BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **241,** 200–204 (1997)
ARTICLE NO. RC977347

Genetic Analysis of Non-insulin-Dependent Diabetes Mellitus in the Otsuka Long–Evans Tokushima Fatty Rat

Yasuo Nara,*[,1] Ming Gao,† Kazumi Ikeda,* Toshiaki Sato,‡
Makoto Sawamura,† Kazuya Kawano,§ and Yukio Yamori†

**Otsuka Department of International Preventive Nutritional Medicine and †Life Science, Department of Enviromental Conservation and Development, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606, Japan; ‡Masuda Red Cross General Hospital, Masuda 698, Japan; and §Tokushima Research Institute, Otsuka Pharmaceutical Co., Tokushima 771-01, Japan*

Received July 22, 1997

The Otsuka Long–Evans Tokushima Fatty (OLETF) rat is an animal model for obese NIDDM. We performed a genome wide scan in F2 progenies obtained by crossing OLETF rats with two control strains, Long–Evans Tokushima Otsuka (LETO) and Fisher-344(F-344) rats. Since diabetes develops only in male progenies, we used only male F2 rats for the linkage studies.Highly significant linkage was observed between the phenotype, postprandial hyperglycemia and P-450ald locus on chromosome 1 and D7Mit 11 locus on chromosome 7. In addition, suggestive linkage was found between fasting glucose level and body weight and these two loci. Four other regions (D1Mit12, D2Mit11, D5Mgh14, and D17Arb1) on chromosome 1, 2, 5, and 17 were detected to influence body weight, fasting glucose level or postprandial hyperglycemia independently. We concluded that non-insulin-dependent diabetes mellitus(NIDDM) in OLETF rats is regulated by multiple genes which affect fasting, postprandial hyperglycemia, and obesity differently.

The incidence of diabetes is progressively increasing in both industrial and developing countries. The most common form of diabetes is NIDDM, caused by a relative loss of insulin effect induced by decreased insulin secretion and/ or increased insulin resistance in the peripheral organs.

Genetic factors are known to contribute to the development of diabetes.However, it is difficult to establish which genes are involved because, diabetes is a multifactorial disease resulting from the complex interaction of multiple genes and environmental factors such as diet, obesity, stress, etc.This is particularly true for NIDDM, the onset of which is after middle age. Although defects in several putative genes were reported in human NIDDM, they accounted for less than 10% of the incidence of the disease(1-5). Therefore, a suitable model to analyze the genes causing NIDDM is needed.

OLETF rats spontaneously develop NIDDM with polyuria, polydipsia and mild obesity (6). Their pathophysiological features include mild obesity, hyperglycemia, hyperinsulinemia and postprandial hyperglycemia in the early stage, and the gradual development of hypoinsulinemia with atrophic islets as well as typical renal complications like Kimmelstiel-Wilson nodules. Males spontaniously develop overt diabetes, but females develop it only after ovariectomy. OLETF rats can be regarded as a good annimal model of NIDDM.

OLETF rats have been studied from both a physiological and genetic view point. The expression of cholecystokinin-A receptor gene in the pancreas and the hypothalamus is weak or absent in OLETF rats (7-9). Since cholecystokinin-A is a gastrointestinal hormone and plays an important role in the control of satiety in the brain, this defect might contribute to mild obesity in OLETF rats. Hirashima et al mapped a candidate locus for postprandial hyperglycemia on chromosome X in OLETF rats (10). However, candidate regions have yet to be established on autosomal chromosomes in OLETF rats.

In this study, we discovered that the loci on chromosome 1 and 7 significantly cosegregated with postprandial hyperglycemia in OLETF rats.

These results will contribute to the detection of causative genes in human NIDDM.

MATERIALS AND METHODS

OLETF and LETO rats were selectively separated from an outbred colony of Long–Evans rats and maintained at Tokushima Research

[1] Present address: Division of Applied Life Science, Graduate School of Integrated Science and Art, University of East Asia, Shimonoseki, Japan,751.

[2] To whom correspondence should be addressed. Fax: 75-753-2997. E-mail: yamori@helios.jinkan.kyoto-u.ac.jp.

0006-291X/97 $25.00

Institute, Otsuka Pharmaceutical Co. F-344 rats were purchased from Charles River Japan Co.All rats were kept free of specific pathogens in a controlled room where the temperature, humidity and lighting were set at 23 ± 2°C, 55 ± 5% and 07:00 - 19:00, respectively.

They were fed a CRF-1 diet (Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum.

We independently produced two sets of F2 progenies (100 and 145 rats for each) by crossing OLETF with 2 different control strains, LETO and non-diabetic F-344 strain, and only male progenies were used for linkage analysis, because females had not developed diabetes by the age of 30 weeks.

1. F2 progenies from crosses between OLETF and LETO rats. To remove paternal and maternal effects from the linkage analysis, we produced two sets of F2 progenies, 50 rats in each, from two types of F1 progenies (female OLETF X male LETO and female LETO X male OLETF). There was no significant difference in postprandial hyperglycemia between the two sets, but body weight and fasting glucose level were significantly higher in the F2 progenies from the crosses between female OLETF and male LETO than those from the crosses between female LETO and male OLETF.

In parent rats at the age of 30 weeks, plasma glucose levels at 120 min after oral glucose tolerance test (2g glucose/ kg body weight) were 16.0 ± 1.8 (n = 14), 8.3 ± 0.4(n = 14) and 7.1 ± 0.3 mmol/l (n=7) for OLETF, LETO and F-344 strain, respectively. Plasma glucose levels were measured at 0, 30, 60, 90 and 120 min after the oral administration.

When we applied a glucose level of over 14.0mmol/l to the diagnostic criterion of diabetes, the incidence of diabetes was the same in the two types of progenies(12% in each). Therefore, linkage analyses were carried out as for one population; in total 100 F2 progenies from crosses between OLETF and LETO rats.

2. F2 progenies from crosses between OLETF and F-344 rats. Diabetes developed in male F2 progenies from crosses between female OLETF and male F-344 rats, but not in male F2 progenies from reciprocal crosses. We produced 145 male F2 progenies by mating F1 obtained from crosses of female OLETF X male F-344, with each other 2% of which developed diabetes.

3. Oral glucose tolerance test. Each animal at the age of 30 weeks was orally administered glucose solution (2g/kg body weight) after 16hr fasting, and blood was taken at 0, 30, 60, 90 and 120 min. Plasma glucose levels were measured by a glucose oxidase method with a Glucose-B Test Kit (Wako, Osaka, Japan).

Insulin levels were not measured in this study, since fasting and postprandial hyperglycemia are more sensitive indices with which to characterize glucose intolerance in OLETF rats.

4. Genotyping. Animals were sacrificed under anesthesia to remove the liver which was immediately frozen in liquid nitrogen. Genomic DNA was extracted from the liver by a standard method using phenol-chloroform.

Firstly, we carried out linkage analysis in the F2 progenies from OLETF X LETO. Since OLETF and LETO strains, derived from the same out-bred Long–Evans colony, share a similar genetic background, polymorphisms between the strains were detected only by 14% of 600 microsatellite markers used. Primers for microsatellite markers were synthesized based on published papers and Gene bank data, or purchased from Research Genetics (11-14). At least one marker was assigned to each chromosome except for chromosome 15 and X.

Since candidate loci were detected on chromosome 1 and 7 in the F2 progenies from OLETF X LETO, we focused on these regions and performed linkage analysis on another set of 145 F2 progenies from crosses between OLETF and F-344 which had different genetic backgrounds. To elucidate linkage region(s) on other chromosomes in this second set, we chose 24 F2 progenies, 12 each with high and low postprandial glucose levels at 120 min, and screened microsatellite markers covering all chromosomes. Since probative values between diabetic phenotypes and the candidate loci on chromosome 1 and 7 by ANOVA were less than 0.05% in these 24 F2 progenies, we expected to detect similarly or more strongly linked loci on other chromosomes by this screening.

More polymorphic markers could be used for the second than the first set, since 52% of the markers so far examined showed polymorphisms. We examined 155 markers so that adjacent distance was at least 1 cM as estimated from published rat genomic maps and the polymorphic markers covered about 70% of the chromosomes (11, 12).

Of the 155 markers screened, 13 showing possible candidate regions based on the above criterion ($p<0.05$) were genotyped to 145 F2 progenies.

Polymerase chain reaction (PCR) was performed under the following conditions: denaturing at 94°C for 3 min, 25-30 cycles consisting of 94°C for 1 min, 50-60°C for 1 min, 72°C for 1 min, and final extension for 3 min at 72°C. The products were electrophoresed on a 7.5 or 9% polyacrylamide gel and stained with ethidium bromide.

An autosequencer (ABI PRISM 377, PerkinElmer, JAPAN) was used when the difference in length detected between the products was small. P-450ald locus was mapped 1.3 cM from ATP1A3S locus on chromosome 1 by using the F2 progenies produced from crosses between stroke-prone spontaneously hypertensive rats and Wistar Kyoto rats.

5. Statistical analysis. We used raw and transformed values (natural logarithm) for physiological parameters and performed statistic analysis with the JMP program (SAS Institute Inc. SAS Campus Drive Cary, NC 27513) for linear correlation and one way ANOVA and MAPMAKER/QTL for mapping and calculation of LOD score to traits (15). Since we obtained similar statistic results, we expressed the results only using the raw values. We used the criteria applied by Galli et al' on Goto-Kakizaki NIDD rats, that is, suggestive ($p=0.003$) and significant ($p \leqq 5 \times 10^{-5}$) (16).

RESULTS

Pre and postprandial glucose levels at 30, 60, 90 and 120 min were significantly higher in the F2 progenies from OLETF X LETO than OLETF X F-344 at the age of 30 weeks (Table 1). The incidence of diabetes was 12% and 2% in the F2 progenies from OLETF X LETO and OLETF X F-344, respectively. LETO rats share some diabetogenic loci with OLETF rats.

The higher incidence in the former might be attributable to this.

Eight out of 70 markers on chromosome 1 showed polymorphisms in the F2 progenies from OLETF X LETO. Two suggestive regions, each for fasting and postprandial hyperglycemia, respectively, were observed on chromosome 1.ATP1A3S locus suggestively linked with postprandial hyperglycemia at 60, and 90 min and summated glucose level ($p<0.0009$, Lod score=2.68), but not with fasting glucose level.

Also, ATPA3X1 located 2.3 cM from ATP1A3S suggestively linked with postprandial hyperglycemia at 60 min($p<0.0027$). In contrast, D1Mit 12 suggestively linked only with fasting glucose level, ($p<0.0012$). The maximum Lod score to fasting glucose level among the three loci (D1Mit8- D1Mit12- MAC.D12) was 2.94. ATP1A3S and D1Mit12 mapped about 70 cM apart. These results suggest that different loci on the same chromosome control fasting glucose and postprandial hyperglycemia.

TABLE 1

Phenotypic Characteristics of F2 Progenies from Crosses between OLETF X LETO or OLETF X F-344 at the Age of 30 Weeks

	Body weight (g)	Plasma glucose level (mmol/l)					
		Fasting	30 min	60 min	90 min	120 min	SUM
OLETF X LETO(100)	622 ± 76	6.7 ± 0.7	12.4 ± 2.3	11.8 ± 2.5	11.5 ± 2.5	10.6 ± 2.3	53.0 ± 9.3
OLETF X F-344(145)	570 ± 62	6.3 ± 0.8	11.4 ± 2.4	10.3 ± 2.2	10.2 ± 2.1	9.4 ± 1.8	47.6 ± 8.3

Note. Values are expressed as the mean ± s.d.m. ( ): Number of animals. SUM: summated plasma glucose level pre- and post-oral glucose administration. Significant differences were observed in all phenotypes between the F2 progenies ($p < 0.001$).

Seven out of 24 markers on chromosome 7 showed polymorphisms between OLETF and LETO strains. There was some linkage between D7Mit2 on chromosome 7 and postprandial hyperglycemia at 30 min ($p<0.0058$). This region did not cosegregate with body weight.

Obesity is a risk factor for NIDDM. D2Mit11 on chromosome 2 suggestively cosegregated with body weight ($p<0.0017$), but not with fasting and postprandial hyperglycemia. These results helped to elucidate candidate loci of diabetes in the other set of F2 progenies.

Since OLETF and LETO strains share about 86% of the genome of the out bred Long–Evans rat, the polymorphic regions between the strains were the result of heterogeneity in the original rats. The candidate regions established in the F2 crosses (OLETF X LETO) might be located near diabetogenic genes.

Polymorphic rate in microsatellite markers between OLETF and F-344 strains was higher than that between OLETF and LETO strains; 11 of 70 markers on chromosome 1. There was no polymorphism at ATP1A3S and ATPA3X1 between OLETF and F-344 strain. P-450ald located about 1.3 cM from ATP1A3S significantly cosegregated with postprandial hyperglycemia at 60 min and summated glucose level, and suggestively cosegregated with fasting and postprandial hyperglycemia at 30, 90 and 120 min (Table 2). Lod scores were 4.6 and 4.3 for 60 min and summated glucose level, respectively. Since there was no polymorphic marker within 30 cM of P-450ald, we could not specify a candidate region to these traits on chromosome 1.

Further, the linkage suggested between fasting glucose level and D1Mit12 on chromosome 1 was confirmed in the F2 progenies from OLETF X F-344 crosses and this region was a candidate for the trait (Table 2).

Nine out of 24 markers on chromosome 7 showed polymorphisms between OLETF and F-344 strains. D7Mit11 mapped at the same site as D7Mit2 significant linked with postprandial hyperglycemia and suggestive linked with body weight and fasting glucose level (Table 2).

The highest Lod score for summated glucose level was 4.8 and was found near D7Mit11 locus. Since body weight significantly correlated with fasting glucose level and postprandial hyperglycemia ($r^2=0.24$, 0.34, 0.24, 0.26, 0.25 and 0.33 for fasting, 30, 60, 90, 120 min and summated glucose level, respectively), this region on chromosome 7 appeared to contain obese and diabetrogenic gene(s). The mean values of body weight, and pre and postprandial hyperglycemia in the D7 Mit11 hetero genotype group were between those of the homo genotype groups (Table 3). Thus, the locus acts codominantly or recessively.

D2Mit11 on chromosome 2 suggestively cosegregated with body weight in the F2 progenies from OLETF X LETO crosses but did not show any significant linkage with body weight in F2 progenies from OLETF X F-344 cross.

We detected a further three regions, on chromosomes 5, 17 and X, in F2 progenies from OLETF X F-344 cross. D17Arb1 on chromosome 17 sub-significantly cosegregated with postprandial hyperglycemia at 60, 90, 120 min and summated glucose level ($p<0.0035$, 0.0036, 0.0038 and 0.0033, respectively).

D5Mgh14 on chromosome 5 sub-significantly correlated with summated postprandial hyperglycemia ($p<0.0061$), while DXMgh2 on chromosome X suggestively cosegregated with postprandial hyperglycemia except at 30min ($p<0.0018$, 0.0024, 0.0008 and 0.0007 for 60, 90, 120 and summated glucose level, respectively, Lod score=3.0 at 120min).

DISCUSSION

We discovered candidate loci for NIDDM in OLETF rats on chromosomes 1 and 7 in two different sets of F2 progenies and found these loci partly influenced fasting glucose level and obesity.

Further, a candidate for fasting glucose level was detected on chromosome 1 and was independent of postprandial hyperglycemia.

Recently, linkage analyses in Goto-Kakizaki (GK) rat, a model for non-obese NIDDM (16, 17) have

TABLE 2

Linkage Analysis between Several Loci and Phenotypes of F2 Progenies from Crosses between OLETF and F-344

Chr.	Marker	N	cM	Body weight	Fasting	Oral glucose tolerance test				
						30 min	60 min	90 min	120 min	SUM
1	P-450ald	144		<0.0143	<0.0004	<0.0009	$<3.5 \times 10^{-5}$	<0.0008	<0.0013	$<4.8 \times 10^{-5}$
	D1Mit12	141	70.0		<0.0022					
7	D7Mgh1	144		<0.0128	<0.0049	<0.0313	<0.0059		<0.0061	<0.0059
	D7Mit11	137	19.5	<0.0011	<0.0007	$<1.2 \times 10^{-5}$	$<1.1 \times 10^{-5}$	<0.0001	$<1.0 \times 10^{-5}$	$<2.0 \times 10^{-5}$
	D7Mit16	144	6.1	<0.0208	<0.0122	<0.0023	<0.0001	<0.0065	<0.0032	<0.0003
	D7Mgh3	143	1.9	<0.0368	<0.0165	<0.0295	<0.0011	<0.0200	<0.0065	<0.0025
	D7Mit4	139	16.4			<0.0763	<0.0052	<0.0502	<0.0983	<0.0239
	D7Mit5	145	13.0	<0.0095			<0.0027	<0.0020	<0.0065	<0.0034
	D7Mit6	143	14.8	<0.0268	<0.0298	<0.0312	<0.0065	<0.0051	<0.0065	<0.0034

Note. Each value is expressed as a probative value calculated by one way ANOVA analysis. N: Number of animals. SUM: Summated plasma glucose level pre- and post-oral glucose administration.

mapped regions for postprandial hyperglycemia to chromosome 1.

These regions were independent of fasting glucose level. We mapped a region for postprandial hyperglycemia in OLETF rats more than 100 cM from a previously mapped locus for fasting glucose level(16, 17). It is interesting that a candidate diabetogenic gene(s) was mapped to chromosome 1 in different NIDDM models. Our P-450ald region does not possess any putative gene(s) for diabetes and there is no synteny proposed for the candidate region for diabetes in model animals and human.

The highest significant linkage was observed between the region on chromosome 7 and postprandial hyperglycemia and the region suggestively cosegregated with fasting glucose level and body weight.

The regions on chromosome 7, named bw/gk1 and Weight1 were proposed as candidates for body weight in GK rats but not glucose intolerance (16,17).The bw/gk1 region was mapped about 20 cM from D7Mit11, but there was no comparative map for the Weight1 region. These results indicate that multiple genes contribute to obesity, some of which also relate with glucose intolerance. Further, D2Mit11 on chromosome 2 suggestively cosegregated with body weight in OLETF rats and was located near a locus affecting subsignificantly body weight in GK rat (16).

Cholecystokinin-A receptor is a possible candidate gene, however, the locus did not cosegregate with body weight in the F2 progenies at the age of 30 weeks (personal communication).The receptor was mapped on chromosome 14.We screened two markers on chromosome 14, but there was no linkage between the markers and the phenotypes. Even though the gene relates with body weight, its effect would be small. Leptin gene is specifically expressed in adipose cells and supposed to be a causative gene for obesity (18). However, there was no change in the coding region, the gene structure and expression of mRNA in adipose tissue between OLETF and LETO strains (19). We can rule out the role of ob-gene in obesity of OLETF rats.

In OLETF rats, D17Arb1 on chromosome 17 was a considerable candidate for postprandial hyperglycemia. On the other hand, Nidd/gk6, on chromosome 17

TABLE 3

Effect of Genotype of D7 Mit11 on Phenotypes of F2 Progenies from Crosses between OLETF and F-344 Rats

Phenotype	OLETF/ OLETF (24)	OLETF/F-344 (77)	F-344/F-344 (36)	p value	Percent expl. genetic variance
Body Weight(g)	605 ± 58	573 ± 60	545 ± 55	<0.0011	8.3
Fasting(mmol/l)	6.7 ± 1.0	6.3 ± 0.7	5.9 ± 0.7	<0.0007	8.9
30 min(mmol/l)	13.5 ± 2.8	11.3 ± 2.2	10.6 ± 1.8	$<1.2 \times 10^{-5}$	14.3
60 min(mmol/l)	12.2 ± 3.5	10.3 ± 1.9	9.5 ± 0.9	$<1.1 \times 10^{-5}$	14.4
90 min(mmol/l)	11.9 ± 3.6	10.0 ± 1.7	9.7 ± 1.0	<0.0001	11.8
120 min(mmol/l)	10.9 ± 2.9	9.3 ± 1.4	8.8 ± 0.9	$<1.0 \times 10^{-5}$	14.6
SUM	55.1 ± 12.8	47.1 ± 6.9	44.3 ± 4.2	$<2.0 \times 10^{-6}$	16.8

Note. Each value is expressed as the mean $\pm$ m.s.d. ( ): Number of animals. SUM: Summated plasma glucose level pre- and post-oral glucose administration.

was proposed as a candidate for fasting glucose in GK rat (17). Since we had no comparative map of the area around the loci, we could not estimate the distance between them. It is interesting that a candidate for NIDDM was mapped on the same chromosome in different NIDDM models.

There was no significant linkage between D5Mit8 on chromosome 5 and postprandial hyperglycemia. The locus was mapped between two markers, the nearest of which was 8.1 cM away and showed no linkage with any trait. This region should be studied in the future, because Nidd/gk4 on chromosome 5 was proposed as a cordidate locus for glucose intolerance in GK rat, and the fatty locus in Zucker strain, a model for obesity, was assigned also to chromosome 5 (17, 20).

A region on chromosome X was recently proposed as a candidate for postprandial hyperglycemia in the F2 progenies from OLETF X F-344 (10). The F1 progenies that possesed X chromosome derived from OLETF showed higher postprandial glucose levels at 120 min than those that possesed X chromosome derived from LETO or F-344 (10).

The region on chromosome X appeared to contain a genetic factor for diabetes in OLETF rats. Since female F2 progenies did not develop diabetes, the region may act recessively or be regulated by other factor(s) such as male sex hormones. Our data supported a previous report on chromosome X in OLETF rats (10).

We detected several candidate regions for phenotypes, that is obesity, fasting glucose level and postprandial hyperglycemia, in NIDDM.

These results confirm that NIDDM is regulated by multiple genes. Some regions influence only a single phenotype, while others affect several. As none of these regions appear to contain putative gene(s) for glucose metabolism or appetite, our results will hopefully contribute to the elucidation of candidate gene(s) for NIDDM in human.

ACKNOWLEDGMENT

We thank S. Miyazaki, T. Matsumoto T. Mashimo, and T. Tamada for their cooperative laboratory works.

REFERENCES

1. Julier, C., Hyer, R. N., Davies, J., Merlin, F., Soularue, P., Briant, L., Cathelineau, G., Deschamps, I., Rotter, J. I., Froguel, P., Boitard, C., Bell, J. I., and Lathrop, G. M. (1991) *Nature* **354,** 155–159.
2. Yano, H., Kitano, N., Morimoto, M., Polonsky, K. S., Imura, H., and Seino, Y. (1992) *J. Clin. Invest.* **89,** 1902–1907.
3. von den Ouweland, J. M., Lemkes, H. H., Ruitenbeek, W., Sandkuijl, L. A., de Vijlder, M. F., Struyvenberg, P. A., van de Kamp, J. J., and Maassen, J. A. (1992) *Nat. Genet.* **1,** 368–371.
4. Froguel, P., Zouali, H., Vionnet, N., Velho, G., Vaxillaire, M., Sun, F., Lesage, S., Stoffel, M., Takeda, J., Passa, P., Permutt, M. A., Beckmann, J. S., Bell, G. I., and Cohen, D. (1993) *N. Engl. J. Med.* **328,** 697–702.
5. Byrne, M. M., Sturis, J., Fajans, S. S., Ortiz, F. J., Stoltz, A., Stoffel, M., Smith, M. J., Bell, G. I., Halter, J. B., and Polonsky, K. S. (1995) *Diabetes* **44,** 699–704.
6. Kawano, K., Hirashima, T., Mori, S., Saitoh, Y., Kurosumi, M., and Natori, T. (1992) *Diabetes* **41,** 1422–1428.
7. Miyasaka, K., Kanai, S., Ohta, M., Kawanami, T., Kono, A., and Funakoshi, A. (1994) *Neurosci. Lett.* **180,** 143–146.
8. Funakoshi, A., Miyasaka, K., Jimi, A., Kawanai, T., Takata, Y., and Kono, A. (1994) *Biochem. Biophys. Res. Commun.* **199,** 482–488.
9. Funakoshi, A., Miyasaka, K., Shinozaki, H., Masuda, M., Kawanami, T., Takata, Y., and Kono, A. (1995) *Biochem. Biophys. Res. Commun.* **2105,** 787–796.
10. Hirashima, T., Kawano, K., Mori, S., Matsumoto, K., and Natori, T. (1995) *Diabetes Res. Clin. Pract.* **27,** 91–96.
11. Serikawa, T., Kuramoto, T., Hilbert, P., Mori, M., Yamada, J., Dubay, C. J., Lindpaintner, K., Ganten, D., Guenet, J.-L., Lathrop, M., and Beckmann, J. S. (1992) *Genetics* **131,** 703–723.
12. Jacob, H. J., Brown, D. M., Bunker, R. K., Daly, M. J., Dzau, V. J., Goodman, A., Koike, G., Kren, V., Kurtz, T., Lernmark, A., Levan, G., Mao, Y.-P., Pettersson, A., Pravenec, M., Simon, J. S., Szpier, C., Szpier, J., Trolliet, M. R., Winer, E. S., and Lander, E. S. (1995) *Nat. Genet.* **9,** 63–69.
13. Mukai, K., Imai, M., Shimada, H., Okada, Y., Ogishima, T., and Ishimura, Y. (1991) *Biochem. Biophys. Res. Commun.* **180,** 1187–1193.
14. Du, Y., Remmers, E. F., Goldmuntz, E. A., Zha, H., Mathern, P., Ding, Y.-P., Kotake, S., Szpirer, J., Szpirer, C., and Wilder, R. L. (1996) *Genomics* **32,** 113–116.
15. Lander, E., and Botstein, D. (1989) *Genomics* **121,** 185–199.
16. Galli, J. L., Li, S., Glaser, A., Ostenson, C. G., Jiao, H., Fakhrai-Rad, H., Jacob, H. J., Lander, E. S., and Luthman, H. (1996) *Nat. Genet.* **12,** 31–37.
17. Gauguier, D., Froguel, P., Parent, V., Bernard, C., Bihoreau, M. T., Portha, B., James, M. R., Penicaud, L., Lathrop, M., and Ktorza, A. (1996) *Nat. Genet.* **12,** 38–43.
18. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994) *Nature* **372,** 425–431.
19. Murakami, T., and Shima, K. (1995) *Biochem. Biophys. Res. Commun.* **209,** 944–952.
20. Truett, G. E., Bahary, N., Friedman, J. M., and Leibel, R. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7806–7809.