3 portrays a summary of the location of the breakpoints for subcongenic strains and a summary of significant additive effects on diabetes-associated phenotypes within *Niddm1i*.

DISCUSSION

Development of type 2 diabetes involves variations in a large number of genes, and although the identities of some have been elucidated, several more are likely to contribute to the loss of normal glucose control and type 2 diabetes. By studying animal models of type 2 diabetes, environmental factors affecting expression of disease-associated phenotypes can be better controlled. Construction of congenic strains and substrains has become a widely used method to isolate and narrow chromosomal regions containing susceptibility genes for genetically complex diseases. NIDDM1I harboring 16 Mb from the diabetic GK strain, provides a unique possibility to combine mechanisms causing altered glucose homeostasis with detailed genetic information (Fakhrai-Rad *et al.* 2000; Galli *et al.* 1999; Lin *et al.* 2001). However, the map resolution obtained from congenic strains are often not appropriate for identifying individual genes underlying a QTL. Theoretical approaches to improve the precision estimates of QTL position by inclusion of co-segregation at many genomic segments affecting the trait of interest have been reported (Jansen 1993; Zeng 1993). Also, improved map resolution can be achieved by selecting recombinants in the chromosome region (Darvasi 1998; Jin *et al.* 2004; Ronin *et al.* 2003; Xu *et al.* 2005). To refine genomic segments within *Niddm1i* coding for phenotypes relevant to type 2 diabetes, we performed multiple QTL analyses incorporating the effect of co-segregating QTLs as cofactors using genetically selected progeny. *In toto*, one highly significant locus (*Niddm1i4*), and two suggestive loci (*Niddm1i2–Niddm1i3*) were identified for postprandial glucose levels. The two suggestive QTLs for glucose and one significant locus (*Niddm1i1*) for body weight were confirmed by independent characterization of the same phenotypes in subcongenics. The mapping data in this study confirm the theoretical predictions that data analyses based on more precise statistical genetic models for QTL analysis and a large number of recombinants are crucial factors to improve QTL mapping resolution for dissection of the genetic basis of

quantitative traits such as the type 2 diabetes susceptibility (Visscher *et al.* 2000). The mapping resolutions of *Niddm1i1-Niddm1i4* were all less than 1 Mb (Figure 1, Table 2).

The considerable genetic complexity displayed in *Niddm1i* is presumably a reflection of the selection protocol used to establish the diabetic GK strain (Goto Y 1975). Allelic fixation in genomic regions responding to strong selection is expected during establishment of GK. Closely linked QTLs with opposite effects encoded by the same haplotype could occur, since selection operates on the net genotypic effect of several linked genes. Two inbred strains like GK and F344 will only represent a limited fraction of the naturally occurring genetic variation (polymorphism) in the original population (Flint and Mott 2001). Therefore, it is tantalizing to note the species conserved character of *Niddm1i*, emphasizing the relevance of genetic investigations of the region as a major type 2 diabetes locus. Despite the substantial complexity of the *Niddm1i* QTL, it is still readily amenable to achieve high-resolution mapping of QTLs and identification of genes regulating mechanisms behind phenotypic variation associated with common disease like type 2 diabetes.

A number of genes within *Niddm1i* are involved in pathways that may be important to energy metabolism, apoptosis, and insulin secretion, which are critical factors associated with the risk for diabetes. This high-resolution study has narrowed down the number of diabetes-associated candidate genes considerably. The 800 kb-genome segments corresponding to *Niddm1i1* is gene rich and harbor approximately 13 annotated genes, with *USMG5* (*upregulated during skeletal muscle growth 5, or DAPIT / LZAP*) as a possible candidate (Paivarinne and Kainulainen 2001). The two loci with opposing effect on glucose levels encoded by the GK alleles (*Niddm1i2* and *Niddm1i3*) have exceptionally few identified protein coding genes: *Sorcs3* and a transposase from an L1 repeat in *Niddm1i2*, and *Sorcs1* within *Niddm1i3*. *Sorcs1* was recently identified as a type 2 diabetes susceptibility gene in the mouse (Clee *et al.* 2006). It is therefore conceivable that also *Sorcs3* might be involved in the pathogenesis of diabetes. The *Niddm1i4* hyperglycemia locus, covers a 700 kb-genome

interval with 7 known genes, including the gene for programmed cell death 4, three genes without known functions, one microRNA gene, the leucine-rich repeat protein SHOC2 (Selfors *et al.* 1998), and the alpha-2-adrenergic receptor ADRA2A (Devedjian *et al.* 2000). The gene encoding TCF7L2 is located 1.4 Mb distally of *Niddm1i4* and is not a probable candidate for the phenotype encoded by this QTL based on our cofactors selected by the forward selection-backward elimination stepwise regression procedure.

In conclusion, the combined analysis of genotypically selected F2-progeny and subcongenic rat strains have revealed an intricate pattern of genetic effects, which are amenable to experimental dissection and subsequent molecular identification of disease mechanisms. Four QTLs for phenotypes highly relevant to type 2 diabetes were mapped to intervals less than 1 Mb and several positional candidate genes have been selected for studies of their relevance to this disease. The complex genetic interplay between several diabetes susceptibility loci under controlled environmental conditions, as reported here, emphasizes the need for caution when attempting to identify disease mechanisms and risk alleles in the genetically and environmentally heterogeneous human population.

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FIGURE LEGENDS

FIGURE 1. Single- and multiple-marker QTL analyses of diabetes associated phenotypes within *Niddm1i* on chromosome 1. LR scores at each individual marker are indicated as thin lines for single-marker regression, and as thick lines for multiple-marker regression.

Experiment-wise significance thresholds ($\alpha=0.05$) are indicated as horizontal lines (thin line for single-marker and thick lines for multiple-marker regression). (A) postprandial glucose at 15 min (G15) during IPGTT (B) postprandial glucose at 30 min (G30), and (C) body weight.

FIGURE 2. The effect of QTLs on the 15-min postprandial glucose levels at *Niddm1i2* (*DISwe2*), *Niddm1i3* (*DIGot244*), and *Niddm1i4* (*DISmu2*) jointly estimated by GLM procedure with MINITAB. Data are presented as least squares mean \pm SEM. Numbers of animals are given within parentheses. F/F denote progeny homozygous for F344, GK/F heterozygous progeny, and, GK/GK progeny homozygous for GK.

FIGURE 3. Congenic strains within *Niddm1i* and locations of mapped loci for diabetes-associated phenotypes. The filled bars designate known homozygous GK segments; the open ends designate intervals containing the recombinant end-points. The locations and confidence intervals of loci influencing bodyweight (*Niddm1i1*, abbreviated *Ni1i1*) and postprandial glucose (*Niddm1i2-4*, abbreviated *Ni2i2-4*) are indicated as striped bars. The positions of the genes *Sorcs1*, *Sorcs3*, and *Tcf7l2* are indicated. The locations of markers and genes were taken from Ensembl Rattus Norvegicus version 40.34j based on RGSC 3.4 (<http://www.ensembl.org>) determined according to release 33.34c from ENSEMBL, based on NCBI assembly 3.4 of the rat genome. The figure is drawn to scale.

FIGURE 1.

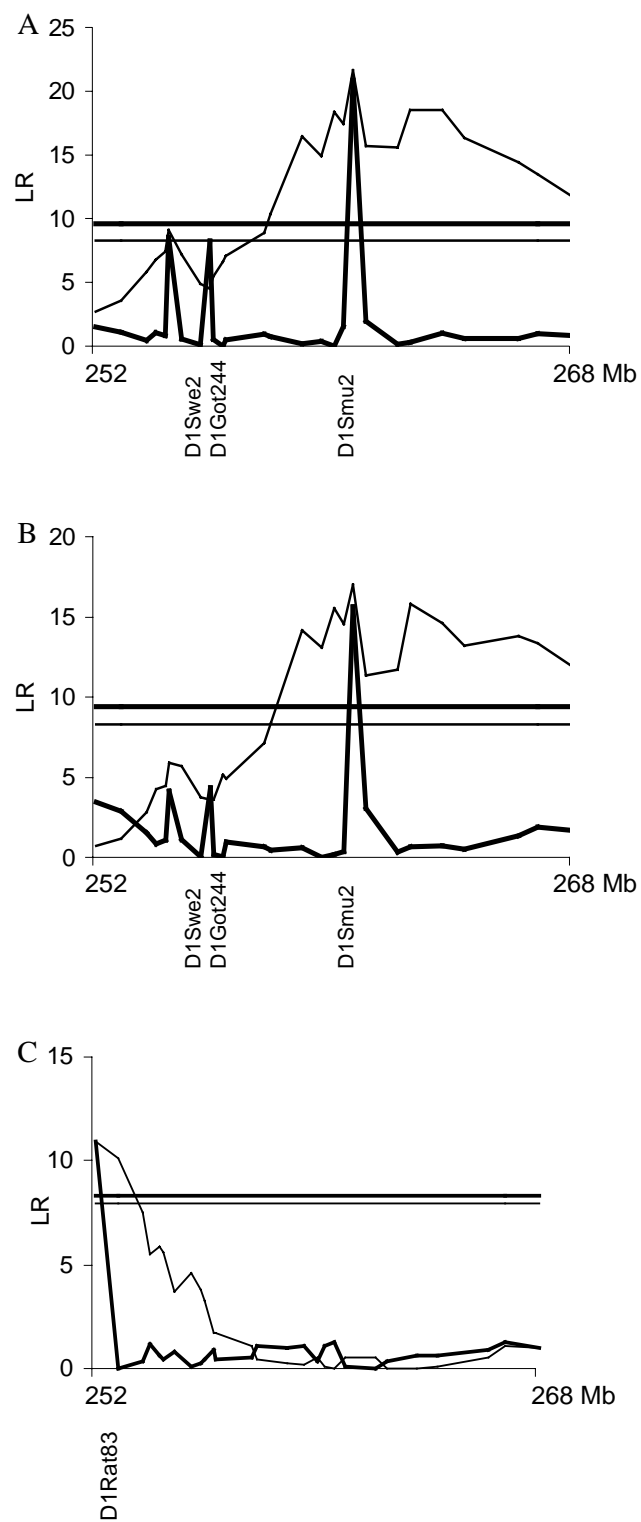


FIGURE 2.

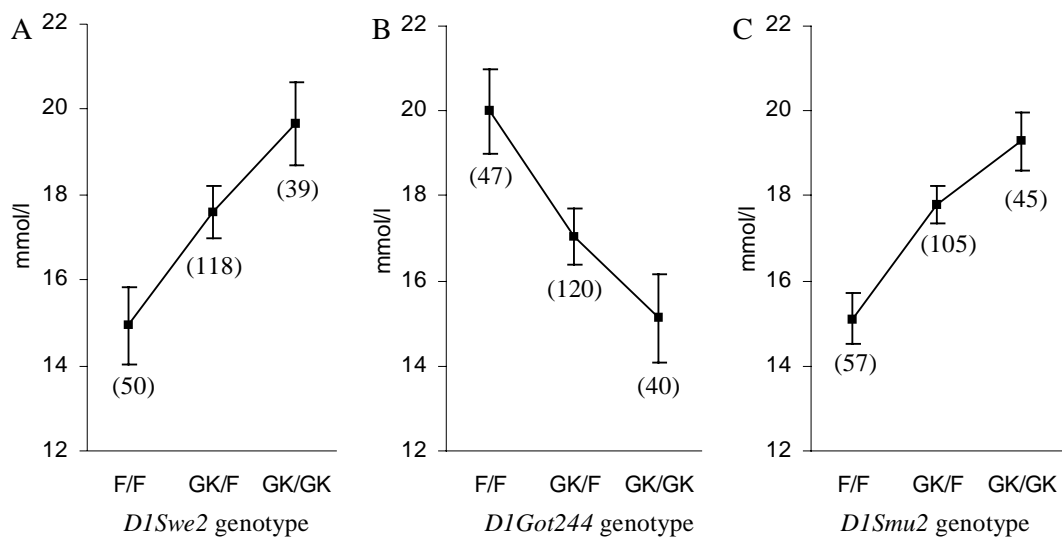


FIGURE 3.

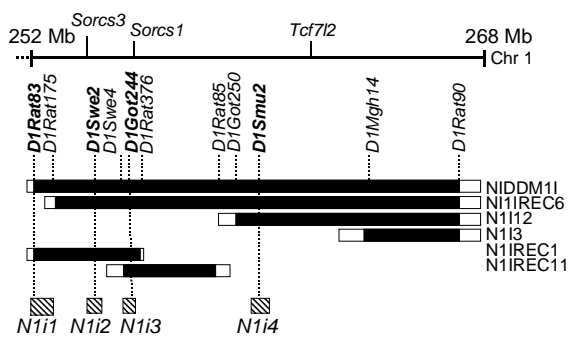


TABLE 1

Microsatellite markers used for *Niddm1i* and genotypes of congenic NIDDM1I and subcongenic strains

Marker	Chr 1 (Mb)	NIDDM1I	N1IREC6	N1I12	N1I3	N1IREC1	N1IREC11
<i>D1Rat83</i>	252.1	GK	F	F	F	GK	F
<i>D1Arb46</i>	252.3	GK	F	F	F	GK	F
<i>D1Rat452</i>	252.6	GK	F	F	F	GK	F
<i>D1Rat175</i>	252.9*	GK	GK	F	F	GK	F
<i>D1Got249</i>	253.7	GK	GK	F	F	GK	F
<i>D1Swe1</i>	254.0	GK	GK	F	F	GK	F
<i>D1Got247</i>	254.3	GK	GK	F	F	GK	F
<i>D1Swe2</i>	254.4	GK	GK	F	F	GK	F
<i>D1Swe3</i>	254.8	GK	GK	F	F	GK	F
<i>D1Swe4</i>	255.4	GK	GK	F	F	GK	GK
<i>D1Got244</i>	255.7	GK	GK	F	F	GK	GK
<i>D1Rat376</i>	255.8*	GK	GK	F	F	GK	GK
<i>D1Swe5</i>	256.1	GK	GK	F	F	F	GK
<i>D1Swe6</i>	256.2	GK	GK	F	F	F	GK
<i>D1Mit8</i>	257.4	GK	GK	F	F	F	GK
<i>D1Rat477</i>	257.6	GK	GK	F	F	F	GK
<i>D1Rat85</i>	258.6	GK	GK	F	F	F	GK
<i>D1Got250</i>	259.2	GK	GK	GK	F	F	F
<i>D1Swe7</i>	259.6	GK	GK	GK	F	F	F
<i>D1Swe8</i>	259.9	GK	GK	GK	F	F	F
<i>D1Smu2</i>	260.2*	GK	GK	GK	F	F	F
<i>D1Got251</i>	260.6	GK	GK	GK	F	F	F
<i>D1Rat456</i>	261.6	GK	GK	GK	F	F	F
<i>D1Mgh15</i>	262.0	GK	GK	GK	F	F	F
<i>D1Smu1</i>	263.0	GK	GK	GK	F	F	F
<i>D1Mgh14</i>	263.7	GK	GK	GK	GK	F	F
<i>D1Got259</i>	263.7*	GK	GK	GK	GK	F	F
<i>D1Rat457</i>	265.4	GK	GK	GK	GK	F	F
<i>D1Mit14</i>	266.0	GK	GK	GK	GK	F	F
<i>D1Rat90</i>	267.1	GK	GK	GK	GK	F	F

Genotypes of congenic strains are indicated as F344-derived genome (F) and GK-derived genome (GK). * Approximate position based of the number of F2 recombinants as the marker was not mapped in NCBI assembly 3.4 of the rat genome.

TABLE 2

Quantitative trait loci for diabetes-associated traits identified by multiple marker QTL analysis

QTL	Marker±SI (Mb) ^a	Trait	LR ^b	Nominal P-value	Effect ^c ±SE	Var ^d percent	Number of genes ^e
<i>Niddm1i1</i>	<i>D1Rat83</i> +0.8	Bw	11.0**	1x10 ⁻³	-6.8±2.0	5.1	13
<i>Niddm1i2</i>	<i>D1Swe2</i> ±0.25	G15	8.5*	3x10 ⁻³	+2.4±0.8	4.0	2
		G30	4.1	0.04	+1.4±0.7	2.0	
<i>Niddm1i3</i>	<i>D1Got244</i> ±0.2	G15	8.3*	4x10 ⁻³	-2.6±0.9	3.9	1
		G30	4.3	0.04	-1.5±0.7	2.1	
<i>Niddm1i4</i>	<i>D1Smu2</i> ±0.35	G15	20.9***	5x10 ⁻⁶	+2.2±0.5	9.6	7
		G30	15.6**	8x10 ⁻⁵	+1.5±0.4	7.2	

Bw, Body weight (g); G15, postprandial glucose at 15 min, and, G30 at 30 min in mmol/l.

a, SI for QTL support intervals estimated by the 1.5-LOD drop method; Mb, mega base pair.

b, LR denotes the likelihood ratio test statistic for QTLs and level of significance: * suggestive experiment-wise significance ($\alpha=0.2$); ** significant experiment-wise significance ($\alpha=0.05$); *** highly significant experiment-wise significance ($\alpha=0.001$).

c, additive effect defined as AA-BB/2, where AA is the genotypic value for GK homozygotes at the QTL, and BB is the genotypic value for F344 homozygotes; SE, standard error.

d, Var is percentage of phenotypic residual variance explained by the QTL

e, the number of positional candidate genes within the QTL interval using NCBI assembly 3.4 of the rat genome.

TABLE 3**Phenotypic characterization of F344, NIDDM1I and subcongenic strains**

Trait	F344	NIDDM1I	N1IREC6	N1I12	N1I3	N1IREC1	N1IREC11
Bw	289±3	286±3	307±2 (<i>P</i> =1x10 ⁻⁶)	303±3 (<i>P</i> =1x10 ⁻³)	286±4	290±5	279±1 (<i>P</i> =6x10 ⁻³)
I15	7.3±0.4	5.5±0.6 (<i>P</i> =8x10 ⁻³)	5.3±0.5 (<i>P</i> =4x10 ⁻³)	7.5±0.8	6.3±0.9	7.2±0.4	9.3±1.0 (<i>P</i> =0.04)
G15	18.1±0.6	20.4±0.5 (<i>P</i> =4x10 ⁻³)	22.0±0.6 (<i>P</i> =3x10 ⁻⁵)	20.5±0.5 (<i>P</i> =3x10 ⁻³)	18.5±0.8	18.5±0.4	17.0±0.5
G30	13.7±0.5	16.5±0.7 (<i>P</i> =1x10 ⁻³)	18.7±0.6 (<i>P</i> =1x10 ⁻⁷)	15.5±0.5 (<i>P</i> =0.01)	13.9±0.6	13.5±0.5	11.4±0.6 (<i>P</i> =5x10 ⁻³)

Values are mean ± SEM at 95 days of age. Bw, body weight (g) for 22-26 rats/strain. I15, postprandial insulin (µg/l) at 15 min, G15, postprandial glucose 15 at min and 30 min (G30) in mmol/l for 12-18 rats per strain during IPGTT. Phenotypes for the congenic strains were compared with those for F344 and evaluated by an unpaired Student t-test. Nominal p-values below 0.05 are indicated.

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