Genet. Res., Camb. (2001), **77**, *pp.* 183–190. *With* 2 *figures.* Printed in the United Kingdom © 2001 Cambridge University Press

Effects of *Dmo1* on obesity, dyslipidaemia and hyperglycaemia in the Otsuka Long Evans Tokushima Fatty strain

SHIRO OKUNO[1], TAKESHI K. WATANABE[1]*, TOSHIHIDE ONO[1], KEIKO OGA[1], AYAKO MIZOGUCHI-MIYAKITA[1], YUKI YAMASAKI[1], YOSHIHIRO GOTO[1], HIROICHI SHINOMIYA[1], HIROSHI MOMOTA[1], HIDEO MIYAO[1], ISAMU HAYASHI[1], TOSHIHIRO ASAI[1], MIKIO SUZUKI[1], YOSUKE HARADA[1], HARETSUGU HISHIGAKI[1], SHIGEYUKI WAKITANI[2], TOSHIHISA TAKAGI[3], YUSUKE NAKAMURA[4] AND AKIRA TANIGAMI[1]

[1] *Otsuka GEN Research Institute, Otsuka Pharmaceutical Co., Ltd, 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-0192, Japan*
[2] *Department of Orthopaedic Surgery, Osaka-Minami National Hospital, 2-1 Kidohigashi-machi, Kawachinagano, Osaka 586-8521, Japan*
[3] *Laboratory of Genome Database, and* [4] *Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai Minato-ku, Tokyo 108-8639, Japan*

(*Received 5 September 2000 and in revised form 5 October 2000*)

Summary

Whole-genome scans have identified *Dmo1* as a major quantitative trait locus (QTL) for obesity and dyslipidaemia in the Otsuka Long Evans Tokushima Fatty (OLETF) rat. We have produced congenic rats for the *Dmo1* locus, using marker-assisted speed congenic protocols, enforced by selective removal of other QTL regions (QTL-marker-assisted counterselection), to efficiently transfer chromosomal segments from non-diabetic Fischer 344 (F344) rats into the OLETF background. In the third generation of congenic animals, we observed a substantial therapeutic effect of the *Dmo1* locus on lipid metabolism, obesity control and plasma glucose homeostasis. We conclude that single-allele correction of an impaired genetic pathway can generate a substantial therapeutic effect, despite the complex polygenic nature of type II diabetic syndromes.

1. Introduction

Dyslipidaemia and obesity are closely associated with type II diabetes in humans (Reaven & Greenfield, 1981; Lewis *et al*., 1991; Howard & Howard, 1994). Our Otsuka Long Evans Tokushima Fatty (OLETF) strain is a useful obese type II diabetes model with accompanying hypertriglyceridaemia, hyperinsulinaemia, hyperglycaemia, insulin resistance and abundant visceral fat (Kawano *et al*., 1992). Our previous studies have demonstrated that the OLETF genome is predisposed to 'type II diabetes' but that full development of the phenotype requires 'obesity' (and probably 'dyslipidaemia'). As in humans, obesity is a strong risk factor for diabetes in the OLETF strain (Okauchi *et al*., 1995*a*, *b*; Ishida *et al*., 1996; Sakamoto *et al*., 1998). Moreover, prior to the relatively late onset of diabetes and apparent insulin resistance, obesity and dyslipidaemia appear fairly early in the strain (Kawano *et al*., 1992; Sato *et al*., 1995; Man *et al*., 1997). Therefore, it is likely that dyslipidaemia and obesity are involved in the development of diabetes in the OLETF strain.

Genome-wide QTL analyses identified twelve statistically significant QTLs (*Diabetes Mellitus OLETF type 1* through *Dmo12*) that are associated with type II diabetes, obesity or dyslipidaemia phenotypes of the OLETF rat. *Dmo1*, assigned around the marker *D1Rat90* on rat chromosome 1, was found to be the major QTL, showing strong effects on body weight and fat weight, plasma free fatty acid, total cholesterol and triglyceride levels (Kanemoto *et al*., 1998; Okuno *et al*., 1999; Watanabe *et al*., 1999*a*; Yamasaki *et al*., 2000).

To evaluate the *Dmo1* effect precisely, we have established congenic lines for the *Dmo1* locus. We have generated animals containing an F344 *Dmo1* allele in place of one OLETF allele (heterozygous state) on the approximately 94% OLETF back-

* Corresponding author. Tel: +81 886 65 2888. Fax: +81 886 37 1035. e-mail: watanabe@otsuka.gr.jp

ground. Third-generation rats (BC3) were used for phenotypic evaluation. The introgressed 28 cM interval for *Dmo1* proved to produce substantial therapeutic effects on obesity (reducing body weight and fat weight), dyslipidaemia (lowering plasma total cholesterol and triglyceride values) and hyperglycaemia (lowering plasma glucose levels 60 min after oral glucose challenge).

2. Materials and methods

(i) *Animals*

Fischer 344/DuCrj (F344) male rats were purchased from Charles River Japan, and OLETF female rats were obtained from Laboratory of Animal Resources, Otsuka Pharmaceutical. Animals were maintained in the facility of CLEA Japan (Shizuoka, Japan) under the following conditions. Rats had free access to sterilized distilled water and a sterilized laboratory diet (CE-2, CLEA Japan; 4·4% fat content and 11·5% of total calorie intake from fat) and were maintained on a 12 h light and dark cycle. Temperature (20–26 °C), humidity (55–70%) and air conditioning were all controlled.

For congenic breeding, female OLETF rats were crossed with male F344 rats to produce F1 hybrids. F1 males were backcrossed to female OLETF rats to produce the first generation (BC1). Selected BCn (n = generation number, 1–3) males were backcrossed to OLETF females, producing succeeding generations. Female OLETF rats crossed with BC2 congenic males were selected based on body weight at 8 weeks to minimize a probable maternal effect in the BC3 progenies. In every generation, only male rats were subjected to genotype and phenotype analyses; females were killed at birth. Tail tips of male rats were cut off at 4 weeks to obtain genomic DNA for genotype assays.

For the genotype analyses, we first analysed all male animals using six markers spanning the *Dmo1* region (see below and Table 1). Animals heterozygous (OLETF/F344) for all markers across this region (Retained) were selected for further genome-wide scanning. To maximize OLETF background genome replacement in succeeding generations, we used animals showing the highest replacement rates for subsequent backcrosses. We also counter-selected animals homozygous (OLETF/OLETF) at other previously reported QTLs (Kanemoto *et al*., 1998; Okuno *et al*., 1999; Watanabe *et al*., 1999*a*) to exclude possible phenotypic effects of these QTLs in generations as early as BC3. Of 66 BC3 male rats, 19 (10 Retained and 9 Control) rats with higher background replacement rates were raised for phenotypic analysis. We also characterized 10 Retained and 10 Control rats from the BC2 generation; these data are summarized in Table 2.

(ii) *Marker-assisted speed congenic protocols*

We estimated a 95% confidence interval for the *Dmo1* QTL using data from three previously genotyped crosses (Kanemoto *et al*., 1998; Okuno *et al*., 1999; Watanabe *et al*., 1999*a*) according to Darvasi & Soller (1997). (95% confidence interval = $530/N\upsilon$, where N = population size and υ = the proportion of variance explained.)

The *Dmo1* Target Interval (Fig. 1, line A) is defined by two terminal markers, *D1Rat169* and *D1Rat459*, including the proposed confidence interval. Supporting genetic linkage maps were constructed using the JoinMap Program as described previously (Kanemoto *et al*., 1998; Watanabe *et al*., 2000).

To genotype the *Dmo1* locus, six markers encompassing a 28 cM interval were used (*D1Rat169*–1 cM–*D1Rat77*–12 cM–*D1Rat306*–10 cM–*D1Rat225*–5 cM–*D1Rat90*, *D1Rat459*) (Steen *et al*., 1999). Whole-genome scanning was performed using 117 markers covering all chromosomes with an average spacing of 13·4 cM (corresponding to about 1500 cM in total). Markers were purchased from Research Genetics or synthesized by International Reagents (Japan) on the basis of published reports (Steen *et al*., 1999; Watanabe *et al*., 1999*b*).

Table 1. *Summary of congenic breeding from BC1 to BC3*

	Male rats[a]	Retained[b]	Replacement (%)[c]	Mated[d]	Replacement (%)[e]
F1	8			8	
BC1	128	75	48·74 (30·19–69·35)	11	61·37 (57·57–69·35)
BC2	128	57	80·53 (71·59–92·10)	17	85·65 (78·37–92·10)
BC3	66	20	92·90 (87·31–99·26)	10	94·38 (91·00–99·26)

[a] Total number of male rats born.
[b] Number of male rats harbouring the target interval as heterozygous.
[c] Average replacement rates and ranges (in parentheses) calculated from *b*.
[d] Number of rats contributing to succeeding generations.
[e] Average replacement rates and ranges (in parentheses) calculated from *d*.

https://doi.org/10.1017/S0016672301004918 Published online by Cambridge University Press

Table 2. *Statistical analyses of obese and diabetic phenotypes of BC2 and BC3 generations*

	BC2				BC3			
Trait/weeks	Control, O/O ($n = 10$)	Retained, O/F ($n = 10$)	P value	% of experimental variance explained by the QTL	Control, O/O ($n = 9$)	Retained, O/F ($n = 10$)	P value	% of experimental variance explained by the QTL
BW/16 weeks (g)	457 ± 9 (407–495)	441 ± 9 (395–470)		8·36	488 ± 13 (430–569)	444 ± 8 (401–492)	*	31·9
BW/20 weeks (g)	477 ± 7 (442–515)	448 ± 10 (378–486)	*	24·3	563 ± 12 (499–627)	496 ± 13 (411–563)	**	44·5
BW/24 weeks (g)	564 ± 8 (530–604)	526 ± 13 (420–564)	*	24·1	626 ± 14 (548–707)	546 ± 12 (499–620)	***	53·4
BW/28 weeks (g)	573 ± 8 (540–622)	530 ± 14 (418–619)	*	26·9	630 ± 17 (534–723)	566 ± 12 (519–638)	**	38·0
BW/29 weeks (g)	570 ± 9 (540–619)	528 ± 14 (422–571)	*	26·3	634 ± 15 (556–724)	568 ± 11 (526–641)	**	43·1
FW/29 weeks (g)	47·12 ± 1·86 (36·75–54·90)	37·78 ± 2·32 (18·72–42·72)	**	38·3	71·07 ± 5·04 (53·10–103·15)	50·74 ± 2·31 (40·70–68·33)	**	45·9
INS/29 weeks (ng/ml)[a]	1·986 ± 0·224 (1·294–3·250)	1·849 ± 0·242 (1·000–3·095)		0·95	4·544 ± 0·569 (2·072–7·063)	3·933 ± 0·393 (2·381–6·423)		5·30
NEFA/10 weeks (mEq/l)	ND	ND			1·144 ± 0·054 (0·899–1·385)	1·186 ± 0·092 (0·760–1·782)		0·83
NEFA/29 weeks (mEq/l)	0·967 ± 0·053 (0·622–1·128)	0·804 ± 0·054 (0·529–1·091)	*	20·4	0·832 ± 0·034 (0·724–1·019)	0·750 ± 0·052 (0·557–0·971)		8·87
TC/10 weeks (mmol/l)	ND	ND			1·384 ± 0·054 (1·159–1·598)	1·311 ± 0·047 (1·096–1·479)		5·75
TC/29 weeks (mmol/l)	2·517 ± 0·100 (1·996–3·015)	2·028 ± 0·066 (1·730–2·356)	***	47·8	2·291 ± 0·162 (1·415–2·917)	1·875 ± 0·106 (1·466–2·511)	*	22·0
TG/10 weeks (mg/dl)	ND	ND			77 ± 10 (36–116)	70 ± 8 (35–123)		1·72
TG/29 weeks (mg/dl)	268 ± 23 (180–385)	158 ± 9 (120–204)	***	52·3	289 ± 34 (146–451)	173 ± 17 (94–282)	*	36·4
G0 (mg/dl)	119 ± 5 (93–136)	125 ± 6 (88–158)		3·18	140 ± 7 (120–172)	144 ± 4 (128–171)		1·38
G30 (mg/dl)	270 ± 13 (213–324)	274 ± 13 (223–338)		0·35	352 ± 15 (292–413)	313 ± 16 (244–366)		15·8
G60 (mg/dl)	307 ± 19 (206–384)	303 ± 25 (210–418)		0·07	396 ± 14 (313–447)	330 ± 18 (225–406)	**	34·4
G90 (mg/dl)	321 ± 21 (229–404)	302 ± 24 (195–405)		1·91	389 ± 14 (324–436)	342 ± 19 (250–415)		18·2
G120 (mg/dl)	310 ± 17 (237–399)	266 ± 27 (146–396)		9·47	346 ± 23 (211–434)	318 ± 17 (215–383)		5·04

Basic statistical data (mean ± SEM) and ranges (in parentheses) are shown. Statistical significance (ANOVA, P values) is marked by asterisks: *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

[a] INS data for two BC3 control animals (nos. 270 and 304) were not available for calculation.

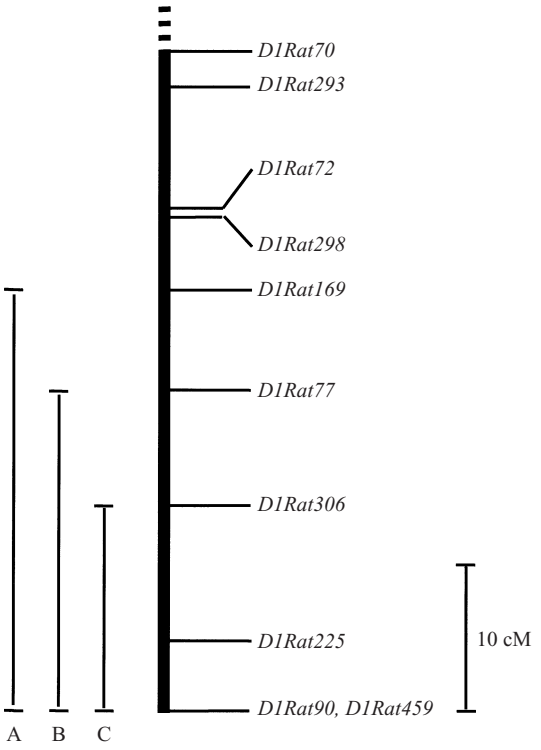

Fig. 1. Estimation of 95% confidence intervals for the *Dmo1* locus on integrated genetic linkage maps. Confidence intervals were calculated based on previous QTL studies. (A) indicates the introgressed interval; (B) indicates confidence intervals calculated from the (OLETF × BN)F2 and (OLETF × F344)F2 studies; (C) indicates confidence intervals calculated from the (OLETF × BN) × OLETF study. The genetic linkage map was constructed using data from the (OLETF × F344)F2 cross ($n = 157$).

(iii) *Phenotype evaluation*

Body weight (BW) was measured at 16, 20, 24, 28 (fasting) and 29 (fasting) weeks. Rats were killed at 29 weeks, and abdominal fat weight (FW) was weighed using visceral fat tissues from mesenteric, epididymal and retroperitoneal adipose tissues.

Plasma insulin (INS) levels were measured at 29 weeks under fasting conditions using a commercially available rat insulin ELISA kit (AKRIN-010, Shibayagi, Gunma, Japan).

Plasma lipids (total cholesterol (TC), triglyceride (TG) and non-esterified fatty acid (NEFA)) were measured at 10 and 29 weeks. Animals were fasted overnight (16 h) before measurements were taken. Lipid values were measured by a peroxidase-coupled method (McGowan *et al.*, 1983), using a COBAS MIRA auto-analytical machine (Roche Diagnostics, Tokyo, Japan) with the following kits: Triglycerides GPO Unimate 5, Unimate Cholesterol kit (Roche Diagnostics, Tokyo, Japan), AutoSera NEFA (Daiichi Pure Chemical, Tokyo, Japan).

At 28 weeks, we performed an oral glucose tolerance test (OGTT) to evaluate glucose tolerance after a 16 h fasting period. Blood samples were collected from tail veins before and during OGTT (0 min (G0), 30 min (G30), 60 min (G60), 90 min (G90) and 120 min (G120)). Plasma glucose levels were measured using the ANTSENSE II system (Bayer Medical, Tokyo, Japan) according to the manufacturer's protocol.

(iv) *Statistical analysis*

Statistical analyses were performed using Statview 4.5 software (Abacus Concepts, CA, and SAS Institute, NC). To evaluate phenotypic differences between Retained and Control groups, we applied one-way analysis of variance (ANOVA). First, we determined whether the variances of the two groups were significantly different using Bartlett's methods (1937, $\alpha = 0{\cdot}25$). If values were not significant, we applied the standard one-way ANOVA test. When values were significant, we applied Welch's methods (1951). Data calculated using either method are shown in Table 2. The significance threshold for ANOVA was set to $\alpha = 0{\cdot}05$ (95% confidence). The correlation for multiple phenotypes using Bonferroni's method has not been applied because the phenotypes examined in this study were closely related.

3. Results

(i) *Evaluation of the confidence interval for QTL* Dmo1

Confidence intervals ($P < 0{\cdot}05$) for the *Dmo1* locus were displayed on the (OLETF × F344)F2 map (Fig. 1). The (OLETF × BN) × OLETF backcross produced a confidence interval extending from *D1Rat306* to the telomere; (OLETF × BN)F2 and (OLETF × F344)F2 studies suggested an interval extending from *D1Rat77* to the telomere (Fig. 1*B*, *C*). We conservatively introgressed a Target Interval between *D1Rat169* and *D1Rat459* (Fig. 1*A*). Four additional internal markers (*D1Rat77*, *D1Rat306*, *D1Rat225* and *D1Rat90*) were used in genotyping assays to exclude double recombination events. We estimated the distance between the markers *D1Rat169* and *D1Rat459* to be 28·8 cM in our two F2 genetic linkage maps (Yamasaki *et al.*, unpublished), which agrees reasonably well with the estimate of 28 cM by Steen *et al.* (1999).

(ii) *Establishment of congenic rats by the BC3 generation*

The congenic rat breeding diagram is summarized in Table 1. Male rats retaining the entire target interval as OLETF/F344 heterozygous (Retained) were selected for whole-genome scanning. To maximize OLETF background genome replacement in succeeding generations, we used animals showing the highest replacement rates for subsequent backcrosses. Third-

https://doi.org/10.1017/S0016672301004918 Published online by Cambridge University Press

generation rats (BC3) were used to evaluate the *Dmo1* effect: we analyzed 10 animals that were heterozygous at the target interval (Retained) and 9 animals that were homozygous in this region (Control). Replacement efficiencies were averaged for each generation (61·37% for BC1 and 85·65% for BC2); these observed values are significantly higher than the calculated theoretical values (50% for BC1 and 75% for BC2). The average replacement rate for the 10 Retained BC3 animals was 94·38% (range = 91·00–99·26%).

(iii) *Genotype–phenotype associations*

Table 2 summarizes the results of ANOVA for BC2 and BC3. Since 14·32% of the BC2 genome remained OLETF/F344 heterozygous and the number of progeny ($n = 20$) was not large enough to normalize such an effect, the data should be treated as preliminary. In the analysis of 10 Retained and 10 Control BC2 rats, we observed significant differences in BW/20–29 weeks, FW, TC, TG and NEFA, but not in BW/16 weeks and INS/29 weeks. We also found no significant differences in G0–G120 values.

In the BC3 generation, approximately 94% of the genome was OLETF homozygous, making these animals useful for evaluating the *Dmo1* function independently of other loci. BW of Retained and Control groups differed significantly at all ages examined ($3{\cdot}81 \times 10^{-4} < P < 0{\cdot}016$), explaining as much as 31·9–53·4% of the phenotypic variance (EXP). The reduction in BW/24 weeks in the Retained group is as large as 80 g, corresponding to a 12·8% reduction in BW relative to Control rats. This difference explained as much as 53·4% of the phenotypic variance, with the remaining 46·6% attributed to environmental factors.

We also observed highly statistically significant differences for FW/29 weeks (20·33 g), accounting for approximately 31% of the total BW reduction at 29 weeks. This indicates that 45·9% of the total variance could be explained by the locus ($P = 3{\cdot}59 \times 10^{-3}$).

We observed statistically significant differences in plasma lipid values in BC3 rats at 29 weeks, but not at 10 weeks. At 29 weeks, TC levels between the Control and Retained groups differed by 0·416 mmol/l ($P = 0{\cdot}043$), indicating that 22·0% of the variance could be explained by genetic effects of the locus. We observed the same trend for TG. At 29 weeks, we observed a 116 mg/dl difference ($P = 0{\cdot}011$, EXP = 36·4%), consistent with a strong correlation between TC and TG values. We saw no difference between genotypes for NEFA values.

We also wanted to assess the differences in plasma glucose levels. G0 and INS are not significantly different between the two groups. However, we observed a highly significant (16·7%) reduction in G60 values for the Retained group relative to Control animals at BC3 ($P = 8{\cdot}34 \times 10^{-4}$, and EXP = 34·4%). We also observed the same tendency for G30 (39 mg/dl difference, $P = 0{\cdot}092$) and G90 (47 mg/dl difference, $P = 0{\cdot}069$) although the differences were not statistically significant.

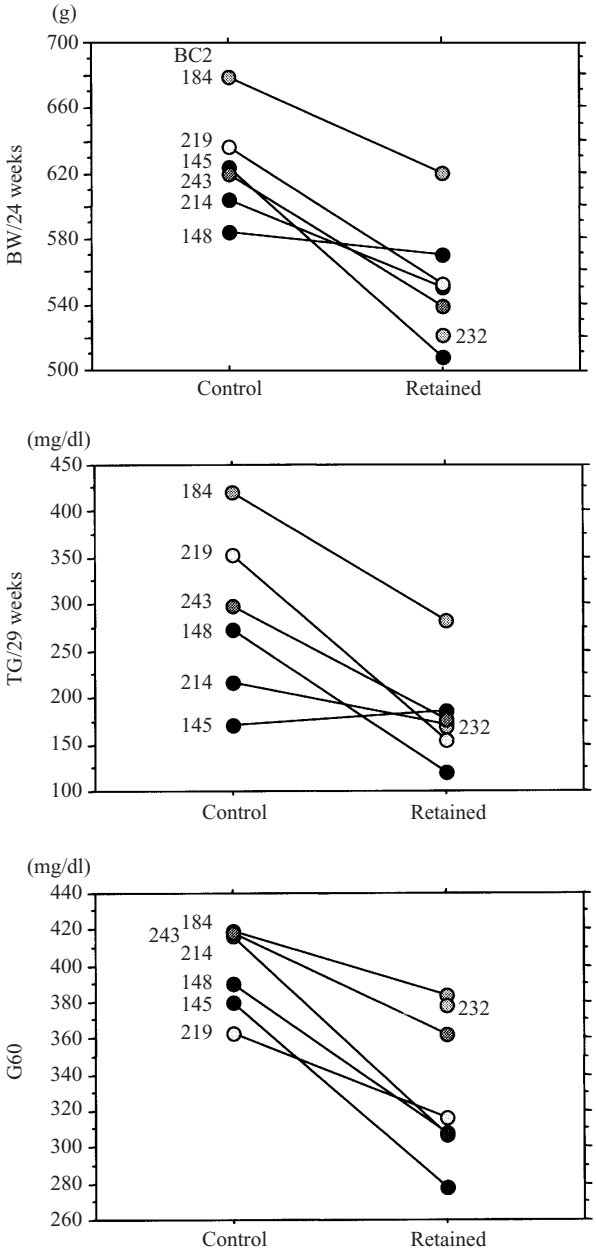

Fig. 2. Inter- or intra-family differences for three representative phenotypes. Mean values of BW/24 weeks, TG/29 weeks and G60 of Retained and Control BC3 rats are shown for each family (derived from the same BC2 parents). Number represents the ID for founder BC2 males.

Fig. 2 compares mean values of measured phenotypes for BC3 Retained and Control rats grouped by 'family' (i.e. BC3 males derived from the same BC2 parents). Phenotypic trends within each family were similar to those observed in all animals (Table 2).

https://doi.org/10.1017/S0016672301004918 Published online by Cambridge University Press

4. Discussion

In our previous QTL scanning studies, we identified 12 highly statistically significant QTLs (Lander & Kruglyak, 1995) that are involved in diabetes, obesity and dyslipidaemia phenotypes of the OLETF rat. Effects of the *Dmo1* locus on body weight, abdominal fat weight, plasma lipid values (TG and TC) and plasma glucose values have been confirmed repeatedly in distinct genetic analyses. (Kanemoto *et al.*, 1998; Okuno *et al.*, 1999; Watanabe *et al.*, 1999*a*; Yamasaki *et al.*, in press).

We have bred congenic rats to isolate predisposing genes and to determine whether obesity, type II diabetes and other clinical phenotypes of the OLETF strain might be treatable by partially correcting one of their multiple defects (St Lezin *et al.*, 1998). In this study, we describe congenic rats carrying a *Dmo1* allele from the non-obese and non-diabetic F344 rat in the context of the OLETF background. Through marker-assisted speed congenic protocols (Markel *et al.*, 1997), augmented by QTL-marker-assisted counter-selection (Bennett & Johnson, 1998), we have targeted genomic intervals of approximately 28 cM, defined by two terminal markers, *D1Rat169* and *D1Rat459*. This region is statistically likely to harbour the genetic component(s) of *Dmo1*.

BC3 congenic animals contain 5·62% of the F344 genome outside the targeted *Dmo1* region. This value is much smaller than the theoretical value of 12·5% for the BC3 generation and is instead more comparable to the 6·25% calculated for the BC4 generation. These data suggest that the marker-assisted speed congenic protocols worked efficiently. We characterized ten Retained and nine Control BC3 rats with regard to several different phenotypes (BW, FW, TG, TC, NEFA, INS, G0, G30, G60, G90 and G120) at various ages (10, 16, 20, 24, 28, 29 weeks).

(i) *Obesity* (*BW and FW*)

We have confirmed the effects of *Dmo1* on obesity; our congenic strains reflect the strong effect of *Dmo1* on BW at 16–29 weeks. At 29 weeks, introduction of a single F344 *Dmo1* allele caused a 66 g BW reduction compared with the Control group. The difference in FW is more dramatic: a 20·3 g reduction that accounts for 30·8% of the decrease in BW/29 weeks (Table 2). The large contribution of abdominal FW to BW reduction implies that the majority of the differences observed in BW might be attributed to overall fat reduction. The phenotypic variance explained by the QTL is as large as 53·4% for BW/24 weeks, implying that half the variance could be explained by genetic factors and half by environmental factors (Table 2).

(ii) *Dyslipidaemia* (*TGs, TCs and NEFAs*)

In the BC3 generation of congenic rats, the effect of the *Dmo1* locus accounts for 22·0% of the difference in TC/29 weeks ($P = 0{\cdot}043$) and 36·4% in TG/29 weeks ($P = 0{\cdot}011$). For TG/29 weeks, the Retained group showed a reduction of over 100 mg/dl compared with Control animals. Thus, the therapeutic effect of the F344 *Dmo1* allele on dyslipidaemia is large after introduction of a single allele. This effect will be confirmed by homozygous (F344/F344) congenic strains, in which both OLETF alleles are corrected.

(iii) *Glucose intolerance* (*G0, G30, G60, G90, G120 and INS*)

We have found a significant difference in G60 values between the Retained and Control groups ($P = 8{\cdot}34 \times 10^{-3}$) and a similar tendency for G30 and G90 values (Table 2). The decrease in G60 was as large as 66 mg/dl in Retained animals, potentially accounting for 34·4% of the variance – that is, one-third of the variance can be explained by the *Dmo1* genetic effect. G0 and G120 values were not significantly different between the groups, however. Although the glucose-lowering effect is substantial, plasma glucose levels in the Retained group did not fall to the levels characteristic of non-diabetic strains.

The substantial improvement in diabetes, obesity and dyslipidaemia phenotypes achieved by single-allele correction of the *Dmo1* pathway is promising in that these specific gene pathways could be targets of effective and wide-ranging therapeutic interventions.

(v) *Future prospects: comparison with other animal models and man*

This is the first report of congenic rats derived from an important model of obese type II diabetes, the OLETF rat. EXPs for *Dmo1* increased significantly compared with those observed in previous QTL studies (BC1 or F2 crosses) by normalizing other genetic factors: $\sim 35\%$ versus 10% (G60), $\sim 53\%$ versus $\sim 16\%$ (BW), $\sim 36\%$ versus $\sim 14\%$ (TG) (Watanabe *et al.*, 1999*a*). Phenotypes for which approximately 50% of the variance can be explained by genetic factors are amenable to simple positional cloning. Therefore, heterozygous congenic resources with pronounced genetic effects are feasible primary resources for gene identification.

Data derived from *Dmo1* congenic animals can be compared with those obtained from another diabetic model, the Goto Kakizaki (GK) rat (Goto *et al.*, 1975). GK rats display apparent fasting hypergly-

https://doi.org/10.1017/S0016672301004918 Published online by Cambridge University Press

caemia and impaired secretion of insulin in response to glucose and insulin resistance, but no apparent obesity and dyslipidaemia is observed in the strain. Two QTL studies from 1996 (Galli *et al*., 1996; Gauguier *et al*., 1996) identified a major postprandial glucose QTL on chromosome 1, which co-localized with *Dmo1*. Galli *et al*. (1999) also reported the first congenic lines for the QTL. The target region overlapped with our *Dmo1* QTL, but differences in observed phenotypes might reflect differences in genetic predispositions between OLETF and GK rats.

In spontaneously diabetic BB/OK rats, a region overlapping with *Dmo1* conferred significant linkage to TC/20 weeks and TG/20–28 weeks (Kovacs *et al*., 1998). The diabetic BB/OK alleles correlated with higher TC and TG values. Taken together, data from these three animal models have confirmed that the *Dmo1* region influences lipid homeostasis and is involved in diabetes development.

We have defined the interval for the *Dmo1* region within a 28 cM interval between *D1Rat169* and *D1Rat459*. This interval is syntenic to human chromosome 10q24–26 and mouse chromosome 19 (Watanabe *et al*., 1999*b*). We are currently characterizing inter-strain polymorphisms of the candidate gene(s).

The potential contribution of the homologous locus in humans to diabetic phenotypes has also been examined. Duggirala *et al*. (2000) reported a susceptibility locus near the marker of *D10S587* on the long arm of human chromosome 10, influencing the onset and incidence of type II diabetes in Mexican Americans. We have constructed a fine syntenic map of the relevant region between rat and human (Watanabe *et al*., 1999*b*, and unpublished data) and found that *Dmo1* is likely to overlap with this chromosomal segment in human.

Further characterization of several different rodent diabetic models and human populations will shed light on the genetic predisposition to diabetes.

We would like to thank A. Tsuji (present address: Institute for Experimental Animals, Hamamatsu University School of Medicine, Japan) for his contribution to the congenic study. We also would like to thank Drs M. Horie and N. Kanemoto (Otsuka Pharmaceutical Co., Ltd) for fruitful discussions. We would like to express our sincere appreciation to Dr K. Kawano (Department of Research Management, Laboratory of Animal Resources and the First Institute of New Drug Research, Otsuka Pharmaceutical Co., Ltd), Drs T. Hirashima and T. Suzukubo (Department of Research Management, Laboratory of Animal Resources, Otsuka Pharmaceutical Co., Ltd) for providing OLETF rats and useful suggestions, and Drs Y. Irie (New Technology Evaluation Division, Otsuka Pharmaceutical Co., Ltd) and E. Takahashi (Otsuka GEN Research Institute, Otsuka Pharmaceutical Co., Ltd) for their continuous support of the project. We also acknowledge the important contributions of individuals at CLEA Japan Inc., especially Drs F. Mochizuki and S. Kimoto.

References

Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London*, *Series A* **160**, 268–282.

Bennett, B. & Johnson, T. E. (1998). Development of congenics for hypnotic sensitivity to ethanol by QTL-marker-assisted counter selection. *Mammalian Genome* **9**, 969–974.

Brown, M. B. & Forsythe, A. B. (1974). The small sample behavior of some statistics which test the equality of several means. *Technometrics* **16**, 129–132.

Darvasi, A. & Soller, M. (1997). A simple method to calculate resolving power and confidence interval of QTL map location. *Behavior Genetics* **27**, 125–132.

Duggirala, R., Blangero, J., Almasy, L., Dyer, T. D., Williams, K. L., Leach, R. J., O'Connell, P. & Stern, M. P. (2000). A major susceptibility locus influencing plasma triglyceride concentrations is located on chromosome 15q in Mexican Americans. *American Journal of Human Genetics* **66**, 1237–1245.

Galli, J., Li, L. S., Glaser, A., Ostenson, C. G., Jiao, H., Fakhrai-Rad, H., Jacob, H. J., Lander, E. S. & Luthman, H. (1996). Genetic analysis of non-insulin dependent diabetes mellitus in the GK rat. *Nature Genetics* **12**, 31–37.

Galli, J., Fakhrai-Rad, H., Kamel, A., Marcus, C., Norgren, S. & Luthman, H. (1999). Pathophysiological and genetic characterization of the major diabetes locus in GK rats. *Diabetes* **48**, 2463–2470.

Gauguier, D., Froguel, P., Parent, V., Bernard, C., Bihoreau, M. T., Portha, B., James, M. R., Penicaud, L., Lathrop, M. & Ktorza, A. (1996). Chromosomal mapping of genetic loci associated with non-insulin dependent diabetes in the GK rat. *Nature Genetics* **12**, 38–43.

Goto, Y., Kakizaki, M. & Masaki, N. (1975). Spontaneous diabetes produced by selective breeding of normal Wister rats. *Proceedings of the Japan Academy* **51**, 80–85.

Howard, B. V. & Howard, W. M. J. (1994). The pathogenesis and treatment of lipid disorders in diabetes mellitus. In *Joslin's Diabetes Mellitus*, 13th edn, pp. 372–396. Philadelphia: Lea and Febiger.

Ishida, K., Mizuno, A., Murakami, T. & Shima, K. (1996). Obesity is necessary but not sufficient for the development of diabetes mellitus. *Metabolism* **45**, 1288–1295.

Kanemoto, N., Hishigaki, H., Miyakita, A., Oga, K., Okuno, S., Tsuji, A., Takagi, T., Takahashi, E., Nakamura, Y. & Watanabe, T. K. (1998). Genetic dissection of 'OLETF', a rat model for non-insulin-dependent diabetes mellitus. *Mammalian Genome* **9**, 419–425.

Kawano, K., Hirashima, T., Mori, S., Saitoh, Y., Kurosumi, M. & Natori, T. (1992). Spontaneous long-term hyperglycemic rat with diabetic complications: Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes* **41**, 1422–1428.

Kovacs, P., van den Brandt, J. & Kloting, I. (1998). Effects of quantitative trait loci for lipid phenotypes in the rat are influenced by age. *Clinical and Experimental Pharmacology and Physiology* **25**, 1004–1007.

Lander, E. & Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics* **11**, 241–247.

Lewis, G. F., O'Meara, N. M., Soltys, P. A., Blackman, J. D., Iverius, P. H., Pugh, W. L., Getz, G. S. & Polonsky, K. S. (1991). Fasting hypertriglyceridemia in noninsulin-dependent diabetes mellitus is an important predictor of

postprandial lipid and lipoprotein abnormalities. *Journal of Clinical Endocrinology and Metabolism* **72**, 934–944.

Man, Z. W., Zhu, M., Noma, Y., Toide, K., Sato, T., Asahi, Y., Hirashima, T., Mori, S., Kawano, K., Mizuno, A., Sano, T. & Shima, K. (1997). Impaired beta-cell function and deposition of fat droplets in the pancreas as a consequence of hypertriglyceridemia in OLETF rat, a model of spontaneous NIDDM. *Diabetes* **46**, 1718–1724.

Markel, P., Shu, P., Ebeling, C., Carlson, G. A., Nagle, D. L., Smutko, J. S. & Moore, K. J. (1997). Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains. *Nature Genetics* **17**, 280–284.

McGowan, M. W., Artiss, J. D., Strandbergh, D. R. & Zak, B. (1983). A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clinical Chemistry* **29**, 538–542.

Okauchi, N., Mizuno, A., Zhu, M., Ishida, K., Sano, T., Noma, Y. & Shima, K. (1995*a*). Effects of obesity and inheritance on the development of non-insulin-dependent diabetes mellitus in Otsuka-Long-Evans-Tokushima fatty rats. *Diabetes Research and Clinical Practice* **29**, 1–10.

Okauchi, N., Mizuno, A., Yoshimoto, S., Zhu, M., Sano, T. & Shima, K. (1995*b*). Is caloric restriction effective in preventing diabetes mellitus in the Otsuka Long Evans Tokushima fatty rat, a model of spontaneous non-insulin-dependent diabetes mellitus? *Diabetes Research and Clinical Practice* **27**, 97–106.

Okuno, S., Watanabe, T. K., Ono, T., Yamasaki, Y., Goto, Y., Miyao, H., Asai, T., Kanemoto, N., Oga, K., Mizoguchi-Miyakita, A., Takagi, T., Takahashi, E., Nakamura, Y. & Tanigami, A. (1999). Genetic determinants of plasma triglyceride levels in (OLETF × BN) × OLETF backcross rats. *Genomics* **62**, 350–355.

Reaven, G. M. & Greenfield, M. S. (1981). Diabetic hypertriglyceridemia: evidence for three clinical syndromes. *Diabetes* **30**, 66–75.

Sakamoto, S., Minami, K., Niwa, Y., Ohnaka, M., Nakaya, Y., Mizuno, A., Kuwajima, M. & Shima, K. (1998). Effect of exercise training and food restriction on endothelium-dependent relaxation in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous NIDDM. *Diabetes* **47**, 82–86.

Sato, T., Asahi, Y., Toide, K. & Nakayama, N. (1995). Insulin resistance in skeletal muscle of the male Otsuka Long-Evans Tokushima Fatty rat, a new model of NIDDM. *Diabetologia* **38**, 1033–1041.

St Lezin, E., Liu, W., Wang, N., Wang, J. M., Kren, V., Zidek, V., Zdobinska, M., Krenova, D., Bottger, A., van Zutphen, B. F. & Pravenec, M. (1998). Effect of renin gene transfer on blood pressure in the spontaneously hypertensive rat. *Hypertension* **31**, 373–377.

Steen, R. G., Kwitek-Black, A. E., Glenn, C., Gullings-Handley, J., Van Etten, W., Atkinson, O. S., Appel, D., Twigger, S., Muir, M., Mull, T., Granados, M., Kissebah, M., Russo, K., Crane, R., Popp, M., Peden, M., Matise, T., Brown, D. M., Lu, J , Kingsmore, S., Tonellato, P. J., Rozen, S., Slonim, D., Young, P., Knoblauch, M., Provoost, A., Ganten, D., Colman, S. D., Rothberg, J., Lander, E. S. & Jacob, H. J. (1999). A high-density integrated genetic linkage and radiation hybrid map of the laboratory rat. *Genome Research* **9**, AP1–8.

Watanabe, T. K., Okuno, S., Oga, K., Mizoguchi-Miyakita, A., Tsuji, A., Yamasaki, Y., Hishigaki, H., Kanemoto, N., Takagi, T., Takahashi, E., Irie, Y., Nakamura, Y. & Tanigami, A. (1999*a*). Genetic dissection of 'OLETF', a rat model for non-insulin-dependent diabetes mellitus: quantitative trait locus analysis of (OLETF × BN) × OLETF. *Genomics* **58**, 233–239.

Watanabe, T. K., Bihoreau, M. T., McCarthy, L. C., Kiguwa, S. L., Hishigaki, H., Tsuji, A., Browne, J., Yamasaki, Y., Mizoguchi-Miyakita, A., Oga, K., Ono, T., Okuno, S., Kanemoto, N., Takahashi, E., Tomita, K., Hayashi, H., Adachi, M., Webber, C., Davis, M., Kiel, S., Knights, C., Smith, A., Critcher, R., Miller, J., Thangarajah, T., Day, P. J. R., Hudson, J. R., Irie, Y., Takagi, T., Nakamura, Y., Goodfellow, P. N., Lathrop, G. M., Tanigami, A. & James, M. R. (1999*b*). A radiation hybrid map of the rat genome containing 5255 markers. *Nature Genetics* **22**, 27–36.

Watanabe, T. K., Ono, T., Okuno, S., Mizoguchi-Miyakita, A., Yamasaki, Y., Kanemoto, N., Hishigaki, H., Oga, K., Takahashi, E., Irie, Y., Bihoreau, M. T., James, M. R., Lathrop, G. M., Takagi, T., Nakamura, Y. & Tanigami, A. (2000). Characterization of newly developed SSLP markers for the rat. *Mammalian Genome* **11**, 300–305.

Wei, S., Wei, K., Moralejo, D. H., Ogino, T., Koike, G., Jacob, H. J., Sugiura, K., Sasaki, Y., Yamada, T. & Matsumoto, K. (1999). Mapping and characterization of quantitative trait loci for non-insulin-dependent diabetes mellitus with an improved genetic map in the Otsuka Long-Evans Tokushima fatty rat. *Mammalian Genome* **10**, 249–258.

Welch, B. L. (1951). On the comparison of several mean values: an alternative approach. *Biometrika* **38**, 330–336.

Yamasaki, Y., Watanabe, T. K., Okuno, S., Ono, T., Oga, K., Mizoguchi-Miyakita, A., Goto, Y., Shinomiya, H., Momota, H., Miyao, H., Hayashi, I., Asai, T., Suzuki, M., Harada, Y., Haretsugu, H., Wakitani, S., Takagi, T., Nakamura, Y., Tanigami, A. (2000). Quantitative trait loci for lipid metabolism in the study of OLETF × (OLETF × F344) backcross rats. *Clinical and Experimental Pharmacology and Physiology* **27**, 881–886.