ORIGINAL

Fine mapping of the hyperglycemic and obesity QTL by congenic strains suggests multiple loci on rat chromosome 14

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Abstract : Linkage analysis previously identified a hyperglycemic quantitative trait locus (QTL), *Nidd 2/of,* on rat Chromosome 14 in crosses utilizing OLETF (Otsuka Long Evans Tokushima Fatty) rat, a model for type 2 diabetes. A separate QTL study mapped an obesity QTL, *Obs5*, to the same chromosomal region. A congenic strain placing ca. 38 cM OLETF-derived segments containing both *Nidd2/of* and *Obs5* on the F344 background was shown to possess mild diabetic and obese phenotypes, suggesting the presence of mutations affecting the glucose metabolism and fat accumulation. In order to localize the loci more precisely, we generated a series of deletion-subcongenic strains in which OLETF-segments were shortened from either ends. We found that there are at least two hyperglycemic QTLs within the *Nidd2/of* locus. We predict that they are localized towards both ends of the *Nidd2/of* region. In contrast, *Obs5* QTL was further narrowed down to a single region of ca. 10 cM fragment. J. Med. Invest. 52 : 109-113, February, 2005

Keywords *: QTL, congenic strain, OLETF, type 2 diabetes*

INTRODUCTION

Even after the completion of human genome project, it is still increasingly challenging to localize susceptibility genes that are involved in the expression of quantitative traits, such as obesity, hyperglycemia, hypertension (1, 2). This is largely due to the complex nature of interactions among those genes and/or gene-environment associations. Genetic dissection of complex trait in human is compromised by genetic heterogeneity and numerous environmental variations. Therefore, the advantage of alleviating the complexity by utilizing well-defined animal models in constant breeding condition for the study of multigene variance is far more significant than for the study of single-gene trait.

Marker-assisted linkage analyses for quantitative trait loci (QTL) and subsequent congenic strain construction have become a usual measure (3, 4). The *Nidd 2/of* locus mapped to rat chromosome 14 is one of the fourteen hyperglycemia QTLs we identified in our previous linkage analysis using the OLETF rat, a model for the type 2 diabetes (5, 6). In an independent genetic analysis we also mapped an obesity QTL, *Obs 5*, within an overlapping chromosomal segment with *Nidd2/of* (7).With subsequently generated congenic strain, this 38 cM segment was confirmed to contain polymorphisms related to both abnormal glucose regulation and retroperitoneal fat accumulation (8). Here we constructed a series of subcongenic strains, in which OLETF-derived segment is shortened from either ends in order to further narrow down the fragment which includes a QTL down to a few cM. Our result indicated that there are multiple loci in this region and each QTL appears to play a distinctive role.

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MATERIALS AND METHODS

Rat strains and animal procedures

The F.O-*Nidd2/of* congenic strain, a parental strain

containing *Nidd2/of* locus (" of "meaning OLETF rat), was intercrossed with Fischer-344 (F344/Crj, SCL, Inc. Hamamatsu Japan)(8). The resultant F1 progeny male was backcrossed with F344 females. By using microsatellite markers BC1 was screened for chromosomes in which recombination occurred within the *Nidd2/of* region. The progeny with desirable recombinant chromosomes was crossed to F344 rats and the heterozygote offsprings were selectively bred to fix the recombinant chromosome on the F344 background. The established strain was subsequently maintained by brother-sister mating. Each strain was named after the genome structure in the region. For example, F.O-*Ni2/of.rat55* indicates that the OLETF-derived segment extends from the proximal end to the position defined by D14Rat55 microsatellite marker. Similarly, F.O-*Ni2/rat55*.of exchanges the distal portion starting from the locus at D14Rat55.

All rats were kept under specific pathogen-free conditions. The temperature (21±2°C), humidity (55± 10%), and air conditioning were all controlled. Rats had free access to tap water and standard laboratory chow and were maintained at a 12-h light and dark cycle (7 am/7 pm). This study conformed to the guidelines for the care and use of Laboratory animals of The University of Tokushima Graduate School Institute of Health Bioscience.

Genotyping

DNA isolation and polymerase chain reaction (PCR) amplification of microsatellite markers were performed as described previously (5).The primers for microsatellite markers were purchased from Research Genetics, Inc. (Hrinlsville, Ala.).

Phenotyping

Oral glucose tolerance test (OGTT) were performed on male rats of 30 weeks of age as described (5). The animals were fasted overnight ; blood glucose levels were measured with the glucose oxidase method (Arkray, Kyoto, Japan) at 0, 30, 60, 90 and 120 min after oral administration of 2.0 g glucose (in a 2.8 M glucose solution) per kilogram of body weight. Animals were anesthetized with pentobarbital sodium intraperitoneally one week after the OGTT. The abdominal fat pads consisting of mesenteric, retroperitoneal, and epididymal fats were removed and weighted. The adiposity index was calculated from each fat pad and body weight (percentage of fat weight/body weight)(7).

Statistical analysis

All values are expressed as means±SE. The statistical significance of differences was evaluated using ANOVA for comparing all traits among congenic strains and control F344 strains.

RESULTS

Generation of the new congenic strains

The extent of the congenic region for newly established substrains of F.O-*Nidd2/of* is described in Figure 1. Five of these strains have distinctive deletion patterns of OLETF-derived chromosome from the distal side of the region and the other five from the proximal side. This is the same strategy as those for identifying binding site of a transcription factor within a promoter region by making a series of deletion mutants. Therefore assuming that a single QTL is sole determinant for the hyperglycemia or obesity in F.O-*Nidd2/of* strain, common region for all the substrains showing the identical phenotypes to that of the parental strain should newly define the QTL.

OGTT analysis of Nidd2/of *substrains*

The substrains were characterized by an oral glucose tolerance test (OGTT), the same test used for mapping the *Nidd2/of* (Table 1). None of the strains showed significantly increased blood glucose levels at any time point during OGTT. Since the entire congenic region in F.O-*Nidd2/of* is covered by these strains, this result suggested that there are more than one QTL within this segment. It is worth noting that the neither of the two strains which exchange the shortest segment at either end, namely F.O-*Ni2/of.rat10* and F.O-*Ni2/wox1.of,* showed equivalent hyperglycemia to that of the parental stain, suggesting that there are two QTL at both ends of the segment. This is indeed an intriguing observation because camel's hump-like LOD score curve in our original linkage analysis in this region retrospectively infers the presence of QTL at both ends (Figure 1)(9). The middle portion might also contain additional QTLs, because although statistical significance was not achieved, strains with shorter fragment tend to show proportionally lower blood glucose levels (Table 1).

Adiposity analysis of Nidd2/of *substrains*

OLETF rat is genetically susceptible to obese phenotype and obesity is important for the development of diabetes (10, 11). In the previous linkage analyses, we found that in the OLETF rat the distinctive set of loci plays a role for obesity from those for hyperglycemia phenotype. Furthermore each obesity QTL affects on

the adiposity in a body-location specific manner (7). *Obs5* was identified for increased retroperitoneal fat index. In subcongenic strains, only certain strains showed increased retroperitoneal fat index (Table 1). The likely location for the QTL is between D14Rat143 and D14Rat55, based on the fact that all strains in Figure 1 that contain all or part of this region have significant effects on adiposity. In contrast, none of the

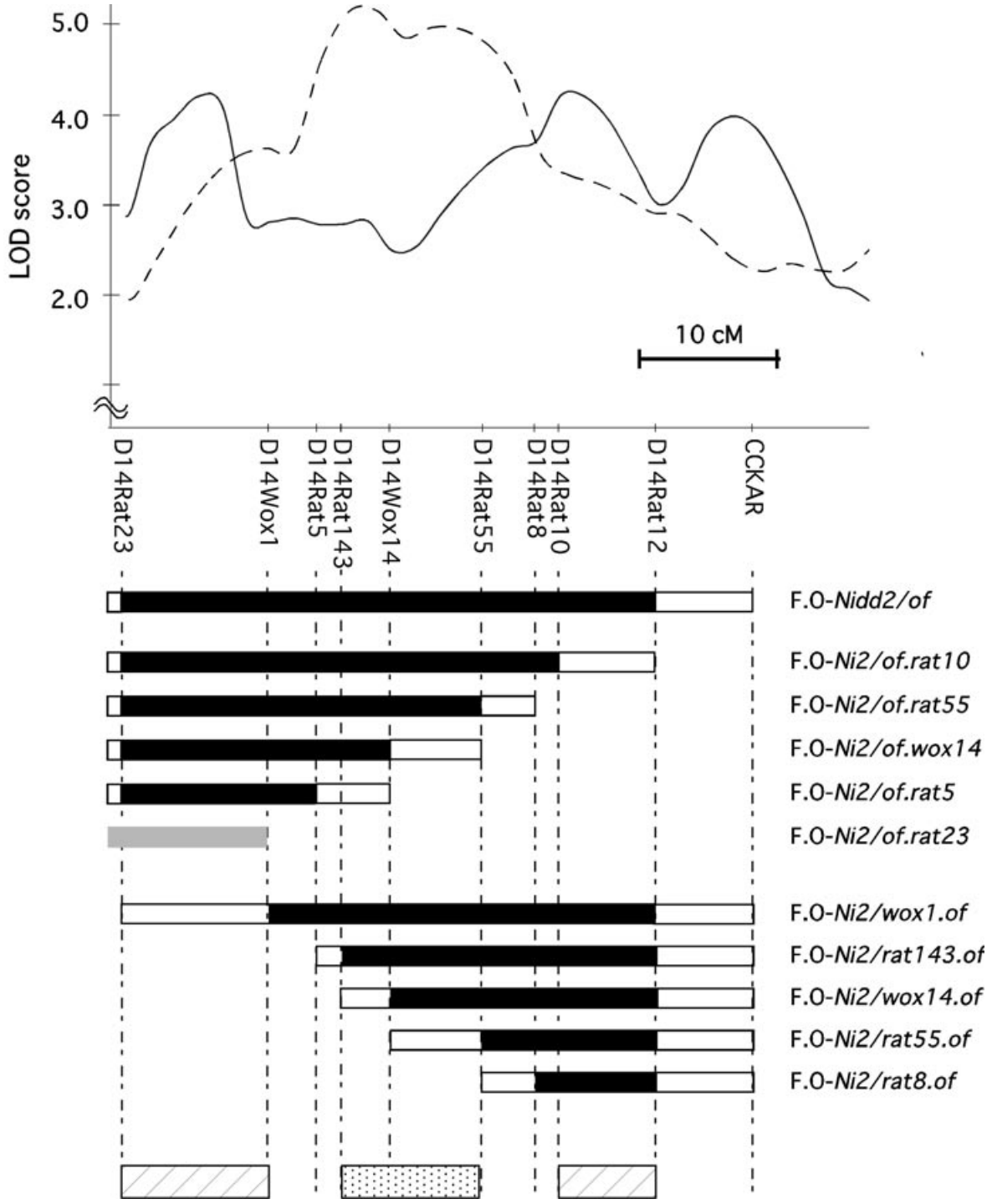

Figure. 1. Genetic map of chromosomal regions exchanged in the congenic substrains. Lod score plots for linkage to glucose level at 30 min in OGTT (solid line) and retroperitoneal fat index (dotted line) are shown (7, 9). The known extent of the OLETF-derived portion carried by each congenic strain is designated by the filled bars; the open ends of this bar designate intervals containing the recombinant end points. The strain represented by gray bar, F.O-*Ni2/of.rat23*, is known to possess OLETF-derived segment at D14Rat23 locus. Thus the interval at either side contains one recombinant end point. The markers appearing in this figure are those that are polymorphic between OLETF and F344 rats and were used for genotype check. Genetic distances represented in centiMorgans were determined by Kosambi map function. Hatched and Stippled bars indicate the fine mapped loci for *Nidd2/of* and *Obs5* QTL, respectively.

Table 1. Phenotypic characterization of congenic substrains

strain	n	0 min	30 min	60 min	90 min	120 min	AUC	BW	Mesenteric fat pad	Retroperitoneal fat pad	Epididymal fat pad	Mesentric fat Index	Retroperitoneal fat Index	Epididymal fat Index
F 344	9	79.2±2.8	105.4±3.9	109.4±2.8	106.3±2.6	102.1±2.1	12357±290	388±5.5	10.65±0.45	8.96±0.29	11.66±0.45	2.74±0.10	2.31±0.07	3.00±0.09
F.O-*Nidd 2/of*	8	81.2±3.3	133.0±8.4†	120.6±5.6¶	119.5±5.9¶	119.3±5.2†	14201±692¶	397±6.6	10.31±0.73	10.36±0.73¶	10.88±0.59	2.59±0.15	2.60±0.15¶	2.73±0.12
F.O-*Ni 2/of.rat 10*	10	78.5±2.2	111.6±1.4	106.6±1.6	107.5±1.6	102.5±0.8	12408±120	411±5.2†	12.24±0.56	11.38±0.34‡	13.91±0.50†	2.97±0.12	2.77±0.07†	3.38±0.11¶
F.O-*Ni 2/of.rat 55*	9	82.6±1.5	102.6±2.3	102.1±1.6	102.4±1.9	104.3±1.9	12016±175	404±3.9¶	12.28±0.46	12.11±0.45‡	14.81±0.37‡	3.04±0.09	2.99±0.09‡	3.66±0.08‡
F.O-*Ni 2/of.wox 14*	8	82.1±1.7	100.4±1.8	105.4±3.1	105.6±2.2	108.0±2.8	12193±255	426±5.3‡	12.53±0.86	12.54±0.62‡	15.08±0.91†	2.93±0.18	2.94±0.13‡	3.53±0.18†
F.O-*Ni 2/of.rat 5*	8	77.1±2.3	101.5±4.5	103.2±3.9	109.5±4.3	103.0±3.4	12126±404	409±7.3¶	9.81±0.68	10.32±0.47	12.56±0.59	2.38±0.15	2.52±0.11	3.06±0.11
F.O-*Ni 2/of.rat 23*	7	79.6±3.4	96.0±4.8	97.3±5.8	97.4±4.8	94.3±4.8	11329±331	408±7.7¶	9.70±0.32	10.58±0.42	12.91±0.69	2.38±0.06	2.59±0.08¶	3.16±0.13
F.O-*Ni 2/wox 1.of*	7	77.3±1.8	111.3±2.4	108.1±3.6	108.0±3.1	105.7±3.2	12567±303	420±6.2†	11.95±0.78	11.84±0.49‡	14.00±0.70†	2.83±0.15	2.82±0.09†	3.33±0.13¶
F.O-*Ni 2/rat 143.of*	6	75.0±3.0	125.7±10.2	109.3±3.0	109.7±4.5	108.3±3.8	13090±501	428±7.0‡	12.25±0.90	10.92±0.57¶	14.36±0.70†	2.85±0.18	2.55±0.11	3.35±0.12¶
F.O-*Ni 2/wox 14.of*	9	74.6±2.6	113.1±7.7	106.1±3.1	105.4±1.3	106.1±1.0	12450±313	409±11.1	11.14±0.59	11.16±0.79¶	12.44±1.0	2.71±0.12	2.70±0.14¶	3.01±0.21
F.O-*Ni 2/rat 55.of*	7	80.1±2.7	111.8±3.5	107.7±2.6	103.9±1.5	108.1±1.8	12527±196	392±11.6	9.51±0.80	10.09±0.58	12.35±0.67	2.41±0.18	2.57±0.11	3.14±0.12
F.O-*Ni 2/rat 8.of*	9	81.4±3.0	109.2±3.6	109.3±2.1	107.2±2.3	105.3±2.1	12575±234	388±6.9	10.55±0.47	9.95±0.25	11.92±0.32	2.72±0.12	2.57±0.06	3.07±0.06

All values are given as means±SE. Fasting glucose and glucose at 30, 60, 90, 120 min. are given in mg/dl, AUC in mg/dl X min, and body weigh, fat pad in gram. Each congenic strain was compared with F344(ANOVA), and significant differences are indicated : ¶ $P<0.05$, † $P<0.01$, ‡ $P<0.001$

substrains examined pronounced any influences on mesenteric fat accumulation, which is consistent with our linkage analysis and with the fact that the parental F.O-*Nidd2/of* strain showed no difference from the F344 rat. Epididymal fat index and body weight were also affected in some substrains (Table 1). This is unexpected because the F.O-*Nidd2/of* shows no significant influence on this type of fat tissue.

DISCUSSION

Among the 14 hyperglycemic QTLs we selected the *Nidd2/of* locus for fine mapping analysis as a next step toward gene finding. This is because our initial characterization of the congenic strain revealed that the locus appears to be more significant for the expression of diabetic phenotypes. Firstly, this locus is responsive to obesity. When obesity was introduced either by high fat diet or leptin receptor deficiency (*fa/fa*), glucose levels were significantly elevated by more than simple addition of the effect from *Nidd2/of* and the effect from the state of obesity (unpublished observation). Given the well-established role of obesity for type 2 diabetes, it stands to reason to expect to find important genes or signaling cascades by studying the QTL like *Nidd2/of*. Secondly, we demonstrated the epistatic interaction between the *Nidd2/of* and *Nidd1/of* by analyzing a double congenic rat possessing the both loci (unpublished observation).

In the past decade, quantitative trait locus mapping using animal models has identified hundreds of chromosomal regions crucial for the expression of atherosclerosis, diabetes, hypertension, obesity and other complex phenotypes (12-16). Indeed in several reports QTL was further dissected by methods similar to what is described in the current study (17, 18). It is rather surprising that in many cases it was found that there were multiple QTL within a single QTL region as initially mapped (17, 19). Thus, the number of genes for complex trait is likely to increase as more elaborate mapping work emerges. Indeed in yeast it was found that underlying genes for a certain quantitative phenotype are tightly linked within a small chromosomal region (20, 21).

Before we began this mapping, we were more inclined to believe that perhaps a single QTL plays a dual role for both hyperglycemia and obesity, because QTL mapping gives only a rough estimation of underlying gene location. However, this study clearly demonstrated that loci for these traits are distinctive and there are likely to be at least two QTLs for hyperglycemic phenotype. Apparent disappearance of diabetic phenotypes among substrains may merely be due to the weak contribution from each QTL, therefore more statistical power may be required for detecting them. Alternatively constructing a double-subcongenic strain introgressing short segments at both ends may achieve the equivalent diabetic effect to that of the parental congenic strain.

It is interesting that at least three QTLs with specific function are clustered and intricate interaction among them is crucial for glucose metabolism. From the point of understanding how obesity causes diabetes and how each QTL associates one another, this region should provide a unique opportunity, as there are limited number of genes thus it represents a model with substantially reduced complexity.

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