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Genetic Interaction between Hyperglycemic QTLs Is Manifested under a High Calorie Diet in OLETF-Derived Congenic Rats

Tomoe FUKUMURA[1, 2], Hiroyuki KOSE[1, 3], Chiyo TAKEDA[2], Yuko KURITA[2], Kazuhiko OCHIAI[1], Takahisa YAMADA[4], and Kozo MATSUMOTO[1, 5]

[1]Division for Animal Research Resources, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, [2]Graduate School of Human Life Science, Osaka City University, 3–3–138 Sugimoto, Sumiyoshi, Osaka 558-8585, [4]Laboratory of Animal Genetics, Graduate School of Science and Technology, Niigata University, Niigata 950-2181, and Present addresses: [3]Department of Life Science, Division of Natural Sciences, International Christian University, Mitaka, Tokyo 181-8585 and [5]Department of Animal Medical Sciences, Faculty of Life Sciences, Kyoto Sangyo University, Kyoto 603-8555, Japan

Abstract: The condition of hyperglycemia results from multiple genetic and environmental factors. In recent years much progress has been made with regards to the search for candidate genes involved in the expression of various common diseases including type 2 diabetes. However less is known about the specific genetic and environmental connections that are important for the development of the disease. In the present study, we used hyperglycemic congenic rats to address this issue. When given a normal diet, two hyperglycemic QTLs (quantitative trait locus), *Nidd2/of* and *Nidd10/of*, showed mild obesity and/or increased blood glucose in the oral glucose tolerance test. In a double congenic strain possessing both loci, these indices were not significantly different from those of either single congenic strain. In contrast, the double congenic strain fed a high-calorie diet showed significantly greater body weight than the single congenic strains or normoglycemic control rats. Although postprandial glucose levels of the double congenic rat were not further aggravated even on the high fat diet, it was notable that the postprandial insulin levels were drastically elevated. From these results, we constructed a novel model animal especially for the study of prediabetic hyperinsulemia, in which two QTLs and an additional dietary condition are involved. This may help to shed light on the genetic basis and gene-to-diet interaction during the early stage of type 2 diabetes.

Key words: congenic rat, epistasis, QTL, type 2 diabetes

Introduction

Recently, the worldwide growth of patient populations of type 2 diabetes is increasingly becoming a major public health problem, because quality of life (QOL) is often severely compromised by diabetic complications such as renal disease, neuropathy and retinopathy [37]. Therefore, establishment of effective preventive measures and

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Address corresponding: K. Matsumoto, Department of Animal Medical Sciences, Faculty of Life Sciences, Kyoto Sangyo University, Motoyama, Kamigamo, Kita-Ku, Kyoto 603-8555, Japan

curative strategies as well as drug development are pressing issues. Type 2 diabetes is a multifactorial disease that is caused by a metabolic and hormonal imbalance between insulin secretion from pancreatic β-cells and insulin resistance in peripheral tissues, and it is profoundly influenced by both genetic and environmental factors, for example, dietary habit and physical activity [7, 9]. Furthermore, genetic heterogeneity and enormous variations in exposure levels to environmental agents make it difficult to identify type 2 diabetes susceptibility loci in humans. Nonetheless, as high performance sequencing and gene expression techniques have become progressively affordable, much effort has been expended in the attempt to search for the common forms of the genetic variants which underpin "common" type 2 diabetes or obesity in human [22]. There are several common alleles that are generally accepted as being associated with type 2 diabetes [17, 28, 29, 36]. Interestingly, most of the single nucleotide polymorphisms (SNPs) are located in proximity to genes strongly expressed in the pancreas [8, 24, 25]. However, the genetic architecture and/or pathophysiological molecular mechanisms involving these diabetes-causing genes remain to be elucidated by animal models. Animal models play a complementary role to human research. They also help to facilitate 1) the identification of quantitative loci QTLs, 2) the understanding of their modes of inheritance and 3) their influence on glucose metabolism and specific QTL–QTL interaction. The insights from such studies may translate into understanding the common form of type 2 diabetes [5].

Many rodent diabetes models have been developed [4]. The inbred Otsuka Long-Evans Tokushima Fatty (OLETF) rat is a widely used model in the study of spontaneous type 2 diabetes [10, 11, 30]. Previously we and other groups identified QTLs responsible for hyperglycemia using a genome-wide scan of the OLETF rat [20, 33, 35]. Subsequently these QTLs were positively defined by observing hyperglycemic phenotypes in a series of congenic strains [14, 34].

The merits of using congenic strains for the study of polygenic traits should not be understated, since they make it possible to characterize the nature of each QTL without prior knowledge of the gene identity [5, 13, 27]. Here we focus on two hyperglylcemic loci on chromosome 14, *Nidd2/of* and *Nidd10/of*, which are listed as *Niddm20* and *Niddm28*, respectively, in the Rat Genome Database (RGD, http://rgd.mew.edu/). *Nidd2/of* is particularly interesting because a large portion of the locus overlaps with an obesity QTL, *Obs5* or *Bw4* (RGD nomenclature), identified in an independent whole-genome analysis [23]. *Nidd10/of*, on the other hand, coincides with the region including the CCKAR gene, which is essentially deleted from the genome of the OLETF rat [19, 32]. CCKAR is known to play a major role in feeding behavior [12, 15, 21]. Therefore, the objective of the current study was to combine excessive feeding behavior induced by the effect of *Nidd10/of* with the obesity-prone *Nidd2/of* locus in order to examine the epistatic relationship between them. A secondary aim was to expose these strains to a high-calorie diet and test how this particular external condition differentially influenced each QTL. Our results indicate that two QTLs and at least one external stimulus function in a cooperative manner in the expression of the prediabetic, hyperinsulemic state.

Materials and Methods

Animals and experimental design

Congenic strains, F.O-*Nidd2/of*, F.O-*Nidd10/of*, and F.O-*Nidd2&10/of*, were generated by crossing OLETF with F344 rats using the speed congenic method [14, 16]. The inbred F344 rat was obtained from Charles River Japan Inc. (Yokohama, Japan). All animals were housed in metal cages in our animal facilities under the following condition: temperature, 21 ± 2°C; humidity, 55 ± 10%; and a lighting cycle of 12 h (8:00 a.m. to 8:00 p.m.). After a 1-week adaptation period, rats (5 weeks of age) of each strain were assigned to two groups that were fed *ad libitum* with a standard diet (control group) or a high-calorie diet composed of control diet supplemented with 10% safflower oil until 21 weeks age. Tap water was given *ad libitum*. The body weights and the food intakes were measured once a week. All experiments were conducted in compliance with the Ethics Guideline for Animal Experiments of Osaka City University.

Oral glucose tolerance test (OGTT)

At 20 weeks of age, an oral glucose tolerance test was

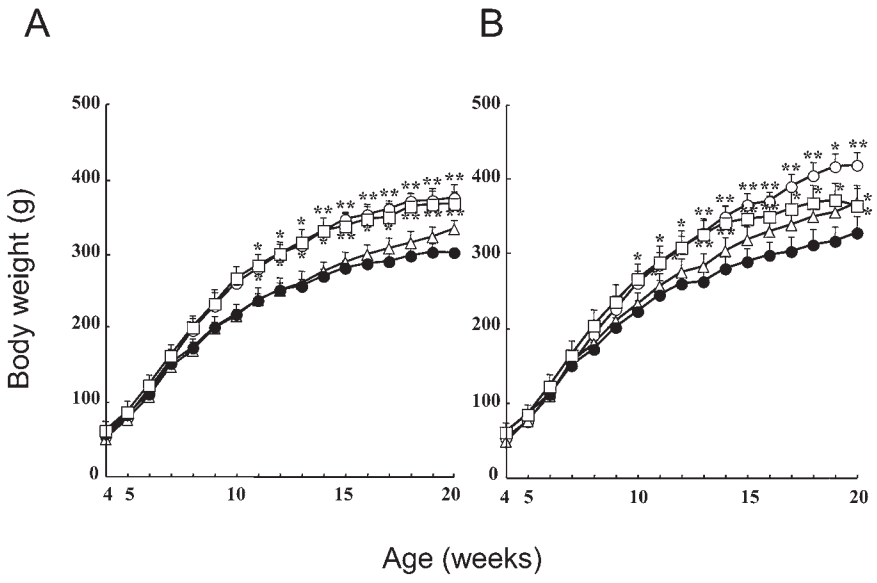

Fig. 1. Chronological body weight changes. Control F344 (close circle) rats, F.O-*Nidd2/of* (open triangle), F.O-*Nidd10/of* (open square) and F.O-*Nidd2&10/of* (open circle) congenic strains, fed control (A) or high-calorie diet (B). Bars represent means ± SD. **P*<0.05, ***P*<0.01 vs. F344 rats fed same diet.

performed after overnight fasting [20]. Two grams of glucose per kilogram body weight was administered orally. Blood glucose and insulin levels were determined at 0, 30, 60, and 120 min after glucose administration using the glucose oxidase method (Arkray, Kyoto, Japan) and immunoreactive insulin with an ELISA kit (Morinaga Insutitute of Biological Science, Yokohama, Japan), respectively. One week after the oral glucose tolerance test at 20 weeks, the rats were fasted for 15 h, were anesthetized with sodium pentobarbital (50 mg/kg) and sacrificed by exsanguination. Abdominal fat tissues were dissected and weighed for mesenteric, epididymal, and retroperitoneal fat [23].

Data analysis

Data are expressed as means ± SDs. Data were analyzed using analysis of variance (ANOVA) with a post hoc test, Scheffe's F test (StatView, SAS Institute, Inc., Cary, NC, USA).

Results

Effect of high-calorie diet on growth and food intake

The body weights of F.O-*Nidd10/of* and F.O-*Nidd2&10/of* congenic strains were significantly higher at 11 weeks or later than the F344 rat on the control diet (Fig. 1A). This is probably due, at least in part, to the increased daily food intake (Table 1). The F.O-*Nidd2/of* rat, on the other hand, showed a tendency of higher body weight without statistical significance. However, on the high-calore diet, the body weight of F.O-*Nidd2/of* at 20 weeks of age was higher than either the control strain on the same diet or F.O-*Nidd2/of* on the control diet (Fig. 1B, Table 1). There was no difference in the body weight of F.O-*Nidd10/of* congenic strain between the control and the high-calorie diets (Table 1). In contrast to the normal diet, the double congenic rat on the high-calorie diet showed drastically increased body weight (Fig. 1B). The body weight difference observed on the control diet was lost on the high-calore diet for the F.O-*Nidd10/of* rat, implying the unexpected high body weight of the double congenic strain was an effect of *Nidd2/of*. This effect was independent of the rate of food intake, since the intake of the double congenic strain was not different from that of F.O-*Nidd10/of* (Table 1).

Effect of high caloric diet on fat depositions

In order to further investigate the obese phenotypes

Table 1. Food intake and body weights of congenic rats fed control or high-calorie diet

Diet	Group	Daily food intake (g/day)	*P* value (vs. F344)	*P* value (vs. F.O-*Nidd 2&10/of*)	*P* value (vs. control diet)
Control diet	F344	13.6 ± 1.1		<0.001	
	F.O-*Nidd 2/of*	14.3 ± 0.6	n.s.	<0.001	
	F.O-*Nidd 10/of*	15.5 ± 0.8	<0.05	<0.01	
	F.O-*Nidd 2&10/of*	17.3 ± 1.0	<0.001		
High-calorie diet	F344	12.6 ± 0.5		<0.001	n.s.
	F.O-*Nidd 2/of*	13.5 ± 0.7	n.s.	<0.001	n.s.
	F.O-*Nidd 10/of*	14.2 ± 1.2	<0.05	<0.05	n.s.
	F.O-*Nidd 2&10/of*	15.8 ± 0.9	<0.001		<0.05

Diet	Group	Body weight gain (g/5–20 weeks)	*P* value (vs. F344)	*P* value (vs. F.O-*Nidd 2&10/of*)	*P* value (vs. control diet)
Control diet	F344	233.1 ± 24.4		<0.001	
	F.O-*Nidd 2/of*	255.6 ± 7.5	n.s.	<0.01	
	F.O-*Nidd 10/of*	279.3 ± 7.8	<0.01	n.s.	
	F.O-*Nidd 2&10/of*	294.5 ± 25.8	<0.001		
High-calorie diet	F344	246.8 ± 11.5		<0.001	n.s.
	F.O-*Nidd 2/of*	292.4 ± 13.8	<0.01	<0.001	<0.01
	F.O-*Nidd 10/of*	279.6 ± 20.7	<0.05	<0.001	n.s.
	F.O-*Nidd 2&10/of*	344.1 ± 15.0	<0.001		<0.001

Values are given as means ± SD. *P* value vs. F344 rat fed same diet or vs. same strain fed control diet.

of these strains, we examined intra-abdominal visceral fats, which here refer to the sum of mesenteric, retroperitoneal and epididymal fats [23]. Consistent with the body weight measurements, upon feeding the high-calorie diet, intra-abdominal fat mass of the F.O-*Nidd2/of* rat increased, and furthermore, the total intra-abdominal fat mass increase observed in the double congenic strain was the most conspicuous (Fig. 2B). A similar tendency was confirmed for each visceral fat type (data not shown). These results suggest that the effect of the F.O-*Nidd2/of* may be partially mediated by efficient lipid accumulation, in contrast to the F.O-*Nidd10/of* strain which did not increase fat deposition on either type of diet (Fig. 2). These results also agree with our previous linkage analysis in which a fat accumulation QTL was found to essentially coincide with *Nidd2/of* locus but not with that of *Nidd10/of* [23]. The unchanged fat mass in the F.O-*Nidd10/of* also indicates that the higher body weight was more likely to have been the result of an increase in lean body mass.

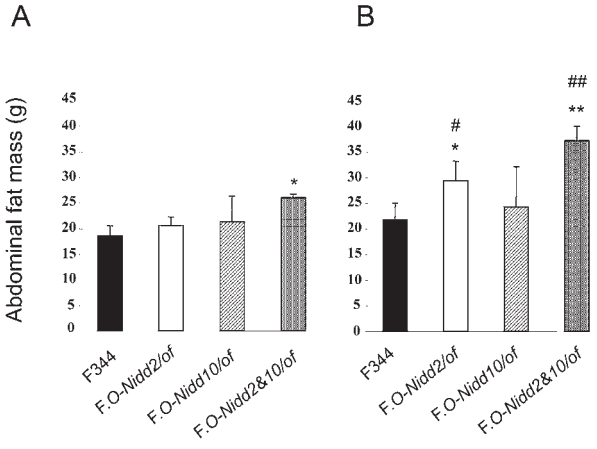

Fig. 2. Abdominal fat weight comparison. Control diet (A), high-calorie diet (B) Error bars represent SD. **P*<0.05, ***P*<0.01 vs. F344 rats fed same diet, $^{\#}P$<0.05, $^{\#\#}P$<0.01 vs. same line fed control diet.

Effect of high caloric diet on glucose metabolism and insulin response

Previously, we reported that at 30 weeks of age the OGTT assay revealed both single congenic strains

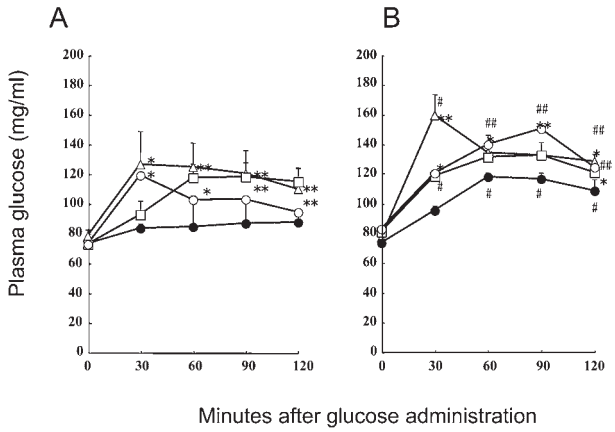

Fig. 3. Blood glucose responses to an oral glucose load. Control F344 (close circle) rats, F.O-*Nidd2/of* (open triangle), F.O-*Nidd10/of* (open square) and F.O-*Nidd2&10/of* (open circle) congenic strains, fed control (A) or high-calore diet (B). Points and bars represent $^{*}P<0.05$, $^{**}P<0.01$ vs. F344 rats fed same diet, $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. same line fed control diet.

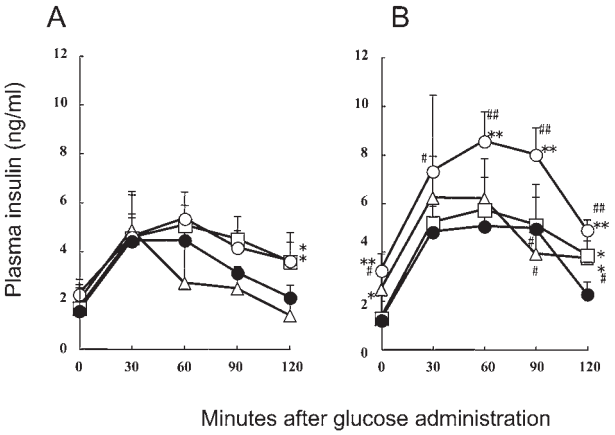

Fig. 4. Blood insulin responses to an oral glucose load. Control F344 (close circle) rats, F.O-*Nidd2/of* (open triangle), F.O-*Nidd10/of* (open square) and F.O-*Nidd2&10/of* (open circle) congenic strains, fed control (A) or high-calore diet (B). Bars represent $^{*}P<0.05$, $^{**}P<0.01$ vs. F344 rats fed same diet, $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. same line fed control diet.

showed mild hyperglycemia [14]. On the control diet the blood glucose levels were consistently and significantly elevated for both single congenic strains (Fig. 3A). However, unlike the obese phenotypes, the double congenic rat did not score the highest. Similarly on the high-calorie diet, although glucose levels of the double congenic rat were significantly higher than those of the control F344 strain, the overall profile of blood glucose responses were not significantly different from either of the single congenic strains (Fig. 3B).

The plasma insulin profile was quite different from that of the glucose metabolism (Fig. 4). On the normal diet, there were hardly any differences among the four groups, suggesting that the mild hyperglycemia observed in the congenic strains were due to insulin resistance in the peripheral tissues, instead of impaired insulin secretion. On the high-calorie diet, only the double congenic strain showed peculiarly high insulin levels both in the fasting state as well as during the postprandial period. We think this is important because the double congenic strain manifested hyperinsulinemia without accompanying obvious, albeit significant, hyperglycemia, only when placed under dietary stress.

Discussion

In the present study we described a conditional genetic interaction in which two hyperglycemic QTLs function cooperatively only when a specific external stress is introduced. In our previous linkage study, *Nidd2/of* was not predicted to interact with *Nidd10/of* but rather with *Nidd1/of* or *Niddm20* (RGD nomenclature) on chromosome 7 [20]. The epistasis with the latter locus was clearly defined in a double congenic strain [13]. In this regard, it is consistent with the linkage data that the F.O-*Nidd2&10* strain did not show clear epistatic interactions on the normal diet. Given the nature of *Nidd2/of* (see below), highly elevated fat mass as well as plasma insulin can be at least partially explained by increased caloric intake due to both elevated feeding behavior (*Nidd10/of*) and higher calories per unit of food mass exerting an effect on the obesity-prone *Nidd2/of* locus. This provides an important indication linking obese sensitive nature to obese-dependent diabetes development in the parental OLETF rat.

We have found *Nidd2/of* to be particularly interesting because 1) it overlaps with obesity QTLs [23]; 2) it interacts with another hyperglycemic QTL [13]; 3) it contains at least two alleles [2]; 4) it has proteomic profiles

that indicate the presence of age-dependent modification of a specific protein [31] and 5) it is highly sensitive to obesity-causing stimuli e.g., a high-calorie diet (this study). Furthermore, this locus alone can lead to diabetes equivalent to that of the parental OLETF rat when the genetic mutation causing much more severe form of obese state is introduced (manuscript in preparation). Therefore, further investigation of this QTL may reveal a new molecular route linking imbalanced lipid metabolism to the common form of type 2 diabetes [18]. Another unique aspect of this strain is that this model rather precisely replicates prediabetic hyperglycemia, and this may help in the effort towards understanding the early stage of the diabetes development and developing new approaches for clinical intervention.

In the light of clear epistasis between *Nidd2/of* and *Nidd10/of*, it is rather difficult explain the postprandial glucose level which was particularly high at 30 min after glucose challenge in the F.O-*Nidd2/of* fed the high-calorie diet, yet the corresponding value for the F.O-*Nidd2&10* was no different from that of the F.O-*Nidd10/of* (Fig. 3). Possibly, the elevated insulin levels in the double congenic strain may help to reduce glucose levels in this particular stage of diabetes development.

In recent years, there has been major progress in the identification of the quantitative trait gene (QTG) variants, in particular for SHR related strains [26]. In comparison to achievements made by genome-wide association studies (GWAS) in the effort of finding genes linked to multifactorial inheritance in human, we would have to say that outcomes from corresponding studies in rodent model animals are quite few [4]. However, the limitation imposed by linkage studies is rather obvious, i.e., it has a correlative nature. Thus, the need for a multi-species platform additionally requires indispensable systems for in-depth characterizing mechanisms of the actions of human disease genes [1]. It is our hope that the new tools that have recently become available for the rat, including the knockout rat technique [6], establishment of embryonic stem cells [3], and the determination of complete sequencing of a single inbred strain (SHR Base, http://shr.csc.mrc.ac.uk.index.cgi), will accelerate the cloning of genes as well as subsequent translational studies using the OLETF rat and its congenic strains.

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