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An OLETF Allele of Hyperglycemic QTL
Nidd3/of *Is Dominant*

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Abstract: *The OLETF rat is a well-established model for the study of type 2 diabetes associated with obesity and has been shown to possess multiple hyperglycemic alleles in its genome. Here we focused on and carefully characterized one of the previously reported congenic strains, F.O-Nidd3/of that carries the OLETF allele of the* Nidd3/of *locus (also known as* Niddm21 *in the Rat Genome Database) in the normoglycemic F344 genetic background. A prominent finding was that the F1 progeny between the congenic and the F344 stain, whose genotype is heterozygote at the* Nidd3/of *locus, showed mild hyperglycemia equal to the parental congenic rat, suggesting that the OLETF allele is dominant. To our knowledge, this is the first study in which a diabetic QTL has been directly demonstrated to be dominant by using congenic strains.*
Key words: *congenic, dominant, QTL*

Given that a recent estimate of diabetes patients throughout the world is over one hundred millions, understanding its basis of molecular genetics is urgently required [7, 22]. From the medical standpoint, the establishment of animal resources of disease models is essential in order to take full advantage of rapidly developing genome pharmacological approaches [18]. The OLETF rat genetically mimics a human condition in which individuals are susceptible to type 2 diabetes [9, 16, 17]. Prominently, there are multiple genomic components that are linked to the expression of a hyperglycemic phenotype in this rat strain [14, 20, 21]. Our ultimate goal is to articulate how each of these disease-causing polymorphisms leads to form the condition which makes the individual rat vulnerable to the diabetes-causing external stimuli. To achieve this, we have generated a series of congenic rat strains, each of which harbors a single hyperglycemic QTL or quantitative trait locus of an average of approximately 30 cM in the genetic background of the wild-type strain [12]. An earlier study demonstrated that most, if not all, of the congenic rats were mildly hyperglycemic, as predicted by the fact that each QTL contributes only small genetic variance. Here we conducted an extensive analysis on one of the strains, F.O-*Nidd3/of*, with an emphasis on testing a hypothesis, suggested by an initial whole-genome scan, that this locus exerts a heterosis effect; that is to say, the heterozygote genotype shows higher blood glucose levels than the homozygote of either one of the parental strains [14]. We attempted to address the

issue directly by making comparisons among the F.O-*Nidd3/of* strain, the F344 normoglycemic strain and the F1 progeny between F.O-*Nidd3/of* and the F344 rat ([F344 × F.O-*Nidd3/of*]F1). If the OLETF allele of *Nidd3/of* is indeed heterotic, then the blood glucose levels of the [F344 × F.O-*Nidd3/of*]F1 rats would be the highest among the strains.

The F.O-*Nidd3/of* strain possesses a 35-cM segment of OLETF-derived chromosome 8 demarcated by *D8Rat58* and *D8Mgh17* [12]. *Nidd3/of* locus is also known as *Niddm21* in the RGD (http://rgd.mcw.edu/). The F1 progenies were produced by intercrossing F.O-*Nidd3/of* males and F344 females (F344/NSlc). The control F344 6-week-old males (F344/NSlc) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). All rats were kept under specific pathogen-free conditions. The temperature (21 ± 2°C), humidity (55 ± 10%), and ventilation were all controlled. Rats had free access to tap water and standard laboratory chow (MF; Oriental Yeast Co., Japan) and were maintained on a 12-h light and dark cycle (7:00/19:00). Animal procedures used in this study were approved by the University of Tokushima Animal Experimentation Committee.

All analyses were performed on 30-week-old males. The oral glucose tolerance test (OGTT) and fat tissue measurements were performed as previously reported [12, 15]. Serum insulin levels were determined with an ELISA kit from Morinaga, Japan. The serum levels of total cholesterol, triglycerides, and non-esterified fatty acids were determined with reagents from Wako, Japan. The statistical significance of differences was evaluated using ANOVA, followed by post hoc analyses with Scheffe's test.

Shown in Fig. 1 is the result of OGTT analysis. Consistent with our previous study, the F.O-*Nidd3/of* rat showed mild hyperglycemia [12]. However, contrary to our expectation, the postprandial plasma glucose levels of F1 progeny, which is a heterozygote at the *Nidd3/of* locus, were not any higher than those of the F.O-*Nidd3/of* rat. There was no statistically significant difference between the congenic and F1 rats, suggesting that the mode of inheritance of the locus is dominant at least in this particular genetic setting. The discrepancy can perhaps most readily be explained by the fact that there is a high degree of heterogeneity in an F2 population in a
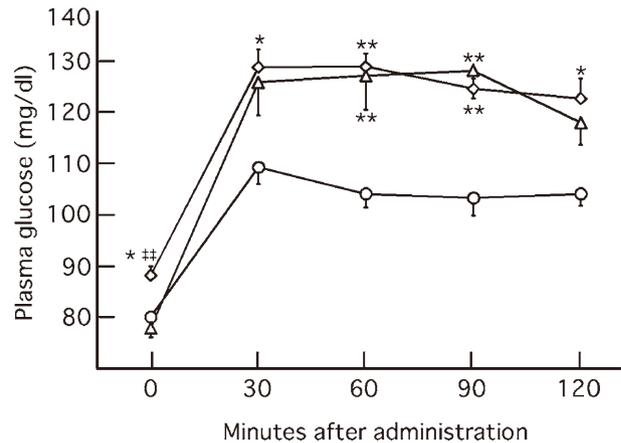

Fig. 1. OGTT result for F.O-*Nidd3/of* congenic (n=20, diamond), [F344 × F.O-*Nidd3/of*]F1 (n=14, triangle) and the F344 rat (n=9, circle). Bars represent the mean ± SE. *$P<0.05$, **$P<0.01$ vs F344, ‡‡$P<0.01$ vs F.O-*Nidd3/of* rats.

whole genome study. On the other hand the genomic milieu is essentially homogeneous in the congenic strain. In theory, greater than 99.9% of the genome outside the introgressed segment is identical between the congenic and its host strain [13]. Therefore, given that the observed heterosis was not some form of artifact, the resultant hypothesis would predict the epistatic interaction, that is, yet another genetic component(s) that empowers specifically the heterozygote genotype at the *Nidd3/of* locus to exert greater influence on glucose metabolism. Indeed, we recently demonstrated that epistatic interaction among OLETF alleles of hyperglycemic QTLs does exist [11]. Though our data did not support heterosis, finding that a QTL exerts the dominant mode of inheritance is quite novel. Previously, we tested the mode of inheritance of *Nidd1/of* and *Nidd2/of* and found OLETF alleles at both loci to be recessive [12]. Recently, Doung *et al.* examined 10 hypertensive QTLs of the DSS (Dahl salt sensitive) rat, similarly using the congenic strains, and found that only one of the hypertensive alleles was dominant [4].

To gain insight into the mechanisms of dominant inheritance, we measured various biochemical parameters for the fasting state (Table 1). As indicated in Fig. 1, fasting glucose levels of F.O-*Nidd3/of* are higher than those of both the control F344 rat and the F1 rat. This is rather difficult to interpret since apparently the OLETF allele in the transheterozygote with the corresponding

Table 1. Comparison of metabolic parameters

	F344 (n=9)	F.O-*Nidd3/of* (n=20)	[F344 × F.O-*Nidd3/of*]F1 (n=14)
Glucose (mg/dl)	80.0 ± 3.1	88.1 ± 1.8*	77.8 ± 1.8‡‡
AUC	$1.22 \times 10^4 \pm 174$	$1.46 \times 10^4 \pm 354$***	$1.43 \times 10^4 \pm 471$**
Insulin (ng/ml)	3.01 ± 0.40	4.08 ± 0.35	3.99 ± 0.44
TCHO (mg/dl)	52.4 ± 5.7	59.7 ± 2.8	63.7 ± 2.8
TG (mg/dl)	122.4 ± 25.3	167.8 ± 15.3	140.2 ± 15.4
NEFA (mEq/l)	0.64 ± 0.058	0.85 ± 0.052	0.71 ± 0.055
Fat weight (g)			
Mesenteric fat	8.9 ± 0.6	9.7 ± 0.4	10.4 ± 0.5
Retroperitoneal fat	10.3 ± 0.7	10.9 ± 0.3	11.1 ± 0.4
Epididymal fat	10.2 ± 0.9	10.6 ± 0.3	13.3 ± 0.4** ‡‡‡
Adiposity index (%)¶			
Mesenteric fat	2.33 ± 0.19	2.35 ± 0.08	2.48 ± 0.11
Retroperitoneal fat	2.65 ± 0.12	2.66 ± 0.07	2.64 ± 0.10
Epididymal fat	2.62 ± 0.22	2.57 ± 0.05	3.16 ± 0.08*
Body weight (g)	387.4 ± 10.3	409.9 ± 5.3	419.3 ± 5.2** ‡‡‡

Thirty-one-week-old fasted males were used for all measurements except glucose, insulin and body weight, which were measured during OGTT analysis at 30 weeks of age. Data are shown as means ± SE. TCHO, total cholesterol; TG, triglycerides; NEFA, non-esterified fatty acids. *$P<0.05$; **$P<0.01$; ***$P<0.001$; vs F344 rats, ‡‡$P<0.01$; ‡‡‡$P<0.001$; vs F.O-*Nidd3/of* rats. ¶: Adiposity index was determined using each fat pad and body weight (percentage of fat weight/body weight).

F344 allele tended to lower the plasma glucose, yet in the homozygote it exerted the opposite effect. In our previous report, statistical significance was not demonstrated between the congenic and the control for the fasting condition [12]. In contrast, postprandial hyperglycemia was reproduced in the current study, suggesting that it is possible that the genetic variance, if any, of the *Nidd3/of* locus is highly vulnerable to some unknown external conditions. Therefore, we think it is too speculative to discuss how the OLETF allele at this locus is involved in the fasting glucose levels.

Consistent with the OGTT data, AUC or area under curve was higher for both the congenic and the F1 rats. Concerning other parameters, none of the factors examined showed any differences among the strains, except epididymal fat mass and its adiposity index. Only the F1 rats increased the fat mass, and this might reflect some aspect of heterosis we initially anticipated. However, increased fat mass is unlikely to be associated with *Nidd3/of*-caused hyperglycemia because the fat mass is normal for F.O-*Nidd3/of* congenic rats. Our independent whole genome scan searching for obesity QTL identified a locus, *Obs3* in the chromosome 8 [15]. However, *Obs3*

barely overlaps with *Nidd3/of* and this locus influences mesenteric rather than epididymal fat, making it an unlikely candidate for explaining the observation. Although central (visceral) obesity is known to be more closely associated with glucose metabolism than peripheral (subcutaneous) obesity, it is unknown whether the increased epididymal fat mass in the F1 rats has any major physiological effect [19].

Most of the *Nidd3/of* locus corresponds to 11q21–q25 of the human chromosome 11. It is intriguing that several studies have identified either obesity or diabetic loci in this region [2, 3, 5, 6]. Indeed, among the 14 hyperglycemic QTLs we identified, the *Nidd3/of* is one of the most profound loci in terms of numbers of diabetes-related QTLs reported in human studies. According to the NCBI database, approximately 130 genes or ESTs are annotated in this region. Unfortunately, none of the genes closely linked to *D8Rat49*, localized to the LOD score peak, is considered to be a candidate from their predicted or known gene functions. However, a series of new discoveries, such as non-coding RNA or epigenetics, has challenged the traditional central dogma of the last several years. Therefore, we think that ar-

ticulate genetic analyses using refined polygenic models, such as QTL congenic strains, will become important in the understanding of the intricate genomic network of quantitative traits.

In conclusion, we provide evidence that the *Nidd3/of* is inherited in a dominant fashion. We should, however, keep in mind that the genetic homogeneity of the congenic strain underlies the definitive revelation of the Mendelian inheritance. From the characterization of monogenic traits, it is believed that the dominant trait is the result of 1) haploinsufficiency [10]; 2) ectopic function of a protein product, more commonly known as the dominant negative mutant [1]; and 3) ectopic expression of a gene, the most famous example of which is a homeotic mutation in *Drosophila* [8]. It is yet to be elucidated whether or not these paradigms apply to the polygenic trait. We expect that the findings of this study will aid identification of the causative gene as well as hint at molecular networks which might lead to the understanding of the hidden heterosis initially implied.

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