

NIDDM Genes in Mice

Deleterious Synergism by Both Parental Genomes Contributes to Diabetogenic Thresholds

Edward H. Leiter, Peter C. Reifsnyder, Kevin Flurkey, Hans-Joachim Partke, Erika Junger, and Lieselotte Herberg

We used mouse genetics to model how polygenic thresholds for the transition from impaired glucose tolerance (IGT) to NIDDM are reached. NON/Lt and NZO/HI are inbred mouse strains selected for IGT and polygenic obesity, respectively. Their F1 male progeny consistently developed NIDDM. Genetic analysis of F2 males from both cross directions identified an NON-derived diabetogenic locus, *Nidd1*, on chromosome (Chr) 4 near the leptin receptor. This locus was associated with reduced plasma insulin, increased non-fasted blood glucose, and lower body weight. Another NON-derived diabetogenic locus on Chr 18 (*Nidd2*) that controls blood glucose was identified. An NZO-derived diabetogenic region on Chr 11 (*Nidd3*), possibly comprising two separate loci, reduced ability to sustain elevated plasma insulin and significantly reduced weight gain over time. Thus, the diabetogenic synergism between genetic loci from strains separately exhibiting subthreshold defects perturbing glucose homeostasis underscores the likely complexity of the inheritance of obesity-associated forms of NIDDM in humans. *Diabetes* 47:1287–1295, 1998

NIDDM, the most prevalent form of diabetes in humans, is a complex, multifactorial disease with multiple etiologies. Genetic and environmental factors, notably dietary, are thought to interact deleteriously to produce an increasingly severe insulin resistance and β -cell dysfunction in an age-dependent fashion, which results in impaired glucose tolerance (IGT). Whether individuals make the transition from this prodromal state of latent NIDDM to overt hyperglycemia may depend in part on the ability of muscle, liver, and adipose tissue to modulate the level of insulin resistance and of pancreatic β -cells to make the necessary adaptations to provide enough insulin to compensate for insulin resistance. Familial predisposition for upper-body obesity is the major phe-

notypic risk factor associated with development of NIDDM in humans (1), as is whole-body obesity in mice (2). NIDDM-predisposing obesity is usually accompanied by development of insulin resistance as manifested by increased hepatic glucose output as well as decreased peripheral glucose utilization and consequent IGT. Development of an obesity-induced diabetes ("diabesity") appears to represent a threshold liability effect requiring the interaction of additional genes and an environmental component (1). Such thresholds may pertain to humans, considering that there are many more individuals in the general population with android obesity than there are individuals with NIDDM. In the present study, we use the power of mouse genetics to model how polygenic thresholds for the transition from IGT to overt NIDDM are reached and exceeded through diabetogenic contributions from both parental genomes. We employ an outcross of two inbred strains selected for either IGT or obesity, NON (nonobese non-diabetic) and NZO (New Zealand obese).

NON is an inbred strain produced by selection at each generation for high fasting blood glucose (BG) (3). Males of the NON/LtHI substrain maintained on a 4.5% fat-containing diet exhibit IGT and develop a mild, postpubertal obesity primarily associated with increases in visceral fat depots (4). However, they do not make the transition to overt NIDDM. Interestingly, serum immunoreactive insulin (IRI) levels in NON mice are at the low end of the normal range ($< 25 \mu\text{U/ml}$) throughout life, and a potential defect in β -cell stimulus-secretion coupling is indicated (4). This strain may therefore represent a mouse counterpart of the Goto-Kakizaki (GK) rat, a model of latent NIDDM associated with low glucose-stimulated insulin secretion by β -cells coupled with IGT (5).

NZO is an inbred mouse strain initially selected for polygenic obesity (6). Defects in glucose-stimulated insulin secretion, in hepatic control of glucose production, and in insulin clearance have been postulated (7). Recent biochemical analyses further suggested that metabolic anomalies in carbohydrate metabolism are secondary to defects in lipid metabolism (8). NZO/HI mice of both sexes exhibit unusually high birth weights, are large at weaning, and rapidly develop severe obesity even when maintained on a standard diet containing 4.5% fat. Although the rapid postweaning increase in body weight (including large increases in visceral and subcutaneous fat depots [9]) resembles that produced by mutations in either the leptin (*Lep*) or leptin receptor (*LepR*) genes, NZO mice differ from mice with mutations at the *Lep* locus in that NZO mice have elevated serum leptin levels. They

From The Jackson Laboratory (E.H.L., P.C.R., K.F.), Bar Harbor, Maine; and the Diabetes Research Institute (H.-J.P., E.J., L.H.), Düsseldorf, Germany. Address correspondence and reprint requests to Dr. Edward H. Leiter, The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609. E-mail: eh@aretha.jax.org.

Received for publication 6 March 1998 and accepted in revised form 29 April 1998.

BG, blood glucose; BW, body weight; Chr, chromosome; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; LOD, logarithm of odds; PCR, polymerase chain reaction; QTL, quantitative trait locus.

also differ from mice with mutations at the *Lepr* locus in that the function of the NZO variant of the leptin receptor is apparently normal (10). Nevertheless, NZO mice appear to exhibit peripheral resistance to leptin (11,12). As illustrated below, only a subset of NZO/HI males makes the transition from a state of IGT to overt NIDDM.

RESEARCH DESIGN AND METHODS

Mice. NZO/HI and NON/LtHI parental strains were maintained by mating sib pairs in a specific pathogen-free (barrier) facility at the Diabetes Research Institute, Düsseldorf, Germany. F1 and F2 generations were produced and maintained in the same facility. Mice were given access ad libitum to autoclaved drinking water and a diet containing 4.5% fat, 51.5% carbohydrate, and 21% protein (SSNIFF, Soest, Germany). Mice were weaned at 3 weeks of age. Same-sex littermates were caged in groups of 3–5.

Analytic procedures for phenotype determination. Body weight (BW) recording and blood harvesting from the tip of the tail were performed between 8:00 and 9:00 A.M. on nonfasted mice. Blood glucose (BG) was determined by an automated glucose oxidase method (Care Diagnostica, Voerde, Germany) on 20 μ l blood. Nonfasting serum IRI was assayed with a radioimmunoassay kit (Pharmacia Insulin RIA 100; Pharmacia AB, Uppsala, Sweden) on 100 μ l (undiluted) or 50 μ l (diluted) serum. BW and BG were determined at monthly intervals beginning at 4 weeks of age through 52 weeks. Serum IRI was determined at 16, 24, 36, and 52 weeks. Mice were necropsied at 36 or 52 weeks.

Genotypic analysis. Tail DNA was prepared from all mice at week 16 by a salt-out procedure. As a first screen for potential linkages, a modification of the weighted DNA pooling technique of Taylor and Phillips (13) was used. Equal aliquots of individual DNAs from mice representing phenotypic extremes were pooled based on data from the 24-week and 36-week sampling intervals. From the first 80 (NZO \times NON) F2 mice, 7 pools were made: low BG (<225 mg/dl), low BG–high IRI (>100 μ U/ml), low BG–low IRI (<50 μ U/ml), high BG (>300 mg/dl), high BG–low IRI, high BG–high IRI, and medium BG (225–300 mg/dl). Most pools consisted of at least 19 DNA samples, with the largest totaling 25 and one with only 5 (low BG–high IRI, which was not given much weight). Some DNA samples were in more than one pool, e.g., both the high BG pool and the high BG–high IRI pool. The pools were typed by polymerase chain reaction (PCR) on 4% Metaphor (FMC, Rockland, ME) agarose gels for 83 polymorphic microsatellite markers (Research Genetics, Huntsville, AL) spanning most of the genome at roughly 20-cM intervals. From the first 61 (NON \times NZO) F2 DNA samples, four pools were made: low BG, high BG, low IRI, and high IRI. These pools, ranging in size from 19 to 27 DNA samples each, were typed for 52 polymorphic markers spanning more than half the genome. This more limited genomic scan was based on prior information obtained from the scan of the reciprocal (NZO \times NON) F2 pools. Markers were typed for all individuals in either cross where a genotypic skewing (quantifiably more of one allele product amplified than the other) in a particular pool or pools was observed. An F1 DNA was always typed with the pools to ascertain that both alleles for each marker were equally amplifiable. *Lepr* was typed as previously described (14) using PCR primers (forward) 5'-GCAACGATAAC-TAGTGG-3' and (reverse) 5'-TTGAGGCTTCTGGATGA-3'. A 541-bp product is produced after 34 cycles of amplification at 94°C for 60 s, 50°C for 90 s, and 72°C for 90 s (Perkin Elmer 2400 Thermal Cycler; Norwalk, CT). This product was purified by ethanol precipitation and then digested by 5 U of *RcaI*. (Boehringer Mannheim, Indianapolis, IN). The product of the NZO allele was digested into two fragments of 374 and 167 bp, whereas the NON allele product was not cut.

Statistical analysis. Because NZO parental males were not characterized by a single phenotype but rather fell into various subphenotypic classes, cluster analysis was performed on the phenotypic data (crosses combined) and for each generation separately to assign mice to diabetic or prediabetic syndromes in an unbiased manner. For each variable with repeat measures (BW, BG, and serum IRI), the mean, standard deviation, standard error of the mean, sum, and minimum and maximum values for each mouse over the period of sampling were entered into the analysis. A hierarchical cluster analysis (15) was performed using the squared Euclidian distance between the points; among the various methods of clustering, we used the method termed linkage between the cluster groups (15). Variables associated with BW were poor discriminators. Mean BG, maximum BG, mean serum IRI, and minimum serum IRI over the sampling period were most useful for the designation of physiologically meaningful subgroups (see Fig. 2).

A total of 193 (NZO \times NON) F2 and 201 (NON \times NZO) F2 mice was generated for DNA typing. Quantitative trait locus (QTL) analysis was performed using MapManager QTb16 (16). A nonparametric analysis of ranked data was used because of the highly skewed distribution for BG and IRI. Analysis was performed both for the combined data set and for each cross separately, because effects of the direction of the cross on the phenotypes were indicated. Each mouse was ranked for its BG or IRI value at 24 and 36 weeks within each cross.

To remove variance associated with the direction of the cross when the full data set was analyzed, the raw data were first ranked within a cross before the data from both crosses were combined. To adjust for multiple tests (two phenotypes at each of two time points), the criterion logarithm of odds (LOD) score used for formal designation of a *Nidd* locus was increased to 4.9 using the full data set and to 5.2 for the tests within individual crosses. Linkage markers on chromosomes giving LOD scores >3.0 are reported as suggestive of additional *Nidd* loci.

Associations of significant BG or IRI QTLs with BW were tested using repeated measures analysis of variance, which permitted, in a single analysis for each QTL, estimation of overall allelic effects on weight as well as estimation of effects on growth rates. Analyses of BW were performed on log-transformed data to eliminate the positive association of age with the variance of BW. Separate analyses were performed for the developmental period of rapid growth (weeks 4–12) and for the period of slower maturational growth (weeks 16–36). A Bonferroni adjustment was made for the number of analyses (5) run for both crosses combined to account for the multiple comparisons problem, so that the overall chance of incorrectly rejecting any of the null hypotheses was 0.05.

RESULTS

Diabetes exacerbation in F1 males. Figure 1 describes a longitudinal profile for mean BG and BW in aging NON/LtHI and NZO/HI males. As described above, NON parental males maintain postprandial normoglycemia despite exhibiting IGT (Fig. 1A). These males develop a progressive and moderate maturity-onset obesity, attaining weights in excess of 40 g by 20 weeks of age (Fig. 1B). Weaning weights of NZO males are significantly higher ($P < 0.0001$) than those of NON males, and NZO males remain heavier than NON males at all ages (Fig. 1B). Obesity development in postweaning NZO males is remarkable, with weights between 40 and 50 g attained by 12 weeks of age. Although obesity development in NZO mice of both sexes is rapid and predictable, male-limited NIDDM development is not. Various subphenotypic classes of NZO/HI males can be discriminated when cluster analysis is applied, using the means of lifetime BG, lifetime IRI, the highest BG, and the lowest IRI during lifetime as an iteratively evaluated set of optimal discriminators. The resulting cluster groups illustrate that progression from IGT (common to all NZO males) to overt NIDDM is a complex threshold phenomenon (Fig. 2). This phenotypic heterogeneity was not a reflection of underlying genetic inhomogeneity, because the highly inbred status of NZO/HI mice was confirmed by reciprocal skin graft acceptance and absence of heterozygosity in a genome-wide scan with simple sequence repeat markers.

Interestingly, those NZO/HI males progressing from IGT to NIDDM between 12 and 20 weeks of age showed greater mean weight gain between 4 and 8 weeks than did those males that remained normoglycemic (mean BW change \pm SE was 16.9 ± 0.6 , $n = 9$, vs. 14.6 ± 0.7 , $n = 20$, respectively, $P = 0.05$). After 16 weeks, those males progressing from IGT into overt diabetes gained weight more slowly than did normoglycemic mice, such that the difference between the groups diminished. Mean serum IRI levels were within a normal range for all males sampled at 16 weeks of age (36.4 ± 5.1 μ U/ml, $n = 31$). By 20 weeks, mean IRI had risen above normal levels (50.9 ± 4.8 μ U/ml, $n = 25$). It should be noted that this progressively developing hyperinsulinemia is moderate relative to that produced by single gene obesity mutations such as *Lepr^{db}* and *Lep^{ob}*.

Differential severity of NIDDM in F1 males from reciprocal outcross. Comparisons of longitudinal profiles of mean BW and BG show that the NZO and NON genomes contributed synergistically to provoke a predictable NIDDM syndrome in F1 males. F1 males produced in reciprocal out-

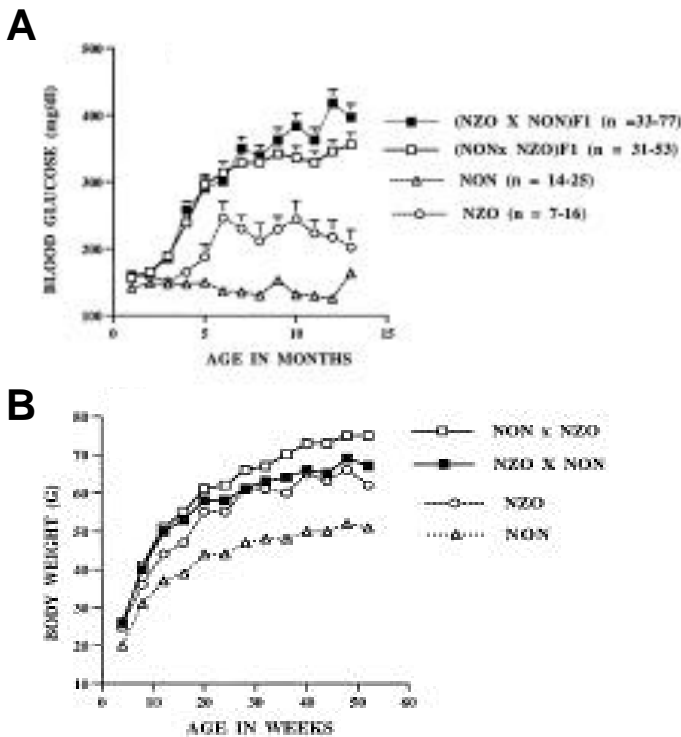


FIG. 1. Synergism of NON and NZO parental genomes to produce a more severe NIDDM syndrome in reciprocal F₁ males. Note that the NZO characteristic obesity pattern is transmitted to F₁ progeny. **A:** Synergism of NON and NZO parental genomes to elicit maturity-onset NIDDM in F₁ males. **B:** F₁ males develop NZO-type obesity.

crosses resembled NZO parental males in terms of the rapid development of obesity after weaning. However, both (NZO × NON)F₁ and (NON × NZO)F₁ males differed from NZO males in the development of a more severe maturity-onset diabetes syndrome. F₁ females exhibited the rapid NZO parental pattern of weight gain and, like parental NZO females, did not develop overt hyperglycemia (data not shown).

A comparison of the mean data for reciprocal outcrosses presented in Table 1 versus the data summaries presented by cluster grouping (Table 2) illustrates the value of cluster analysis for delineating differences in subdiabetogenic phenotypes that are not obvious when group mean statistics are presented. Analogous to the NZO males at a given sampling interval, clusters of F₁ males representing multiple subphenotypes were identified (Fig. 2 and Table 2). Reciprocal F₁ males showed the rapid weight gain pattern typical for NZO males, yet F₁ males more consistently attained diabetogenic thresholds for development of NIDDM. Hyperglycemia (BG >250 mg/dl) predictably developed between 12 and 20 weeks and became more severe with age in most individuals. Interestingly, the clustering of phenotypes depicted in Fig. 2 indicated that a higher percentage of (NZO × NON)F₁ individuals fell into the more severely diabetic phenotypic clusters. The most severely diabetogenic cluster group, i.e., cluster 7 (Fig. 2 and Table 2), was limited to the NZO parentals (7.7%) and the (NZO × NON)F₁ males (21.2%). The most severely diabetic cluster group in the reciprocal (NON × NZO) males (cluster 6) was distinguished by maintenance of a higher mean lifetime serum IRI than the most severely diabetic group in the NZO × NON cross. Similarly, cluster groups 3 and

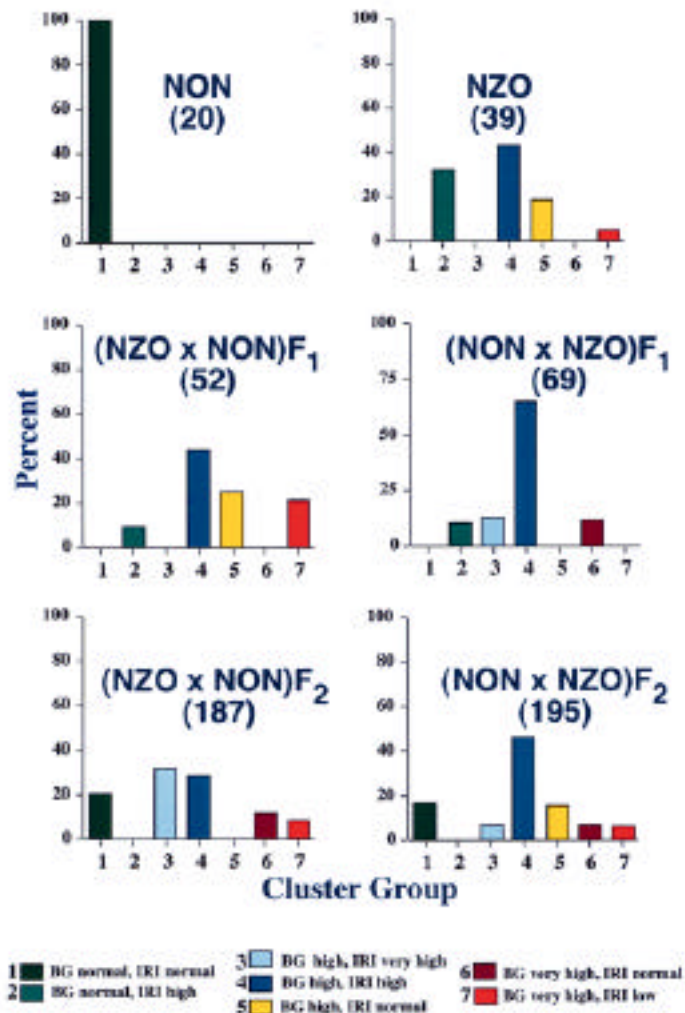


FIG. 2. Cluster analysis of parental, F₁, and F₂ genotypes illustrates 1) physiological heterogeneity among NZO mice, 2) heterosis among F₁ mice (emergence of more severely affected genotypes), 3) recovery of the parental phenotype in F₂ mice, and 4) an effect of the direction of the cross. The cluster limits are BG normal = <200 mg/dl; BG high = 200–300 mg/dl; BG very high = >300 mg/dl; IRI low = <40 μ U/ml; IRI normal = 40–80 μ U/ml; IRI high = 80–100 μ U/ml; IRI very high = >100 μ U/ml. Mean individual BG and IRI based on sampling from 4–52 weeks of age were used as the classification criteria. The number of mice of each genotype is given in parentheses.

4, describing less severe diabetogenic classes characterized by elevated BG with concomitant high to very high serum IRI, comprised 78% of (NON × NZO)F₁ males. The most hyperinsulinemic cluster group (cluster 3), comprising 13% of the (NON × NZO)F₁ males, was not represented in the reciprocal (NZO × NON)F₁ male cluster groupings (Fig. 2 and Table 2). Persistence of a hyperglycemic state in the face of increasing serum IRI indicates an increasingly severe insulin-resistant state. However, it probably also indicates existence of a compensatory mechanism to limit glucose levels. Hence, examination of cluster categories identified a higher percentage of males with the more diabetogenic phenotypes in the (NZO × NON) outcross compared with the reciprocal (NON × NZO) outcross.

Segregation of diabetogenic subphenotypes in reciprocal F₂ males. Because diabetes was limited to NZO and reciprocal F₁ males, genotypic analysis was limited to F₂

TABLE 1
Mean glucose and insulin levels for P1, F1, and F2 males at 24- and 36-week sampling intervals

Strain (n)	BW (g)	BG (mg/dl)	Serum insulin (μ U/ml)
NON			
24 weeks (20)	44.1 \pm 5.0 \pm 1.1	132 \pm 14 \pm 3	26 \pm 15 \pm 4
36 weeks (20)	47.5 \pm 3.6 \pm 0.8	158 \pm 41 \pm 9	29 \pm 15 \pm 4
NZO			
24 weeks (38)	56.7 \pm 7.2 \pm 1.2	256 \pm 98 \pm 16	73 \pm 57 \pm 9
36 weeks (33)	62.0 \pm 8.8 \pm 1.6	279 \pm 118 \pm 21	91 \pm 63 \pm 11
(NZO \times NON)F1			
24 weeks (46)	58.3 \pm 5.0 \pm 0.7	300 \pm 120 \pm 18	93 \pm 62 \pm 10*
36 weeks (41)	63.5 \pm 4.6 \pm 0.7	363 \pm 98 \pm 15	110 \pm 76 \pm 12
(NON \times NZO)F1			
24 weeks (61)	62.3 \pm 4.2 \pm 0.5	316 \pm 79 \pm 10	107 \pm 48 \pm 7
36 weeks (40)	69.2 \pm 4.8 \pm 0.8	343 \pm 99 \pm 16	174 \pm 81 \pm 13
(NZO \times NON)F2			
24 weeks (193)	55.0 \pm 6.4 \pm 0.5	298 \pm 149 \pm 11	89 \pm 89 \pm 6
36 weeks (190)	58.6 \pm 9.0 \pm 0.7	355 \pm 168 \pm 12	93 \pm 107 \pm 8†
(NON \times NZO)F2			
24 weeks (203)	53.4 \pm 6.6 \pm 0.5	332 \pm 164 \pm 12	78 \pm 71 \pm 5
36 weeks (194)	56.4 \pm 9.4 \pm 0.7	383 \pm 211 \pm 15	98 \pm 124 \pm 9‡

Data are means \pm SD \pm SE. * n = 42; † n = 171; ‡ n = 181.

males. Group means of parental versus F1 and F2 males are shown in Table 1 for 24- and 36-week sampling intervals. With regard to BW, F2 males resembled F1 males in that the former also exhibited the NZO parental pattern of high weaning weight and rapid weight increases between 4 and 12 weeks that is shown for F1 males in Fig. 1B. The lack of a subpopulation exhibiting the more moderate NON weight-gain pattern over this maturational period indicates the presence of directional dominance of high BW determinants from NZO. As in F1 males, cluster analysis based on the same set of discriminators used for NZO and F1 males did partition the (NZO \times NON)F2 males into five subphenotypic classes and the (NON \times NZO)F2 males into at least six classes (Fig. 2 and Table 2), indicating that direction of the cross affected the phenotype. The NON parental cluster group (cluster 1), absent in the F1 male generation, reappeared in the F2 generation in addition to the spectrum of prediabetic/diabetic cluster groups present in the NZO parental male population (Fig. 2 and Table 2).

QTL analysis in the F2 populations. Potential linkage of genes to the NIDDM subphenotypes of BG and IRI were indicated using the set of 83 microsatellite markers on DNA pools from individuals at each extreme of the F2 distribution, as described in METHODS. This scan method guided in-depth genomic analysis of chromosomal regions to which potential linkage was indicated (Table 3). Appropriate statistical corrections for multiple comparisons were applied. Significant linkage of a NON-derived diabetogenic locus on Chr 4 contributing to low IRI and elevated BG was indicated when analyzing both F2 crosses separately (LOD scores >4.0). When both crosses were combined, power to detect linkage increased so that LOD scores became highly statistically significant (P value of 1.2×10^{-5} , the level proposed as the genome-wide threshold for linkage in an F2 cross [17] adjusted for multiple comparisons). This locus, representing a dominant susceptibility contribution from NON, and provisionally designated *Nidd1*, yielded peak LOD scores for

BG and IRI within the *D4Mit58-Lepr* interval at both the 24- and 36-week sampling intervals (Fig. 3). Although currently listed as mapping between *D4Mit166* and *D4Mit58* (18), the recombination data in Table 3 support a previous report locating *Lepr* distal to *D4Mit58* (19). In addition to linkage to both of the two NIDDM subphenotypes (BG and serum IRI), *Nidd1* further contributed a significant main effect ($P < 0.0005$) to BW, with homozygosity for the NZO allele associated with greater weight gain (and higher serum IRI) during the maturational phase after 16 weeks of age. This locus accounted for 11% of the variance in BW at 24 weeks.

A second highly significant NON-derived diabetogenic allele was detected on the proximal region of Chr 18. This locus, provisionally designated *Nidd2*, differed from *Nidd1* in affecting BG but not serum IRI in a dominant or additive fashion. The effect of this linkage was time dependent, being strongest at the 24-week sampling interval and giving a LOD score for BG of 5.0 at *D18Mit60* (Fig. 3 and Tables 3 and 4). Unlike *Nidd1*, *Nidd2* did not exert significant main effects on BWs of F2 males.

As expected, given that spontaneous NIDDM was observed in the NZO parental males, diabetogenic contributions from the NZO genome were also detected. At least one—and likely two—NZO genomic contributions affecting serum IRI at 24 weeks in an additive fashion on the medial region of Chr 11 were indicated in the combined F2 population (Table 3 and Fig. 3). A peak at *D11Mit261* (LOD score of 8.9) was separated from a second peak at *D11Mit 41* (LOD score of 11.1) by 15 recombination units. We provisionally designate a locus within this interval as *Nidd3* with the expectation that interval-specific congenics will ultimately allow identification of more than one *Nidd* locus in this region. This locus exhibited highly significant linkage to serum IRI, but not BG, at 24 weeks. A temporal component affected ability to detect linkage, because the LOD score was drastically diminished at 36 weeks (Table 3). There was a significant main effect ($P = 0.02$) of *D11Mit261* on BW, with

TABLE 2
Diabetogenic subphenotypes arranged according to cluster groups depicted in Fig. 2

Cluster group, description (n)	24-week BW (g)	24-week BG (mg/dl)	24-week IRI μ U/ml)	36-week BW (g)	36-week BG (mg/dl)	36-week IRI (μ U/ml)
NON						
1, BG normal, IRI normal (20)	44.1 \pm 1.1	132 \pm 3	26 \pm 4	47.5 \pm 0.8	158 \pm 9	29 \pm 4
NZO						
2, BG normal, IRI high (13)	51.3 \pm 1.6	171 \pm 9	50 \pm 11	57.8 \pm 1.5	189 \pm 10	92 \pm 16
4, BG high, IRI high (16)	58.9 \pm 1.3	259 \pm 16	100 \pm 16	66.4 \pm 1.3	267 \pm 15	110 \pm 16
5, BG high, IRI normal (7)	63.9 \pm 1.2	324 \pm 22	81 \pm 15	67.3 \pm 2.7	366 \pm 12	60 \pm 22
7, BG very high, IRI low (3)	50.9 \pm 5.1	431 \pm 83	14 \pm 4	40.7 \pm 6.1	615 \pm 64	15 \pm 8
(NZO \times NON)F1						
2, BG normal, IRI high (5)	53.3 \pm 2.0	221 \pm 36	88 \pm 28	59.6 \pm 2.5	239 \pm 27	73 \pm 22
4, BG high, IRI high (23)	57.8 \pm 0.7	253 \pm 14	124 \pm 13	64.7 \pm 0.5	337 \pm 12	163 \pm 12
5, BG high, IRI normal (13)	61.6 \pm 1.6	367 \pm 30	71 \pm 10	66.6 \pm 0.9	439 \pm 13	70 \pm 9
7, BG very high, IRI low (11)	58.7 \pm 1.2	425 \pm 40	39 \pm 8	58.2 \pm 1.3	509 \pm 27	22 \pm 4
(NON \times NZO)F1						
2, BG normal, IRI high (7)	59.2 \pm 1.8	220 \pm 28	84 \pm 19	64.6 \pm 2.3	208 \pm 30	109 \pm 16
3, BG high, IRI very high (9)	64.8 \pm 1.0	313 \pm 28	140 \pm 13	69.7 \pm 1.2	314 \pm 24	297 \pm 35
4, BG high, IRI high (45)	62.2 \pm 0.6	330 \pm 11	102 \pm 7	68.8 \pm 0.6	386 \pm 12	162 \pm 13
6, BG very high, IRI normal (8)	61.7 \pm 1.0	448 \pm 23	41 \pm 6	62.9 \pm 2.0	538 \pm 38	32 \pm 4
(NZO \times NON)F2						
1, BG normal, IRI normal (36)	50.0 \pm 0.7	154 \pm 4	36 \pm 4	55.0 \pm 0.8	176 \pm 7	48 \pm 8
3, BG high, IRI very high (59)	56.6 \pm 0.7	230 \pm 10	153 \pm 15	62.5 \pm 0.8	274 \pm 9	179 \pm 19
4, BG high, IRI high (54)	58.6 \pm 0.8	328 \pm 14	99 \pm 8	63.6 \pm 1.1	396 \pm 13	81 \pm 7
6, BG very high, IRI normal (21)	53.8 \pm 0.9	418 \pm 23	34 \pm 5	52.1 \pm 1.7	570 \pm 12	23 \pm 5
7, BG very high, IRI low (17)	48.6 \pm 1.1	578 \pm 36	26 \pm 10	43.6 \pm 1.2	677 \pm 25	8 \pm 1
(NON \times NZO)F2						
1, BG normal, IRI normal (32)	49.2 \pm 1.1	163 \pm 6	34 \pm 5	54.1 \pm 1.4	173 \pm 5	58 \pm 7
3, BG high, IRI very high (14)	60.0 \pm 1.1	354 \pm 18	205 \pm 18	65.3 \pm 1.3	379 \pm 26	346 \pm 51
4, BG high, IRI high (88)	56.1 \pm 0.6	277 \pm 9	109 \pm 7	60.6 \pm 0.7	299 \pm 10	124 \pm 11
5, BG high, IRI normal (29)	54.2 \pm 0.9	414 \pm 17	43 \pm 5	54.1 \pm 1.3	533 \pm 15	33 \pm 5
6, BG very high, IRI normal (18)	48.8 \pm 1.2	579 \pm 30	20 \pm 3	46.0 \pm 1.2	721 \pm 26	10 \pm 2
7, BG very high, IRI low (14)	43.9 \pm 1.5	635 \pm 32	11 \pm 3	40.0 \pm 1.5	873 \pm 22	6 \pm 1

Data are means \pm SD.

the NZO allele associated with greater weight gain and accounting for 7% of the variance at 24 weeks. The direction of the cross affected expression of the NON allele; mice homozygous for the NON allele weighed less if their grandparental sire was an NZO male ($P = 0.04$).

Whereas the NZO-derived *Nidd3* susceptibility allele on Chr 11 affected serum IRI but not BG, at least one, and possibly two, more NZO diabetogenic factors determining high BG but not serum IRI may exist on Chr 5. Power to detect this linkage was reduced by the finding that the NZO contributions to elevated BG were exclusively obtained in the (NZO \times NON)F2 cross. As shown in Table 3, at the 24-week sampling interval, a moderately high LOD score (4.2) for BG was linked to an NZO locus marked by *D5Mit81* (28 cM). At 16 and 20 weeks, LOD scores were 2.8 and 3.5, respectively, whereas at 36 weeks, no significant linkage to *D5Mit81* was detectable. Genetic complexity of the Chr 5 contribution was further underscored by a second, discrete peak (LOD score = 2.9) detected at *D5Mit7* (Table 3), sited at least 18 cM distal to *D5Mit81*. There was suggestion of association of BW with both markers at 24 weeks ($P = 0.02$), accounting for 4.5% of the variance in BW. Another cross direction-specific NZO diabetogenic contribution was suggested on Chr 9, where a LOD score of 3.2 for *D9Mit28* (19 cM), potentially controlling serum IRI, was detected exclusively in the NON \times NZO outcross (Table 3).

DISCUSSION

The present study is the first to use genome-wide scan techniques to identify NIDDM susceptibility QTLs in the mouse. Our analysis identifies a minimum of three mouse *Nidd* loci with complex relationships to diabetes-related subphenotypes. In a human population, inheritance of sufficient numbers of polygenes to trigger clinical NIDDM in the majority of cases very likely represents contributions from both maternal and paternal progenitors. A threshold model in mice, wherein deleterious combinations of genes are acquired from both parents, has recently been simulated by introduction in the heterozygous state of one copy of a genetically targeted insulin receptor gene and one copy of a targeted mutation in an unlinked insulin receptor substrate-1 gene (20). This multigenic simulation, designed to impair insulin signaling, indeed produced a progressively more severe hyperinsulinemia and insulin resistance in F1 mice of both sexes on a mixed genetic background, with NIDDM developing in 39%, predominantly the males. An insulin resistance syndrome in the absence of hyperinsulinemia was observed in (BTBR \times C57BL/6J)F1 male progeny that was not present in either parental strain (21). Two previous studies have used segregation analysis to identify more than three *Nidd* genes controlling subphenotypes marking IGT in the GK rat (22,23). Further, a separate GK-derived locus was identified that

TABLE 3
Markers for potential *Nidd* loci and recombination distances (θ)

Locus	cM	θ	<i>n</i>	BG (24 weeks)	BG (36 weeks)	Ins (24 weeks)	Ins (36 weeks)
<i>D4Mit80</i>	37.7	—	—	3.5	4.7	4.9	5.4
<i>D4Mit166</i>	44.5	4.9	391	4.4	6.8	5.4	4.7
<i>D4Mit58</i>	48.5	3.5	394	5.4	5.6	6.9	5.7
<i>Lepr</i>		2.1	394	5.7	6.5	7.0	5.7
<i>D4Mit146</i>	50.8	4.0	394	4.5	4.6	5.8	4.6
<i>D5Mit75</i>	20.0	—	—	2.6*	—	—	—
<i>D5Mit81</i>	28.0	5.4	189	4.2*	—	—	—
<i>D5Mit300</i>	34.0	2.8	192	2.3*	—	—	—
<i>D5Mit134</i>	41.0	6.9	193	1.8*	—	—	—
<i>D5Mit7</i>	50.0	8.7	193	2.9*	—	—	—
<i>D5Mit208</i>	54.0	1.3	193	2.3*	—	—	—
<i>D5Mit158</i>	62.0	7.6	122	1.4*	—	—	—
<i>D9Mit90</i>	9.0	—	—	—	—	1.9†	—
<i>D9Mit128</i>	18.0	3.8	198	—	—	3.2†	—
<i>D9Mit207</i>	33.0	13.1	198	—	—	2.5†	—
<i>D11Mit77</i>	2.0	—	—	—	—	2.3	2.4
<i>D11Mit19</i>	14.0	4.9	392	—	—	2.6	2.0
<i>D11Mit270</i>	20.0	12.6	393	—	—	4.0	2.0
<i>D11Mit131</i>	29.0	4.5	392	—	—	6.8	3.5
<i>D11Mit261</i>	34	4.2	392	—	—	8.9	2.8
<i>Acrb</i>	40	4.0	392	—	—	7.5	2.7
<i>D11Mit41</i>	49	11.0	392	—	—	11.1	3.0
<i>D11Mit70</i>	54	5.0	393	—	—	8.1	2.9
<i>D11Mit126</i>	63	8.2	393	—	—	4.3	—
<i>D12Mit203</i>	38.0	—	—	—	—	2.1	—
<i>D12Mit204</i>	40.0	3.9	388	—	—	3.0	—
<i>D12Mit47</i>	45.0	5.6	388	—	—	2.0	—
<i>D18Mit110</i>	4.0	—	—	2.8	1.8	—	—
<i>D18Mit68</i>	10.0	6.0	387	4.6	2.6	—	—
<i>D18Mit60</i>	16.0	4.2	387	5.0	2.8	—	—
<i>D18Mit236</i>	21.0	4.3	390	3.7	2.2	—	—

LOD scores are presented for a free model and only for those chromosomes showing a marker with a LOD score ≥ 3 . Values under cM are map position data from 1997 Mouse Chromosome Committee reports (39). *Lepr* position based on current recombination data confirming previously published physical mapping data (17). *NZO \times NON F2 only; †NON \times NZO F2 only.

increased BW and interacted with insulin but not glucose levels. These studies show a uniparental (GK) contribution, because no diabetogenic contributions from the "normal" progenitor strains were detected (although they probably exist). More recently, an F2 segregation analysis of diabetogenic background modifiers of the *Lepr^{fa}* gene in rats showed contributions by both parental strains (24). In the present study, we demonstrate that the NON genome contributes at least two loci (*Nidd1* and *Nidd2*) that synergize deleteriously with other NZO-contributed *Nidd* genes (*Nidd3* and possibly additional loci on Chr 5, 9, and 12) to move more individuals across a diabetogenic threshold.

The highly significant linkage for *Nidd1* (Chr 4) derived from the NON genome is within the region containing the leptin receptor (*Lepr*) gene. In addition to linkage to both of the two NIDDM subphenotypes (BG and serum IRI), *Nidd1* further contributed a significant main effect ($P < 0.0005$) to BW, with homozygosity for the NZO allele associated with greater BW gain during the maturational phase after 16 weeks of

age. The NON leptin receptor cDNA has been sequenced and appears normal, although serum and adipocyte leptin levels were deemed disproportionately low given an increased fat depot mass in this strain (25). Hence, it seems unlikely that the diabetogenic contribution from NON in this region from Chr 4 represents a deviant leptin receptor. The NON (and GK) strain characteristic of reduced insulin secretion in response to glucose has been noted above. Insulin is a major regulator of leptin gene expression (26). Islet β -cells express leptin receptors on their surface, and leptin exerts negative feedback on β -cell insulin secretion (27). However, in NON mice, low serum leptin level is not accompanied by elevated IRI secretion. This would suggest that if an adipocyte-insular axis indeed exists, it is not fully operative in NON mice. Among interesting candidate genes in this area are a Janus kinase encoding gene (*Jak1*) and a tyrosine kinase receptor (*Tie1*) gene. A member of the hepatocyte nuclear factor 3 gene family (*Hfh2*) has been mapped in proximity to *Lepr* (28). The support intervals for *Nidd2* on Chr 18 and *Nidd3* on medial

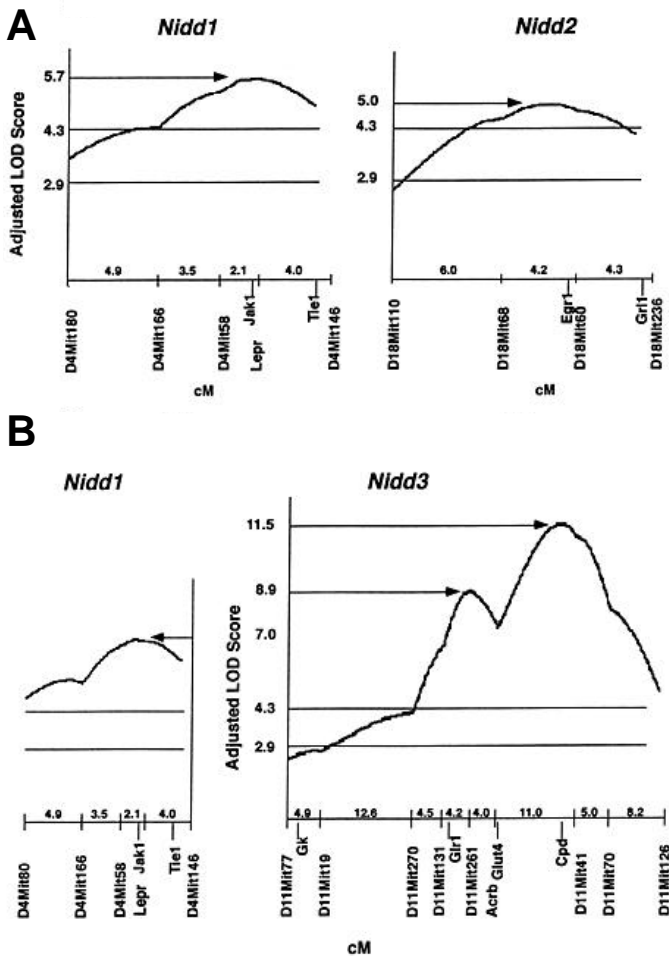


FIG. 3. Support intervals for *Nidd* loci using nonparametric analysis and assuming a free model. Potential candidate genes in the intervals are noted; numbers between microsatellite markers represent recombination distances in cM. **A:** QTL intervals for BG. **B:** QTL intervals for serum insulin.

Chr 11 encompass several interesting candidate genes. On Chr 18, a locus termed early growth response 1 (*Egr1*) and the glucocorticoid receptor-1 (*Grl1*) map within the support interval (29). On Chr 11, several glutamate receptors (*Glr1*), an acetylcholine receptor, β -subunit (*Acrb*), carboxypeptidase D (*Cpd*) (30), and the muscle-adipocyte glucose transporter (*Glut4*) present potential candidate genes for *Nidd3*. Subnormal GLUT-4 activity has been observed in brown adipose tissue of 5-week-old NZO mice (31). Interestingly, a locus contributing to IDDM (*Id4*) has also been found in this interval (32). The *Cpd* gene represents an intriguing candidate gene because we have previously found that a null mutation in the carboxypeptidase E (*Cpe*) locus on Chr 8 produced a male sex-specific obesity-diabetes syndrome on the C57BLKS/J background (33). However, preliminary biochemical analysis failed to indicate any differences in catalytic activity of CPD in brain extracts from NZO compared with NON mice (Dr. Lloyd Fricker, Albert Einstein School of Medicine, personal communication).

Weaker evidence suggesting presence of at least one or more *Nidd* loci on Chr 5 is of interest. We have found that the *D5Mit7* marker for the smaller of two peak LOD scores detected on this chromosome showed no recombinants with

the vitamin D binding protein (*Vdbp*) locus when the latter gene was typed by us in The Jackson Laboratory (B6 \times SPRET) \times SPRET DNA mapping panel (34). Specific variants at this locus, also known in humans as *GC* (group-specific component protein, Chr 4q.12), have recently been associated with elevated plasma glucose levels in both Pima Indians and Japanese NIDDM patients (35–37). Suggestive evidence for linkage on Chr 9 and Chr 12 was also obtained. Development of recombinant congenic stocks of NON mice carrying putative *Nidd* susceptibility alleles identified in Table 3 are currently being developed in an effort to confirm the contributions of the chromosomal regions potentially harboring *Nidd* loci.

These present results in mice are consistent with previous genetic studies in rats showing a significant parental strain/sex effect on phenotypic variables associated with pathophysiology of diabetes in F1 and F2 progeny following outcross. Previous studies indicated that maternal-paternal effects were associated with severity of IGT in reciprocal outcross between GK (derived by selective breeding of glucose-intolerant Wistar rats) and glucose-tolerant Wistar rats, with greater impairment associated with GK as the maternal parent (22). However, in another reciprocal outcross between GK and Fisher 344 rats, a higher level of glucose intolerance was associated with GK as the paternal parent (23). These effects could be explained by a pathogenic point mutation in a mitochondrial gene similar to that reported in diabetes-prone BHE rats (38). Parental genomic imprinting or maternal effects on the fetus peculiar to the gestational environment also represent logical possibilities. Among the latter effects might be perturbations of fetal metabolism if gestational diabetes were present in the dam or differential exposure to fetal sex steroids if a male fetus developed between two male fetuses versus two female fetuses. In the present study, NIDDM was prevalent in both reciprocal F1 male populations, so that matrilineal inheritance of a mitochondrial gene defect affecting energy metabolism seems unlikely. Preliminary results from reciprocal F1 backcrosses to NON/Lt also confirm that NIDDM can develop regardless of whether the mitochondrial donors are NZO or NON females. This same series of backcrosses of reciprocal F1 mice to NON sires or dams also demonstrated that the NZO Y chromosome was not required for NIDDM development. Interestingly, the preliminary backcross data to NON confirmed what was observed in NZO/Hi males regarding the relationship between early weight gain and risk for NIDDM development. Post-hoc analysis of rates of BW gain in both NZO/Hi and first backcross males over an 8-week span from weaning revealed that males that were to make the transition from IGT to NIDDM gained weight more rapidly than did those that remained normoglycemic over this period. Although only a 12.5% difference in mean BW at 12 weeks distinguished future diabetic NZO/Hi males from those destined to remain normoglycemic, the subtle environmental factors affecting these maturational changes in weight (and which probably include litter effects) were clearly important in determining risk for NIDDM development. The actual mechanisms underlying either maternal or paternal diabetogenic effects depending on specific strain combinations remain unresolved, because none of the highly significant *Nidd* intervals identified in this study were dependent on cross direction, nor were they mapped within previously reported imprintable genomic regions. Evidence for cross-dependent linkages

TABLE 4
Nidd loci controlling BG and/or IRI indicated by QTL analysis at 24- and 36-week sampling intervals

Locus	Susceptibility donor	Interval marker	Phenotype/LOD score	BW associated (% variance)
<i>Nidd1</i> (Chr 4)	NON	<i>Lepr</i>	BG ₂₄ = 5.7 IRI ₂₄ = 7.0 BG ₃₆ = 6.5 IRI ₃₆ = 5.7	Yes (11%)
<i>Nidd2</i> (Chr 18)	NON	<i>D18Mit60</i>	BG ₂₄ = 5.0	No
<i>Nidd3</i> (Chr 11)	NZO	<i>D11Mit41</i> <i>D11Mit261</i>	IRI ₁₆ = 11.9 IRI ₂₄ = 11.0 IRI ₂₄ = 8.9	Yes (7%)

Nonparametric LOD score for both crosses were combined (for BG and/or IRI) at 24 or 36 weeks.

were based on LOD scores that were only suggestive, such that a larger population of F2 male segregants would have to be analyzed to establish whether the linkages were spurious or real.

In conclusion, we have demonstrated that F1 males from reciprocal outcrosses between NZO/HI and NON/Lt are more NIDDM-susceptible than are NZO/HI males, establishing the principle that deleterious polygenes from both parental genomes can interact synergistically to increase the numbers of individuals exceeding diabetogenic thresholds. We have only begun, through F2 segregation analysis, the task of identifying the genetic map positions of "diabesity" genes contributed by each parental genome. Studies to validate the putative linkages identified in the present report are in progress, entailing both first-backcross analysis to NON/Lt and construction of interval-directed recombinant congenic stocks at the second backcross.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant RR08911, by the Ministerium für Wissenschaft und Forschung des Landes Nordrhein Westfalen (Düsseldorf), and by the Bundesministerium für Gesundheit (Bonn). Institutional shared services at The Jackson Laboratory were supported by National Cancer Center support grant CA-34196.

We gratefully acknowledge the expert technical assistance of Angela Schraven and Susanne Breitwieser.

REFERENCES

- Bouchard C: Genetics and the metabolic syndrome. *Int J Obes* 19:S52-S59, 1995
- Leiter E: Obesity genes and diabetes induction in the mouse. In *Critical Reviews in Food Science and Nutrition*. Filer L, Ed. Boca Raton, FL, CRC, 1993, p. 333-338
- Makino S, Yamashita H, Kunimoto K, Tsukahara K, Uchida K: Breeding of the NON mouse and its genetic characteristics. In *Current Concepts of a New Animal Model: the NON Mouse*. Sakamoto N, Hotta N, Uchida K, Eds. Tokyo, Elsevier Science, 1992, p. 4-10
- Leiter EH, Herberg L: The polygenetics of diabesity in mice. *Diabetes Rev* 5:131-148, 1997
- Portha B, Serradas P, Bailbe D, Suzuki K-I, Goto Y, Giroix M-H: β -Cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. *Diabetes* 40:486-491, 1991
- Proietto J, Larkins RG: A perspective on the New Zealand obese mouse. In *Lessons from Animal Models of Diabetes IV*. Shafir E, Ed. London, Smith-Gordon, 1993, p. 65-73
- Andrikopoulos S, Proietto J: The biochemical basis of increased hepatic glucose production in a mouse model of type 2 (non-insulin-dependent) diabetes

- Diabetologia* 38:1389-1396, 1995
- Andrikopoulos S, Rosella G, Kaczmarczyk SJ, Zajac JD, Proietto J: Impaired regulation of hepatic fructose-1,6-bisphosphatase in the New Zealand Obese mouse: an acquired defect. *Metabolism* 45:622-626, 1996
- Herberg L: Insulin resistance in abdominal and subcutaneous obesity: comparison of C57BL/6J-ob/ob with New Zealand obese mice. In *Frontiers in Diabetes Research. Lessons from Animal Diabetes II*. Shafir E, Renold A, Eds. London, John Libbey, 1988, p. 367-373
- Igel M, Becker W, Brauers A, Herberg L, Joost H-G: Evidence for resistance to leptin in obese, hyperinsulinemic KK and NZO mice (Abstract). *Diabetes* 45 (Suppl. 1):151A, 1996
- Igel M, Becker W, Joost H-G, Herberg L: Post-receptor resistance to leptin in New Zealand obese mice. *Exp Clin Endocrinol Diab* 105:37, 1997
- Halaas J, Boozer C, Denton D, Blairws J, Friedman J, Fidahuse N: Physiological-response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci U S A* 94:8878-8883, 1997
- Taylor B, Phillips S: Detection of obesity QTLs on mouse chromosome 1 and 7 by selective DNA pooling. *Genomics* 34:389-398, 1996
- Igel M, Becker W, Herberg L, Joost H-G: Hyperleptinemia in the New Zealand obese (NZO) mouse reflects disruption of leptin signaling from its receptor. *Endocrinology* 138:4234-4239, 1997
- Norusis M: *SPSS Professional Statistics 6.1 Handbook*. New York, McGraw Hill, 1994, p. 267-277
- Manley K: A Macintosh program for storage and analysis of experimental genetic mapping data. *Mamm Genome* 4:301-313, 1993
- Lander E, Shork N: Genetic dissection of complex traits. *Science* 45:1-14, 1994
- Mock B, Neumann P, Fiedorek F Jr: Mouse chromosome 4. *Mamm Genome* 7:S60-S69, 1997
- Chua SCJ, Chung W, Wu-Peng X, Zhang Y, Liu S, Tartaglia L, Leibel R: Phenotypes of mouse *diabetes* and rat *fatty* due to mutations in the OB (leptin) receptor. *Science* 271:994-996, 1996
- Bruning J, Winnay J, Bonner-Weir S, Taylor S, Accilli D, Kahn C: Development of a novel polygenic model of NIDDM in mice heterozygous for *IR* and *IRS-1* null alleles. *Cell* 88:561-572, 1997
- Ranheim T, Dumke C, Schueler K, Cartee G, Attie A: Interaction between BTBR and C57BL/6J genomes produces an insulin resistance syndrome in (BTBR \times C57BL/6J)F1 mice. *Arterioscler Thromb Vasc Biol* 17:3286-3293, 1997
- Gauguier D, Nelson I, Bernard C, Parent V, Marsac C, Cohen D, Froguel P: Higher maternal than paternal inheritance of diabetes in GK rats. *Diabetes* 43:220-224, 1994
- Galli J, Li L-S, Glaser A, Östenson C-G, Jiao H, Fakhrai-Rad H, Jacob H, Lander ES, Luthman H: Genetic analysis of non-insulin dependent diabetes mellitus in the GK rat. *Nat Genet* 12:31-37, 1996
- Chung W, Zheng M, Chua M, Kershaw E, Powerkeh L, Tsuji M, Wupeng X, Williams J, Chua S, Leibel R: Genetic modifiers of *Lepr^{fa}* associated with variability in insulin production and susceptibility to NIDDM. *Genomics* 41:332-344, 1997
- Igel M, Becker W, Herberg L, Joost H-G: Evidence that reduced leptin levels, but not an aberrant sequence of leptin or its receptor, contribute to the obesity syndrome in NON mice. *Horm Metab Res* 28:669-673, 1996
- Caro J, Sinha M, Kolaczynski J, Zhang P, Considine R: Leptin: the tale of an obesity gene. *Diabetes* 45:1455-1462, 1996
- Kieffer T, Heller R, Habener J: Leptin receptors expressed on pancreatic beta cells. *Biochem Biophys Res Commun* 224:522-527, 1996

28. Clevidence DE, Overdier DG, Tao W, Qian X, Pani L, Lai E, Costa RH: Identification of nine tissue-specific transcription factors of the hepatocyte nuclear factor 3/forkhead DNA-binding domain. *Proc Natl Acad Sci U S A* 90:3948-3952, 1993
29. Johnson K, Davisson M: Mouse chromosome 18. *Mamm Genome* 7:S295-S304, 1997
30. Xin X, Varlamov O, Day R, Bridgett M, Leiter EH, Fricker LD: Cloning and sequence analysis of cDNA encoding rat carboxypeptidase D. *DNA Cell Biol* 16:897-909, 1997
31. Ferreras L, Kelada ASMK, McCoy M, Proietto J: Early decrease in GLUT4 protein levels in brown adipose tissue of New Zealand obese mice. *Int J Obes* 18:760-765, 1994
32. Ghosh S, Palmer SM, Rodrigues NR, Cordell HJ, Hearne CM, Cornall RJ, Prins J-B, McShane P, Lathrop GM, Peterson LB, Wicker LS, Todd JA: Polygenic control of autoimmune diabetes in nonobese diabetic mice. *Nat Genet* 4:404-409, 1993
33. Naggert JK, Fricker LD, Varlamov O, Nishina PM, Rouille Y, Steiner DF, Carroll RJ, Paigen BJ, Leiter EH: Hyperproinsulinaemia in obese *fat/fat* mice associated with a carboxypeptidase E mutation which reduces enzyme activity. *Nat Genet* 10:135-142, 1995
34. Leiter EH: The vitamin D receptor (*Vdr*) is linked to the vitamin D binding protein (*Vdbp*) on mouse chromosome 5. *Mouse Genome Informatics Database Submission* Accession ID: MG1: 1194727: J45281 (<http://www.jax.informatics.org/>), 1998
35. Baier L, Thuillez P, Dobberfuhr A, Bogardus C: Association of a variant of the vitamin D binding protein with plasma glucose levels in non-diabetic Pima Indians (Abstract). *Diabetes* 46:170A, 1997
36. Pratley R, Thompson D, Prochazka M, Baier L, Pima Diabetes Genes Group: A genome scan for linkage to quantitative traits predicting NIDDM in Pima Indians (Abstract). *Diabetes* 46:170A, 1997
37. Hirai M, Suzuki S, Hinokio Y, Hiral A, Chiba M, Toyota T: Group specific component protein genotype is associated with non insulin dependent diabetes mellitus in Japan (Abstract). *Diabetes* 46:170A, 1997
38. Mathews C, McGraw R, Berdanier C: A point mutation in the mitochondrial DNA of diabetes-prone BHE/cdb rats. *FASEB J* 9:1638-1642, 1995
39. Mouse Chromosome Committee reports. *Mamm Genome* 7 (Special Issue):1997