Genet. Res., Camb. (1999), **73**, *pp.* 29–36. *With* 2 *figures.* Printed in the United Kingdom © 1999 Cambridge University Press

Identification of possible quantitative trait loci responsible for hyperglycaemia after 70% pancreatectomy using a spontaneously diabetogenic rat

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(*Received 8 June 1998 and in revised form 24 August 1998*)

Summary

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat is an animal model for obese-type non-insulin-dependent diabetes mellitus (NIDDM) in humans. The OLETF rat exhibits sustained hyperglycaemia after partial pancreatectomy, while the normal control rat does not. This difference is thought to be genetically determined and to be caused by impairment of β-cell regrowth, a possible event involved in the pathogenesis of NIDDM. Our investigation was designed to identify quantitative trait loci (QTL) responsible for post-pancreatectomy hyperglycaemia by performing a genome-wide scan in an F_2 intercross obtained by mating the OLETF and Fischer-344 (F344) rats. We have identified three possible QTL on rat chromosomes (Chrs) 3, 14 and 19 that account for a total of approximately 75% of the genetic variance in the F_2. For the QTL on Chr 14, the OLETF allele corresponds with increased glucose levels, as expected. Surprisingly, for the QTL on Chr 19, the F344 allele corresponds with increased glucose levels. The Chr 3 QTL exhibits heterosis, heterozygotes showing significantly higher glucose levels than OLETF or F344 homozygotes. We also found evidence for interaction (epistasis) between the QTL on Chrs 14 and 19.

1. Introduction

Pancreatectomy has been recognized as a model allowing investigation of the correlation between limitation of islet number and expression of diabetes (Allen, 1922; Friedman & Marble, 1941; Martin & Lacy, 1963; Bonner-Weir *et al.*, 1983). A 90% pancreatectomy in rats caused hyperglycaemia, and acceleration of β-cell replenishment and secretory abnormalities in the remaining β-cells associated with chronic hyperglycaemia (Bonner-Weir *et al.*, 1983; Weir *et al.*, 1986). In contrast, a study of 60% pancreatectomy in the rats has indicated that plasma glucose levels are unchanged and insulin secretory responses are also normal when adjusted for the lower β-cell mass, but that mild hyperglycaemia and β-cell dysfunction are both unmasked simply by adding sucrose to the water supply (Leahy *et al.*, 1988). Furthermore, it has been reported that there is no discernible or qualitative change in glucose tolerance and insulin secretory responses after 40% pancreatectomy and that regrowth of much of the excised islet tissue in an important compensatory mechanism contributing to this maintenance of normal function (Lee *et al.*, 1988). Thus, it is likely that insufficient β-cell regrowth followed by β-cell dysfunction associated with glucose toxicity after pancreatectomy induces a lasting hyperglycaemia (Weir *et al.*, 1986).

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat, a genetic model of spontaneous development of non-insulin-dependent diabetes mellitus (NIDDM), exhibits hyperglycaemic obesity with hyperinsulinaemia and insulin resistance, along with pathological changes in pancreatic islets, and has been considered as one of the best models for human NIDDM with mild obesity (Kawano *et al.*, 1991, 1992). Zhu *et al.* (1996) have demonstrated that after 70% pancreatectomy at 6 weeks of age, a sustained hyperglycaemia is evident in the OLETF rat but not in its diabetes-resistant counterpart, the Long-

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Evans Tokushima Otsuka (LETO) rat, used as a normal control. The OLETF rat exhibits insulin resistance at around 16 weeks of age or older but not at 10 weeks of age or younger, at which age post-pancreatectomy hyperglycaemia is observed (Ishida *et al*., 1995). Thus, it is predicted that the OLETF rat expresses the sustained hyperglycaemia through impaired β-cell regrowth and/or β-cell function after 70% pancreatectomy, both or either of which may be determined by genetic predispositions by the OLETF but not the LETO rat. Indeed, a poor capacity for proliferation of pancreatic β-cells characterized by a decrease in β-cell mass has been detected in the pancreatectomized OLETF rat, and proposed to be genetically determined and to be the primary cause of the sustained hyperglycaemia in this model (Zhu *et al*., 1996).

Since impaired pancreatic β-cell proliferation is associated with events involved in the pathogenesis of NIDDM, it is important to identify the genetic determinants, if any, contributing to this β-cell defect. As the first step towards this goal we investigated the molecular genetic basis of the sustained hyperglycaemia after 70% pancreatectomy in the OLETF rat, which is thought to derive from this β-cell defect, by performing a genome scan in an F_2 progeny bred from the OLETF rat.

2. Materials and methods

(i) *Rat strains*

An inbred strain of the OLETF rat (OLETF/Otk) was obtained by selective breeding, based on glucose intolerance for 20 generations, of non-diabetic Long-Evans rats, which were purchased from Charles River Canada (St Constant, Quebec, Canada) (Kawano *et al*., 1991, 1992). These OLETF rats have been maintained for more than 40 generations by sister–brother mating in Tokushima Research Institute, Otsuka Pharmaceutical Co. (Tokushima, Japan). Inbred Fisher-344 (F344) rats (F344/Crj) were obtained from Charles River Japan (Yokohama, Japan). We crossed female OLETF rats with male non-diabetic F344 rats to generate F_1 progeny which, in turn, were used to generate 98 male F_2, progeny. All rats were kept under specific pathogen-free conditions. The temperature (21 ± 2 °C), humidity (55 ± 5%) and air conditioning were controlled. Rats had free access to tap water and standard laboratory chow (Oriental Yeast, Tokyo, Japan) and were maintained at a 12 h light and dark cycle (6 a.m./6 p.m.).

(ii) *Animal procedures*

Only male rats 6 weeks of age at the beginning of the study were subjected to 70% pancreatectomy for genetics studies. After an overnight fast, animals were anaesthetized with ether and given additional either if needed during surgery. All pancreatic tissue was removed by gentle abrasion with cotton applicators, except for an automatically well defined remnant bordered by the branch of the hepatic portal vein and the first portion of the duodenal loop. After surgery, the rats had food and water available ad libitum. Non-fasting blood glucose concentrations were measured at 4–5 p.m. before surgery (0 day) and at 3, 7, 14, 21 and 28 days after surgery. Blood samples were obtained by tail snipping, and non-fasting blood glucose values were determined by the glucose oxidase method (Toecho Super, Kyoto Daiichi Kagaku, Kyoto, Japan) (Takezaki *et al*., 1994). In this method, glucose oxidase converts blood glucose to gluconic acid and, at the same time, discharges an electron to the ferricyanide ion, reducing it and converting it to ferrocyanide ion. The electric current is generated by electrochemically oxidizing the ferrocyanide ion. The blood glucose level is obtained by measurement of the electric current that is generated in proportion to the glucose concentration. Liver was dissected and frozen at -80 °C for subsequent isolation of high-molecular-weight DNA. This study conforms to the guidelines for the care and use of laboratory animals of University of Tokushima School of Medicine.

(iii) *Effective number of genes*

A variance-stabilizing transformation (natural logarithm) was applied to the phenotypic data. The effective number (n) of genetic factors controlling each trait was estimated using the classical formula of Wright (1968):

$$n = (\mu_{P1} - \mu_{P2})^2 / 8\sigma_G^2,$$

where μ_{P1} and μ_{P2} are the mean phenotypes of the two parental strains, and σ_G^2 is the genetic variance of the phenotype in the F_2 progeny. The genetic variance was estimated as

$$\sigma_G^2 = \sigma_{F2}^2 - \sigma_E^2,$$

where σ_{F2}^2 is the total phenotypic variance and σ_E^2 is the environmental component of the phenotypic variance in the F_2 progeny. The environmental component of the variance was inferred from the F_1 generation: $\sigma_E^2 = \sigma_{F1}^2$.

(iv) *Genotype analysis by the polymerase chain reaction*

We purchased primer pairs for over 600 microsatellite markers (Jacob *et al*., 1995; Gauguier *et al*., 1996) (Research Genetics, Huntsville, AL), and chose a set of 188 markers that were polymorphic in OLETF and F344 rats. We genotyped 98 (OLETF female × F344

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Table 1. *Phenotypic characteristics of parental strains, F_1 progeny and F_3 progeny*

Phenotype	OLETF	F344	F_1 progeny	F_2 progeny
No. of animals	13	9	16	98
Glucose levels at 0 d (mg/dl)	108·4 ± 2·2	102·6 ± 2·2	113·7 ± 2·1	107·5 ± 0·9
Glucose levels at 3 d (mg/dl)	140·5 ± 11·5	115·8 ± 3·4	207·4 ± 12·2	141·8 ± 7·3
Glucose levels at 7 d (mg/dl)	264·9 ± 22·9	123·4 ± 2·2	266·4 ± 15·2	181·1 ± 7·6
Glucose levels at 14 d (mg/dl)	251·2 ± 27·1	120·1 ± 5·9	261·1 ± 17·6	181·8 ± 8·8
Glucose levels at 21 d (mg/dl)	247·1 ± 22·6	109·9 ± 1·6	246·8 ± 20·8	179·6 ± 10·2
Glucose levels at 28 d (mg/dl)	279·3 ± 41·4	117·8 ± 2·7	286·1 ± 21·9	185·8 ± 11·1

Phenotypes are given as mean ± SE. Only male rats were partially pancreatectomized at 6 weeks of age. Non-fasting glucose concentrations were determined before and after the surgery. Phenotypes in the parental strains and F_1 progeny were compared by a one-way analysis of variance with Scheffé's *F* analysis as *post hoc* test.

male) F_2 progeny and the parental strains by polymerase chain reaction (PCR) amplification of the polymorphic markers. PCR was performed using 200 μl microtubes and a PC-800 thermal cycler (ASTEC, Fukuoka, Japan). The reaction volume was 10 μl, containing final concentrations of 10 mM Tris-HCl (pH 8·8), 50 mM KCl, 1·5 mM $MgCl_2$, 0·1 % Triton X-100 and 200 μM each of dATP, dCTP, dGTP and dTTP. Genomic DNA (50 ng), 0·5 U of *Taq* polymerase (Wako Pure Chemical Industries, Osaka, Japan) and 0·66 μM of each primer were used. The PCR protocol was as follows: 2 min at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1·5 min at 55 °C and 1·5 min at 72 °C. PCR products were mixed with the loading buffer supplemented with xylene cyanol and bromophenol blue dyes in 25 % glycerine, and separated by electrophoresis on 4 % MetaPhor (FMC BioProducts, Rockland, ME) agarose gel. After electrophoresis, the gel was stained with ethidium bromide, and photographed under UV light using Polaroid film type 667 (Polaroid, Cambridge, MA). Some PCR products were fluorescence-labelled and analysed by electrophoresis in standard denaturing sequencing gel using an ABI Prism 377 gene scan system (Perkin Elmer, Emeryville, CA).

(v) *Data analysis*

To identify the quantitative trait loci (QTL) affecting susceptibility to post-pancreatectomy hyperglycaemia, we carried out a total genome scan on the F_2 progeny using a set of 188 informative simple sequence length polymorphisms, covering approximately 80 % of the genome. Genetic markers were mapped relative to each other with the MAPMAKER/EXP computer package, using an error detection procedure (Lander *et al*., 1987; Lincoln & Lander, 1992). QTL affecting phenotypes were mapped relative to genetic markers with the MAPMAKER/QTL computer package (Lincoln *et al*., 1992). A number of methods for QTL mapping in experimental populations are generally performed by finding correlations between the inheritance of particular genetic markers and variation in the phenotype for each individual in the population. MAPMAKER/QTL extends these methods to provide support for 'interval mapping', allowing one fully to exploit the information provided by a genetic linkage map, and to calculate LOD scores for putative QTL, providing a measure of the support for any particular hypothesis. Thus, the program calculates the most likely phenotypic effect of having genotypes F344/F344, F344/OLETF or OLETF/OLETF at a putative QTL and then calculates a LOD score reflecting the strength of evidence for the existence of the QTL and the proportion of the phenotypic variance explained (Lander & Botstein, 1989). The genomic-wide threshold for significance in an F_2 intercross is a LOD score of 4·3, corresponding to a *P* value of $5{\cdot}2 \times 10^{-5}$ (Lander & Kruglyak, 1995). A *P* value of 0·0016 and/or a LOD score of 2·8 was used as the threshold for possible linkage (Lander & Kruglyak, 1995). Once a significant or possible QTL for a trait is identified, it is appropriate to assess whether it has any effect on other traits using a point-wise, rather than a genome-wide, significance threshold of $P = 0{\cdot}05$. Note that the threshold set by Lander & Kruglyak (1995) would be a conservative one in our present study, based on approximately 80 % coverage of the genome by genetic markers. Phenotypic comparisons for different genotypic groups were performed by using a Student's *t*-test or an analysis of variance with a *post hoc* test using a Scheffé's *F*. Interaction between QTL was tested by two-way analysis of variance (Paterson *et al*., 1991). Two-way analysis of variance was performed by multiple linear regression with the PROC GLM routine of the computer program SAS (SAS Institute, Cary, NC). For each pair of QTL tested, the dependent variable of glucose values was regressed on the independent variables of genotype at each QTL individually, plus the interaction of the given pair of QTL (Paterson *et al*., 1991). The average effect of each two-locus genotype was calculated with the PROC GLM routine of the computer program SAS

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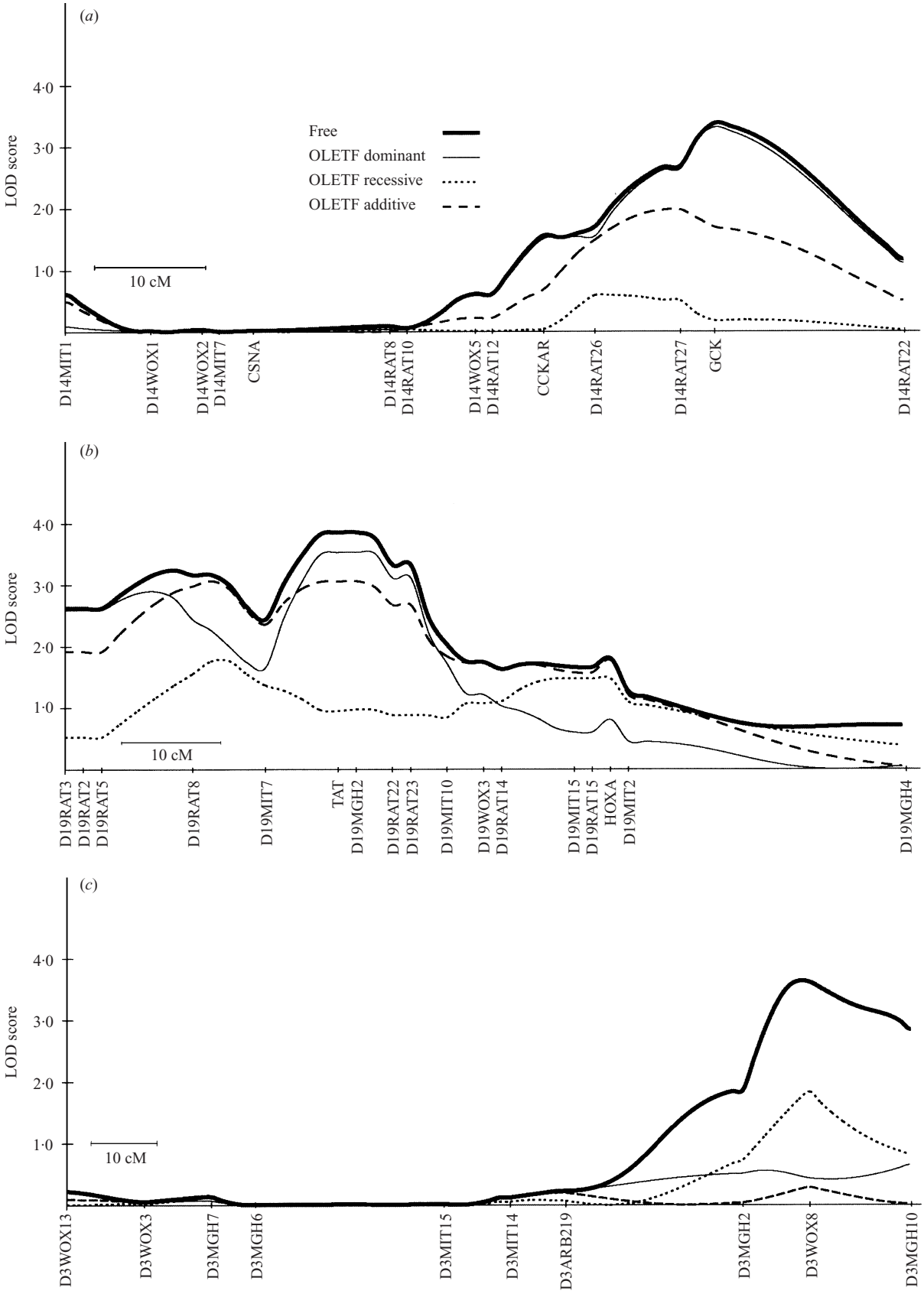

Fig. 1. Scans for LOD scores for linkages to glucose levels 7 days after surgery on Chr 14 (*a*), to glucose levels 21 days after surgery on Chr 19 (*b*) and to glucose levels 21 days after surgery on Chr 3 (*c*). The abscissa labels indicate microsatellite markers used for the linkage analysis, and map distances in centimorgans determined by the Kosambi map

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Table 2. *LOD scores for the Chr 3 QTL, Chr 14 QTI and Chr 19 QTL*

Phenotype	Chr 3 QTL	Chr 14 QTL	Chr 19 QTL
Glucose levels at 0 d	0·11 (NS)	0·15 (NS)	0·54 (NS)
Glucose levels at 3 d	2·54 ($P = 0·003$)	1·25 (NS)	2·70 ($P = 0·002$)
Glucose levels at 7 d	2·03 ($P = 0·009$)	3·37 ($P = 0·0004$)	3·17 ($P = 0·0007$)
Glucose levels at 14 d	2·47 ($P = 0·004$)	1·53 ($P = 0·03$)	1·80 ($P = 0·02$)
Glucose levels at 21 d	3·63 ($P = 0·0002$)	1·13 (NS)	3·86 ($P = 0·0001$)
Glucose levels at 28 d	2·16 ($P = 0·007$)	0·91 (NS)	3·34 ($P = 0·0005$)

Maximum LOD scores at or near the maximum likelihood position for glucose at 21 d in the case of the Chr 3 QTL, for glucose at 7 d in the case of the Chr 14 QTL and for glucose at 21 d in the case of the Chr 19 QTL. Pointwise *P* values were used for evaluating secondary phenotypes of the indicated QTL.
NS, not significant (pointwise $P > 0·05$).

(SAS Institute, Cary, NC). QTL genotypes in individual animals were inferred from genotypes of flanking markers closest to the LOD score peak.

3. Results

Only males, 6 weeks of age, from the parental strains, the 16 F_1 progeny and the 98 F_2 progeny were partially pancreatectomized and phenotyped for non-fasting blood glucose concentrations before surgery (0 day) and at 3 days, 7 days, 14 days, 21 days and 28 days after surgery (Table 1). The glucose levels before the surgery did not differ significantly between the OLETF and F344 rats; however, the glucose levels after the surgery were increased significantly ($P < 0·0001$) in the OLETF rats as compared with the F344 rats. The F_1 progeny showed that hyperglycaemia after pancreatectomy is inherited in a dominant manner. Compared with the F_1 progeny, the F_2 progeny showed slightly lower means and larger variance in the glucose levels after pancreatectomy. The increased variance of post-pancreatectomy glucose levels among F_2 animals reflects the segregation of alleles contributing to the difference between the parental strains.

We estimated the proportion of genetic variance and the number of genes responsible for the post-pancreatectomy glucose levels. The proportion of genetic variance in the F_2 was found to be about 30–54% by comparison of the variances in F_1 and F_2 progenies, and the effective number of genes was estimated as two or three by Wright's formula (Wright, 1968), depending upon the time of measurements. If the genetic variance were divided equally among three loci, each would explain approximately 10–18% of total phenotypic variance. These results indicate that post-pancreatectomy hyperglycaemia in the OLETF rat is clearly polygenic, and suggest that the trait is amenable to genetic dissection.

According to the criteria set by Lander & Kruglyak (1995), we found statistical evidence for three QTL affecting non-fasting glucose levels after pancreatectomy.

A gene on chromosome (Chr) 14, near *GCK*, showed a maximum LOD score of 3·37 for glucose levels 7 d after the surgery (Fig. 1*a*; Table 2), and accounted for 14% of the phenotypic variation in the F_2 progeny. This locus exerted effects on glucose levels 14 d after the surgery (Table 2), and the inheritance pattern at the loci was consistent with OLET F alleles acting in a dominant mode of inheritance to increase the level of plasma glucose (Fig. 1*a*; Table 3).

A QTL on Chr 19, near *D19Mgh2*, was linked (LOD score = 3·86) to the glucose levels 21 d after the surgery (Fig. 1*b*; Table 2), accounting for 16% of the phenotypic variation in the F_2 progeny. This Chr 19 QTL affected glucose levels at all days tested after the surgery (Table 2) and, interestingly, the F344 alleles were associated with increased glucose levels through acting in a recessive or additive manner (Fig. 1*b*; Table 3). This was surprising, given that the F344 rat strain exhibits no significant increase in glucose levels in response to pancreatectomy (Table 1). A tempting hypothesis is that the F344 allele of this QTL has an effect on post-pancreatectomy hyperglycaemia only in combination with OLETF alleles of other loci – that is, epistasis (see below).

A QTL linked to *D3Wox8* on Chr 3 designates a possible locus (LOD score = 3·63) for the level of glucose 21 d after the surgery (Fig. 1*c*; Table 2), and

function are represented. The LOD scores were calculated by the MAPMAKER/QTL program at 2 cm intervals spanning the distance between each pair of polymorphic markers. 'Free' refers to fitting independent values for the mean phenotypes for each of the three possible genotypical classes; the other labels refer to constraining these mean phenotypes according to the indicated model. The resulting LOD scores reflect the log-likelihood of the observed data under each model. We rejected a model in favour of an alternative when the LOD score under the first model was lower than the LOD score under the second model by at least 1 unit. The QTL on Chr 3 conforms to the inheritance pattern of superdominance.

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Table 3. *Additive effect on post-pancreatectomy glucose levels and dominance deviation of the OLETF allele at three OTL*

Locus	Phenotype	Peak LOD	Additive effect[a]	Dominance deviation[a]
Chr 3 QTL	Glucose at 21 days	3·63	−0.779	72·76
Chr 14 QTL	Glucose at 7 days	3·37	27·43	29·52
Chr 19 QTL	Glucose at 21 days	3·86	−56·21	−35·69

[a] Allele effects are of the OLETF allele contrasted with the F344 allele, and on glucose levels at 21 d in the case of the Chr 3 QTL, on glucose levels at 7 d in the case of the Chr 14 QTL and on glucose levels at 21 d in the case of the Chr 19 QTL.

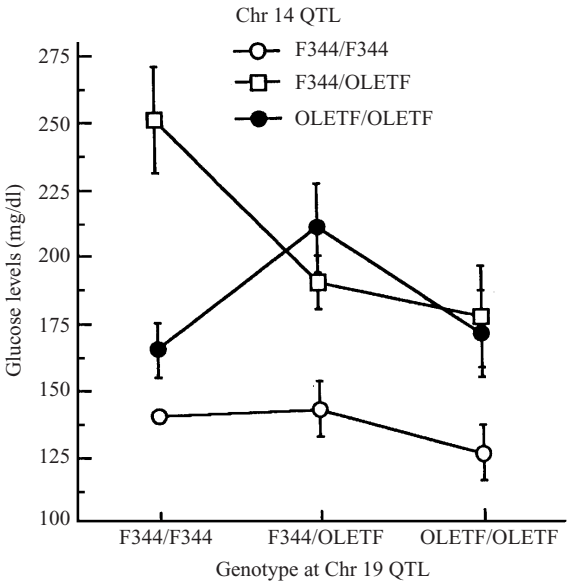

Fig. 2. Interaction between Chr 19 QTL and Chr 14 QTL. To investigate interaction between the two loci, QTL genotype was inferred from the genotype of the closest microsatellite marker. The Chr 19 QTL–Chr 14 QTL interaction was significant ($P = 0{\cdot}001$) when the genotype of the closest microsatellite marker is used as the QTL genotype. Data points are the average of glucose levels at 7 d after pancreatectomy in the F_2 for each possible two-locus genotype, and error bars 1 standard error of the mean.

explains 17% of the phenotypic variation in the F_2 progeny. Interestingly, this locus exhibited overdominance, heterozygotes showing significantly higher glucose levels than OLETF homozygotes or F344 homozygotes, and no difference in effects between the two homozygote classes, reflecting an intra-allelic interaction (Table 3). The Chr 3 QTL had an effect on glucose levels at all days tested after the surgery (Table 2).

Our finding on Chr 19 of a QTL for which the F344 allele is associated with increased glucose levels prompted us to test for interaction between loci. Using two-way analysis of variance we found significant interaction between Chr 19 QTL and Chr 14 QTL ($P = 0{\cdot}001$ for glucose levels at 7 d after pancreatectomy; Fig. 2). This interaction suggests that Chr 19 QTL and Chr 14 QTL might encode components of a signalling cascade involved in post-pancreatectomy β-cell growth and function, and that there might be some interaction between the two components.

Note that possible evidence ($P = 0{\cdot}0008$) for an X-linked locus affecting glucose levels at 7 d after pancreatectomy was found in a region near an X-linked marker, *DXMgh4*, by an analysis of variance.

4. Discussion

In the present study we demonstrated that sustained hyperglycaemia after pancreatectomy in the OLETF rat is clearly polygenic, and identified three QTL influencing susceptibility to post-pancreatectomy hyperglycaemia on Chrs 3, 14 and 19 using the F_2 progeny obtained from the cross of OLETF and F344 rats. The three QTL together explain 22–38% of the total phenotypic variance or 70–82% of the genetic variance, depending on the time of measurements. The OLETF allele at the Chr 14 QTL is associated with increased glucose levels through acting in a dominant manner. Surprisingly, for the Chr 19 QTL, the F344 allele is associated with increased glucose levels through acting in a recessive or additive manner. Further, for the Chr 3 QTL, overdominance was detected, in which the maximal effect of increased glucose levels is exerted by combination of the OLETF and F344 alleles. Identification of the Chr 19 QTL and the Chr 3 QTL, respectively, characterized by susceptible F344 allele and overdominance, might explain the occurrence of the F_1 or F_2 individuals possessing glucose levels outside the range of OLETF rat values.

Besides identification of chromosomal regions, QTL mapping allows us to begin to investigate relationships between genes. We found epistatic interaction between the Chr 14 QTL and Chr 19 QTL, suggesting that the two loci might encode components of a signalling cascade involved in post-pancreatectomy β-cell growth and function, and that there might be some interaction between the two components. F344 rat is an inbred strain that exhibits no significant increase in

https://doi.org/10.1017/S0016672398003644 Published online by Cambridge University Press

glucose levels in response to pancreatectomy. Thus, if F344 alleles of any QTL increase post-pancreatectomy glucose levels in the F_2, they might do so by interacting with OLETF alleles at other loci; that is, the F344 allele of Chr 19 QTL only increases post-pancreatectomy glucose levels, or fails to decrease post-pancreatectomy glucose levels, in a rat that also has an OLETF allele on Chr 14 QTL. This is illustrated in Fig. 2. Our present data indicated that F344 homozygotes at the Chr 19 QTL show increased glucose levels only in a rat that is heterozygous for the Chr 14 QTL.

We have mapped the Chr 3 QTL, Chr 14 QTL and Chr 19 QTL to 30 cM, 27 cM and 38 cM 2-LOD support intervals, respectively. When the three intervals are searched for potential candidate genes based on their roles in mediating β-cell growth and function, there exist the glucokinase (*GCK*)/*MODY2* locus (Froguel *et al*., 1993), the insulin-like growth factor binding protein 1 (*IGFBP*1) locus (Hogg *et al*., 1994) and the insulin-like growth factor binding protein 3 (*IGFBP*3) locus (Hogg *et al*., 1994) that are physiologically relevant candidates for the Chr 14 QTL. In contrast, no obvious candidate genes are known within the intervals of the Chr 3 QTL and the Chr 19 QTL. The human chromosomal regions homologous to the rat genomic intervals of the three QTL and 20q12–q13 for the Chr 3 QTL, 7p15–p13 for the Chr 14 QTL and 4q28–q32 and 16q21–q22 for the Chr 19 QTL (Yamada *et al*., 1994; Jacob *et al*., 1995; Bihoreau *et al*., 1997). These comparative maps suggest the hepatocyte nuclear factor-4 alpha (*HNF-4α*)/*MODY1* locus (Yamagata *et al*., 1996) and the potassium voltage-gated channel (*KCNB1*) locus (Inagaki *et al*., 1995) as positional candidate genes for the Chr 3 QTL and the carboxypeptidase E (*CPE*) locus (Naggert *et al*., 1995), the neuropeptide Y receptor Y2 (*NPY2R*) locus (Herzog *et al*., 1997) and the neuropeptide Y receptor Y5 (*NPY5R*) locus (Herzon *et al*., 1997) as positional candidates for the Chr 19 QTL, all of which are physiologically involved in β-cell growth and function.

The OLETF rat becomes spontaneously hyperglycaemic from 18 weeks of age onwards (Kawano *et al*., 1991, 1992). Nara *et al*. (1997) have recently identified the QTL responsible for spontaneous persistent hyperglycaemia on Chrs 1, 5, 7 and 17 using 145 (OLETF female × F344 male) F_2 progeny and the 100 (OLETF female × LETO male) F_2 progeny at 30 weeks of age. We have also mapped the major spontaneous hyperglycaemia susceptibility loci on Chrs 7, 8, 11 and 14 using 160 (OLETF female × F344 male) F_2 progeny at 30 weeks of age (our unpublished data). Since the spontaneous hyperglycaemia susceptibility locus on Chr 14 lies 40–50 cM distant from the 2-LOD support interval of the Chr 14 QTL for post-pancreatectomy hyperglycaemia obtained in this study, the two loci appear to be different. Thus, it is likely that the mapped loci for post-pancreatectomy hyperglycaemia are distinct from those of spontaneous hyperglycaemia. The sustained hyperglycaemia induced by 70% pancreatectomy in the OLETF rat at 6 weeks of age before the onset of insulin resistance is associated with insufficient proliferation of β-cells in response to severe stress of pancreatectomy (Zhu *et al*., 1996). On the other hand, during the history of spontaneous development of hyperglycaemia in the OLETF rat without pancreatectomy, insulin resistance occurs first in prediabetic states and, subsequently β-cells normally respond to the mild stress of insulin resistance by increasing insulin secretion in an early stage of diabetogenesis, resulting in hyperinsulinaemia (Ishida *et al*., 1995). A hypothesis arises that the post-pancreatectomy hyperglycaemia susceptibility loci detected in this study correspond mainly to the genes involved in β-cell proliferation, while the QTL for spontaneous hyperglycaemia at 30 weeks of age correspond mainly to genes involved in insulin sensitivity. However, it is expected from the data of the response to pancreatectomy that the OLETF β-cells, in themselves, are fragile and then become unable to respond normally to insulin resistance with increasing age. If searching for the QTL for spontaneous hyperglycaemia in rats at older age than 30 weeks, we might identify the genes involved in β-cell proliferation together with insulin sensitivity as the hyperglycaemia susceptibility QTL.

We are grateful to Dr Tetsuya Yoshinaga for providing several programs for data assembly on the UNIX computer. This work was supported in part by research grant from the Ministry of Education, Science and Culture, Japan, and The Japan Diabetes Foundation, Japan.

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