

Genetic Modifiers of the Insulin Resistance Phenotype in Mice

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Insulin resistance can result from genetic interactions among susceptibility alleles. To identify genetic loci predisposing to insulin resistance, we used crosses between different strains of mice with a targeted null allele of the insulin receptor gene. On the genetic background of B6 mice, the insulin receptor gene mutation causes mild hyperinsulinemia. In contrast, on the genetic background of 129/Sv mice, the same mutation causes severe hyperinsulinemia, suggesting that the 129/Sv strain harbors alleles that interact with the insulin receptor mutation and predispose to insulin resistance. As a first step to identify these alleles, we generated an F₂ intercross between insulin receptor heterozygous mutant mice on B6 and 129/Sv backgrounds (B6^{IR} × 129^{IR}) and performed a genome-wide scan with polymorphic markers at a 20-cM resolution. We report the identification of loci on chromosomes 2 (logarithm of odds [LOD] 5.58) and 10 (LOD 5.58) that show significant evidence for linkage to plasma insulin levels as a quantitative trait. These findings indicate that targeted mutations in knockout mice can be used to unravel the complex genetic interactions underlying insulin resistance. *Diabetes* 49:589–596, 2000

Insulin resistance is an important factor in the pathogenesis of type 2 diabetes. It is generally agreed that insulin resistance has a major genetic component (1,2), but efforts to unravel predisposing genes have generally been unsuccessful. In fact, with the exception of the insulin receptor (IR) gene, which is mutated in a subset of patients with extreme insulin resistance, there is no firm evidence in humans of other insulin resistance genes (3). Because of the non-Mendelian nature of type 2 diabetes, several explanations are possible, including genetic heterogeneity, multigenic inheritance, incomplete penetrance, phenocopies, and transmission through mitochondrial DNA (1,4). To complicate matters further, it should be empha-

sized that, in a common disorder such as type 2 diabetes, many predisposing alleles may be represented by polymorphic variants, the functional impact of which may be hard to demonstrate, as shown in studies of the insulin receptor substrate (IRS)-1 gene (5,6).

Genetic studies in rodents enable us to circumvent some of these limitations. Two developments have especially affected our ability to use mice as an experimental system to address the genetics of complex disorders like type 2 diabetes: 1) the increased ability to manipulate the mouse genome by transgenesis and homologous recombination in embryonic stem cells (7) and 2) the development of a comprehensive linkage map of the mouse genome (8). One of the hypotheses that can be tested in mouse models is that unlinked allelic variants may interact to yield a clinical phenotype such as type 2 diabetes (9). In this respect, we have recently shown that combined heterozygosity for *ir* and *irs-1* null alleles gives rise to epistatic interactions to impair insulin action and cause a large number of such double-heterozygous mice to develop a type 2 diabetes-like syndrome (10). An important lesson from these and other studies (11,12) is that even major mutations, such as null *ir* or *irs-1* alleles, can hardly cause insulin resistance by themselves but have major effects in the context of a predisposing genetic background (13).

Whereas studies of *ir/irs-1* mice provide a model for genetic interactions, they do not identify novel diabetes genes. However, we noticed that on an outbred genetic background (B6 × 129/Sv), the *ir* mutation caused diabetes in ~5–10% of mice, suggesting that this mutation may interact in a similar synergistic fashion with naturally occurring alleles to cause insulin resistance and diabetes. Thus, in this study, we set out to map those alleles by way of a whole-genome scan in an F₂ cross between mice congenic for the *ir* mutation on either a B6 or 129/Sv strain. On the background of the B6 genome, the mutation has a mild effect to raise insulin levels and does not cause diabetes. On the background of the 129/Sv genome, the mutation causes severe insulin resistance, as indicated by elevated plasma insulin levels, and causes diabetes in a subgroup (~25%) of mice. Thus, the B6 strain is resistant to the effects of the *ir* null allele, and the 129/Sv strain is more sensitive to the mutation. To better characterize the metabolic effects of this mutation, we performed an outcross of B6 × 129^{IR} mice and measured plasma insulin and glucose levels, as well as body weight, in F₂ mice. The data indicated that the *ir* mutation exerts a strong effect on insulin levels but not on plasma glucose levels or body weight. We therefore carried out a genome-wide scan in F₂ mice and analyzed the data using plasma insulin levels as a quantitative genetic trait. We report the identification of three quantitative trait loci (QTL) exceeding the threshold for

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IACUC, Institutional Animal Care and Utilization Committee; IR, insulin receptor; IRS, insulin receptor substrate; LOD, logarithm of odds; QTL, quantitative trait loci.

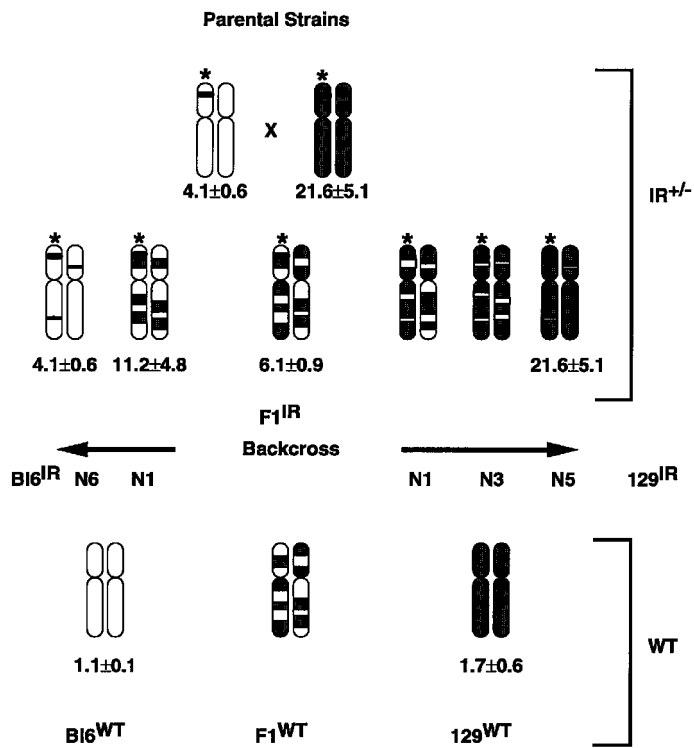


FIG. 1. Generation of recombinant congenic strains and F_2 intercross. The rationale for the experiments described in this article is depicted. To derive recombinant congenic strains on either background, F_1 hybrid mice generated by mating chimeric 129^{IR} and $B6^{WT}$ were repeatedly backcrossed onto B6 or 129/Sv. At each backcross, mice were genotyped for the presence of the null *ir* allele. The null allele is embedded in sequences derived from the 129/Sv genome; therefore, a small region of 129/Sv genome is always present around the *ir* locus on chromosome 8 and is depicted as a black band near the *IR* locus. As the number of generations increases, so does the amount of genome contributed by the donor strain. Insulin levels are indicated at the bottom of the chromosome. Please note that, with progressively increasing generation numbers, insulin levels drop in the B6 backcross and increase in the 129/Sv backcross. The control cross is shown at the bottom. In the absence of the *ir* mutation, insulin levels are not affected. *, *ir* null allele; ●, 129/Sv-derived chromosomes; ○, the B6-derived chromosomes.

significant linkage. Because these interactions are not observed in the same cross in the absence of the null *ir* allele, we conclude that these alleles act epistatically (13) and represent hyperinsulinemia susceptibility loci. Identification of the susceptibility alleles at these loci will provide important novel information on the genetics of insulin resistance.

RESEARCH DESIGN AND METHODS

Animal husbandry. The derivation of the *ir* null allele has been described previously (14). Briefly, the targeted *ir* mutation was derived in J-1 embryonic stem cells and was tested for germ line transmission by crossing with B6 mice. Thus, the genetic background of the *ir* mutation is the 129/Sv Steel substrain (15). Recombinant congenic strains carrying the *ir* mutation in a B6 or 129/Sv background were obtained by repeated backcrosses of F_1 hybrid mice ($B6 \times 129^{IR}$). The strategy is summarized in Fig. 1. Six rounds of backcrosses were used to generate B6 mice congenic for the *ir* null allele, referred to as $B6^{IR}$. Five rounds of backcrosses were used to generate 129/Sv mice congenic for the *ir* null allele, referred to as 129^{IR} . For 129/Sv backcrosses, we used the 129/SvEvTac stock from Taconic Farms (Germantown, NY). This stock derives from the same Steel substrain from which J-1 cells were derived. Thus, this stock provides a close, but not complete, genetic match for the J-1 cells (15). Both male and female animals were used to generate backcrosses, so that Y chromosomes were derived from either strain. At each mating, animals

were genotyped as described to identify the mutant *ir* allele. In addition, limited genotyping of $B6^{IR}$ mice using polymorphic markers confirmed that they are almost entirely B6 derived. We estimate that ~99% of the $B6^{IR}$ mice genome derives from B6 and ~96% of the 129^{IR} mice genome derives from 129/Sv.

Phenotype analysis. Phenotypic analysis was conducted in 6-month-old mice. Blood was drawn from the retroorbital sinus of fed, anesthetized animals between 9:00 and 11:00 A.M. Animals were anesthetized with pentobarbital (40 mg/kg body wt). Glucose levels were measured using a glucometer (Accucheck; Boehringer Mannheim, Indianapolis, IN). Diabetes was defined as random plasma glucose higher than the mean + 2 SD of values on at least two separate occasions in wild-type mice of the same genetic background. Insulin was measured in plasma samples by a radioimmunoassay using a rat insulin standard (Linco, St. Louis, MO). All assays were carried out in duplicate. Each value is the mean of two independent determinations. Hyperinsulinemia was observed in male mice only. Therefore, only males were included in the analysis. All procedures were approved by the Institutional Animal Care and Utilization Committee (IACUC) (National Institute of Child Health and Human Development protocol #96-010; Columbia University IACUC protocol #2715.01).

Statistical analyses. Descriptive statistics, analysis of variance followed by Fisher's test, and regression analysis were performed using Statview software (Abacus Concepts, Berkeley, CA). The variance of the data was calculated as the sample sum of squares divided by its degrees of freedom.

Genome-wide scan in the F_2 progeny. F_2 mice were obtained by intercrosses of F_1 hybrid mice derived from $B6 \times 129^{IR}$ matings. DNA was prepared from tail biopsies as described (14). Eighty-six markers at an average recombination distance of 20 cM were typed. Markers were from Research Genetics (Huntington, AL). Detailed sequence information is available from the corresponding author on request. Polymerase chain reaction conditions were as recommended by the manufacturer. Genotypes were scored using 15% polyacrylamide/urea or 4% agarose gels. Data were analyzed using the scan function of MapMaker/QTL (16–18). A log transformation of the insulin data values was used because of the skewed distribution observed in the F_2 population. Initially, 77 markers were typed in 40 mice at the two extremes of the trait distribution (20 with the highest insulin values and 20 with the lowest insulin values). Thereafter, all F_2 mice were typed at markers showing suggestive or significant logarithm of odds (LOD) scores and at an additional nine markers to narrow candidate intervals. To analyze syntenic regions, we used the National Center for Biotechnology Information Human/Mouse Homology Maps (www.ncbi.nlm.nih.gov). For candidate genes, reference is provided using the Online Mendelian Inheritance in Man catalog reference number (www.ncbi.nlm.nih.gov/omim). For disease loci, we refer to the original report.

RESULTS

The effect of an *ir* null allele varies on different genetic backgrounds. In previous studies, we observed that heterozygosity for a null *ir* allele is associated with a variable pattern of insulin resistance and diabetes in mice of mixed genetic background ($129/Sv \times B6$) (10,14). We tested the hypothesis that genetic factors accounted for this variability. To this end, we generated recombinant congenic strains in which the *ir* null mutation is present in an almost pure B6 or 129/Sv background (19,20) (Fig. 1). As shown in Table 1, 129^{IR} mice develop severe hyperinsulinemia; in contrast, $B6^{IR}$ mice develop mild hyperinsulinemia. These data were obtained in male mice—females did not develop hyperinsulinemia on either background (data not shown). In all the ensuing analyses, only male mice are included. These findings are consistent with the hypothesis that the genetic background influences the susceptibility to insulin resistance (as assessed by plasma insulin levels) of mice carrying a null *ir* allele. The 129 strain is sensitive to the effect of the *ir* null allele to cause hyperinsulinemia, whereas the B6 is resistant to this effect. As a control, hyperinsulinemia is not observed in the same animals without the *ir* mutation (Table 1), indicating that the observed phenotype is due to genetic interactions elicited by the *ir* mutation and not by other deleterious interactions between the two genomes.

Generation of an F_2 intercross between B6 and 129/Sv mice. To begin to map the genetic loci responsible for the variation, we performed a cross between 129^{IR} mice and wild-type

TABLE 1
Effect of backcross on insulin, glucose, and body weight values

	B6 ^{IR}	129 ^{IR}	F ₁ ^{IR}	F ₂ ^{IR}	BC1 ^{IR}	B6 ^{WT}	129 ^{WT}
n	10	12	11	189	9	7	5
Insulin (ng/ml)	4.1 ± 0.6	21.6 ± 5.1	6.1 ± 0.9	9.6 ± 1.1	11.2 ± 4.8	1.1 ± 0.1	1.7 ± 0.6
Glucose (mg/dl)	173 ± 5	201 ± 30	166 ± 9	177 ± 5	166 ± 6	175 ± 8	145 ± 13
Body weight (g)	30.8 ± 0.8	32.8 ± 1.8	33.6 ± 1.2	33.4 ± 0.5	32.9 ± 1.4	32.1 ± 0.9	30.0 ± 2.1
Insulin							
8 Weeks	2.1 ± 0.3	—	—	—	2.1 ± 0.3	0.92 ± 0.1	—
16 Weeks	2.8 ± 0.5	—	—	—	4.5 ± 1.3	1.1 ± 0.1	—
24 Weeks	4.1 ± 0.6	—	—	—	9.65 ± 4.0	0.9 ± 0.1	—
30 Weeks	5.5 ± 1.1	—	—	—	19.1 ± 6.2	1.5 ± 0.2	—

Data are means ± SE. The first three rows represent data derived from Fig. 2 on 6-month-old male mice, and the bottom five rows represent data on animals studied prospectively.

B6 mice (B6^{WT}) and intercrossed the resulting mice to obtain an F₂ generation. We measured plasma insulin levels, blood glucose, and body weight in 189 F₂^{IR} mice. The choice of B6^{WT} as opposed to B6^{IR} mice was designed to avoid perinatal losses due to homozygosity for the *ir* mutation.

In F₁^{IR} (129^{IR} × B6^{WT}) hybrids, insulin levels were intermediate between the parental B6^{WT} and 129^{IR} strains (6.1 vs. 1.1 and 29.1 ng/ml, respectively). In contrast, in F₂^{IR} hybrids and in (129^{IR} × B6^{WT}) × B6^{WT} backcrosses (BC1^{IR}), insulin levels increased 57 and 83% compared with F₁^{IR} mice (9.6 and 11.2 ng/ml, respectively, vs. 6.1 ng/ml) (Table 1). The increase in insulin values in the F₂^{IR} and BC1^{IR} generations was associated with a large increase in the variance of the data compared with the F₁^{IR} generation (F₁^{IR} = 9.3 vs. F₂^{IR} = 213 and BC1^{IR} = 206). In a cross between a susceptible and a resistant strain, the variance of a trait observed in the F₁ generation is attributable to environmental effects, whereas the variance in the F₂ generation and in backcrosses is a function of both the genetic and environmental component of the trait. By comparing the variance of insulin values in the F₂^{IR} generation with the variance observed in the F₁^{IR} generation, we conclude that genetic loci control the effect of the *ir* mutation on insulin levels in this cross. Interestingly, insulin levels in the congenic 129^{IR} strain show a larger variability than in the congenic B6^{IR} strain (Fig. 2A). This difference may be due to genetic heterogeneity among the various 129 substrains (15), the limited number of backcrosses performed to date, or an intrinsic environmental effect on insulin levels.

Effect of the null *ir* allele on plasma glucose levels and body weight. We wanted to determine whether the null *ir* allele would affect other quantitative traits, such as glucose levels and body weight. Analysis of F₂^{IR} and BC1^{IR} mice suggests that the *ir* null allele specifically affects insulin levels. In fact, glucose levels are elevated in the diabetic range only in a subgroup of F₂^{IR} mice (~15%) but not in BC1^{IR} mice (Fig. 2B). These data indicate that the development of hyperglycemia in F₂^{IR} mice requires homozygosity for one or more 129/Sv-derived predisposing alleles. Moreover, as shown in Fig. 3, there are at least two subgroups of hyperglycemic mice: a hyperinsulinemic/mildly hyperglycemic group and a normoinsulinemic/frankly hyperglycemic group. This observation suggests that the pathogenesis of hyperglycemia is likely to be complex in this cross, with contributions from alleles predisposing to either insulin resistance or β -cell failure. The complexity of the genetic interactions leading to hyperglycemia is underscored by the fact that although all recombinant inbred 129^{IR} mice show hyperinsulinemia, only some (~25%) show hyperglycemia. At this stage in the backcross (N5), it is not possible to conclude that the extreme insulin resistance observed in 129^{IR} mice is invariably associated with diabetes (Fig. 2B).

In contrast, the *ir* mutation does not affect body weight in F₂^{IR}, BC1^{IR}, 129^{IR}, and B6^{IR} mice (Fig. 2C). Insulin and glucose values in (129^{IR} × B6^{WT}) F₂ hybrids. To better characterize the metabolic abnormality in F₂ mice, we studied the correlation between insulin and glucose levels. In F₂^{WT} mice, insulin and glucose values are main-

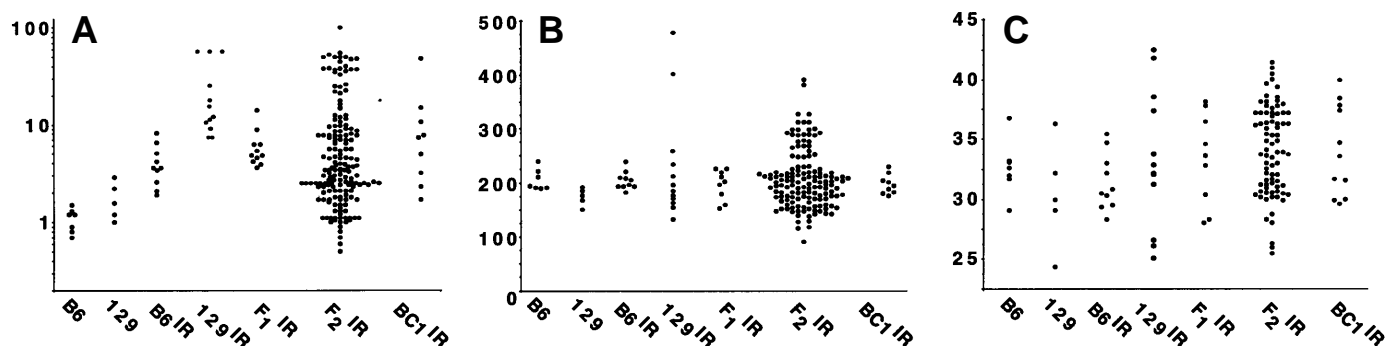


FIG. 2. Backcross analysis of plasma insulin levels (ng/ml) (A), glucose levels (mg/dl) (B), and body weight values (g) (C) in 6-month-old male 129^{IR}, B6^{IR}, F₁^{IR}, F₂^{IR}, and BC1^{IR} mice. The data are presented as scattergrams with individual values.

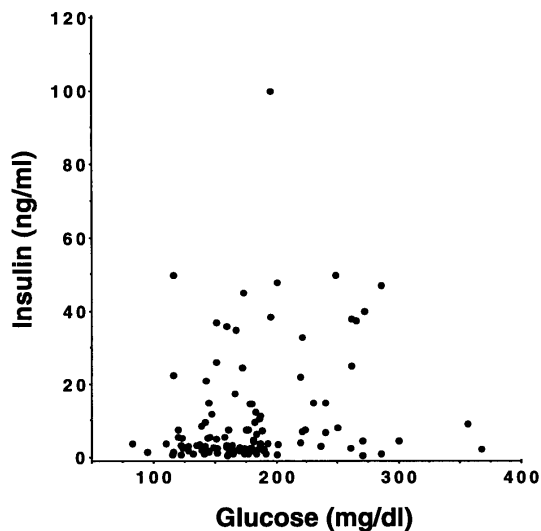


FIG. 3. Scattergram of plasma insulin and glucose values in 6-month-old male F_2 hybrid mice. The results shown represent the average of at least two independent measurements for each mouse. We arbitrarily identified three subclasses of F_2^{IR} mice: normoinsulinemic/normoglycemic (lower left area, insulin <10 ng/ml and glucose <200 mg/dl), hyperinsulinemic/normoglycemic (middle upper area, insulin >10 ng/ml, glucose >200 and <260 mg/dl), and normoinsulinemic/hyperglycemic (lower right area, insulin <10 ng/ml, glucose >260 mg/dl).

tained within a relatively narrow range (not shown). In contrast, as shown in Fig. 3, both insulin and glucose values are scattered over a broad range in F_2^{IR} mice, so that no statistically significant correlation between variations of the two parameters can be established. Interestingly, we can distinguish three categories among F_2^{IR} mice: a normoinsulinemic/normoglycemic group (insulin <10 ng/ml, glucose <200 mg/dl), a hyperinsulinemic/normoglycemic group (insulin >10 ng/ml, glucose >200 – 260 mg/dl), and a normoinsulinemic/hyperglycemic group (insulin <10 ng/ml, glucose >260 mg/dl). This horseshoe-shaped relationship between insulin and glucose levels is typical of the clinical course of type 2 diabetes in humans over many years; however, since all mice shown in this figure were tested at age 6 months, the complex relationship between insulin and glucose levels suggests that the pathogenesis of hyperinsulinemia and hyperglycemia are likely to be complex in this cross.

A genome-wide scan of F_2 mice reveals QTL controlling insulin levels. To map the genetic loci that regulate the susceptibility to hyperinsulinemia in this cross, we genotyped F_2^{IR} mice across the whole genome using simple repeat markers that are polymorphic between 129/Sv and B6 mice. Initially, we genotyped 40 mice at the two extremes of the distribution of insulin levels using 77 markers at an average distance of 20 cM. Five markers showed a departure from the expected Mendelian distribution of alleles (1:2:1 for the three possible genotypes: homozygous B6, heterozygous B6/129, and homozygous 129) (Table 2), with a statistically significant difference between the mice at the top and those at the bottom of the distribution, suggesting that the observed deviation from Mendelian ratios was not due to segregation distortion. One marker on chromosome 1 was nearly significant and was also pursued in more detail.

More mice were genotyped at some of these markers, and 10 markers were added in the vicinity of the most promising

TABLE 2

Distribution analysis (χ^2) of the genotyping data at a selected marker

Marker	Genotype			P
	B6/B6	B6/129	129/129	
D1-Mit19	14	6	0	0.057
D2-Mit151	17	3	0	<0.05
D6-Mit55	12	6	2	<0.05
D10-Mit162	13	7	0	<0.05
D12-Mit231	4	2	14	<0.05
D15-Mit171	9	7	1	<0.05

Summary of marker data showing a deviation from the expected Mendelian frequency among the 20 mice at the top of the trait distribution; χ^2 distribution was calculated assuming two degrees of freedom. P values were obtained by comparing these data with those derived from the 20 mice at the bottom of the distribution, which did not show a departure from the expected Mendelian ratios.

peaks. Analysis of the enlarged genotype set shows significant LOD scores on both chromosomes 2 and 10 (5.58 on each) and suggestive peaks on chromosomes 1, 6, and 12 (with peak LOD scores of 3.58, 2.79, and 3.11) (Table 3). The fact that both high LOD scores are 5.58 appears to be coincidental. The other initially observed peaks disappeared when more markers and genotypes were added. Both the LOD score curves on chromosomes 2 and 10 (shown in Fig. 4, along with the curve for chromosome 1) have high LOD scores over a long interval, and one cannot determine from these data whether the peaks are caused by one or more genes in the region. At four of the five loci with significant or suggestive LOD scores, excess alleles are contributed from the B6 strain, and at one locus by the 129/Sv strain.

DISCUSSION

The genetic basis of insulin resistance has been the object of intense scrutiny for many years. There is general agreement that insulin resistance is an early abnormality in the clinical course of type 2 diabetes (22), but its complex genetic nature has thus far hampered efforts to identify common susceptibility alleles. It is likely that different genes are at fault in different individuals and that many predisposing alleles represent polymorphic variants that impair, rather than ablate, gene function (1,4). Despite substantial progress in our understanding of insulin action and the genetic analysis of complex disorders, these alleles have eluded identification. Previous studies from several laboratories, including ours, have suggested that the failure to identify susceptibility alleles may be due to the synergistic or epistatic nature of the interactions necessary for a predisposing allele to become clinically significant (10,13,23–25). Thus, the present study was undertaken with the aim of providing evidence for naturally occurring alleles that act in a synergistic fashion to cause hyperinsulinemia and diabetes.

We show in this study that differences in the susceptibility to insulin resistance in mice carrying a targeted gene mutation on different backgrounds can be exploited for the purpose of identifying genetic modifier loci that affect plasma insulin levels. The notion that the different susceptibility of rodent strains to certain traits can be exploited for the pur-

TABLE 3
QTL analysis of genotyping data

Peak	Chromosome	Marker	QTL (cM)	LOD score	Variation (%)	Donor strain
1	1	D1 Mit19	38.3	3.58	20.5	B6
2	2	D2 Mit151	27.6	5.58	35.2	B6
4	6	D6 Mit201	56.6	2.79	20.4	B6
6	10	D10 Mit42	48.5	5.58	34.9	B6
7	12	D12 Mit231	44.1	3.11	14.0	129

Genotyping data were analyzed using MapMaker/QTL. The likely QTL position based on interval mapping assuming a free genetic model is indicated in centimorgans. Variation indicates the percentage of the trait variation that can be explained by the individual QTL. Please note that the contribution of individual QTLs cannot be assumed to be additive, and therefore the total amount of variation explained exceeds 100% (49). Moreover, it should be emphasized that all mice analyzed are heterozygous for the *ir* mutation. Thus, the variation explained by each QTL should be considered as the combined effect of the QTL interacting with the *ir* mutation. The donor strain is the strain contributing an excess of alleles at the indicated locus.

pose of genetic analysis using targeted or natural mutations has been demonstrated in elegant studies of epidermal growth factor receptor (26,27) and transforming growth factor- β knockouts (28) as well as the leptin receptor mutation in the fatty rat (29). In fact, the pioneering work of Coleman (30) first led to the recognition of the role of genetic background in determining diabetes susceptibility in the context of obesity. More recently, modifier genes affecting intestinal neoplasia (31,32), obesity due to the *agouti* mutation (33,34), insulin resistance with hypertension (35), and the function of

von Willebrand factor (36) have been identified. Variations of this approach have been used to analyze epistatic interactions in the pathogenesis of type 1 diabetes (37), lung cancer (38,39), atherosclerosis (40), reproductive abnormalities (41), obesity (29), and hypertension in rodents (42). Several outbred strains develop diabetes as a result of polygenic interactions among genes required for either obesity or glucose intolerance (43,44). More recently, two studies have addressed the possibility of using analysis of outbred mice to study epistatic interactions in the pathogenesis of type 2 dia-

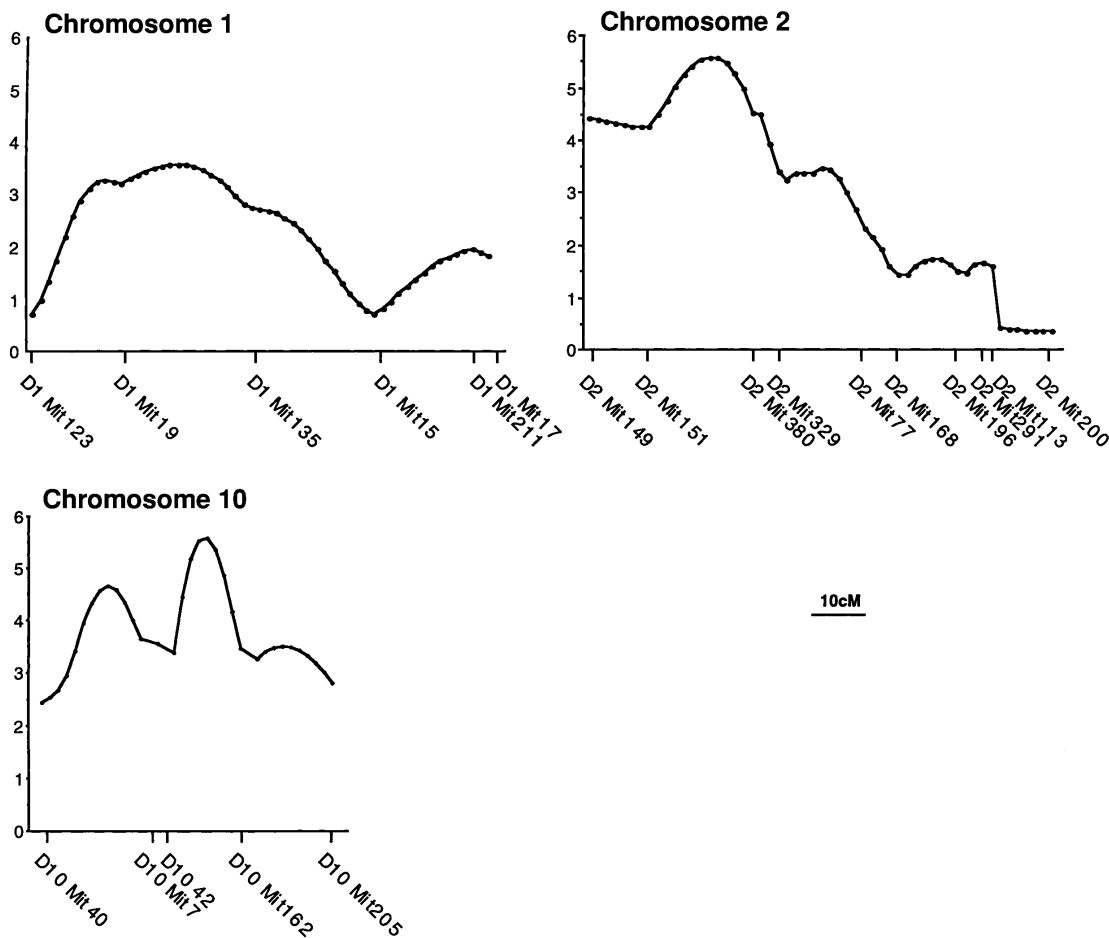


FIG. 4. QTL analysis of genotyping data for selected chromosomes. The map scale is indicated on the right and is similar for all chromosomes shown.

TABLE 4
List of candidate genes in QTL intervals

QTL chromosome	Position (cM from centromere)	Human syntenic region	Candidate genes	OMIM number or reference
10	48.5	12q22-24	IGF-1 Protein phosphatase-2, regulatory subunit B Pro-melanin-concentrating hormone weight 1	147440 601647 176795 (48,49)
10	35.0	10q21-22		
2	62.3	11p13	mahogany/attractin Pax-6	(33,34) 106210
2	27.6	9q33-34	Nidd 5	(50)
6	56.6	12p13	Tumor necrosis factor- α receptor 1A Protein phosphatase-1 γ -subunit	191190 176914
12	44.1	14q24-32	CaM kinase γ -subunit	602123
1	38.3	2q35	IRS-1 Tyrosine phosphatase IA-2 SHIP phosphatase	147545 601773 601582

A partial list of candidate genes detected by searching syntenic regions in human chromosomes using the Human/Mouse Homology Maps available from the National Center for Biotechnology Information Website (www.ncbi.nlm.nih.gov). Reference to candidate genes is provided using the Online Mendelian Inheritance in Man (OMIM) catalog (www.ncbi.nlm.nih.gov/omim). The bibliography number is indicated for disease- or QTL-associated loci. SHIP, SH2 domain-containing inositol phosphatase.

betes (23,25). Our study demonstrates that these interactions can be applied to the study of genes regulating insulin levels, an important quantitative trait in the pathogenesis of type 2 diabetes.

In our study, only male mice were susceptible to diabetes and hyperinsulinemia. The reasons for this sexual dimorphism are unknown, but they are reminiscent of the differences in the susceptibility to hyperglycemia in mice carrying the *db* mutation. In this case, Leiter et al. (45) have shown that the increased prevalence of hyperglycemia in males is related to sex-specific differences in the hepatic sulfurylation of testosterone and estrogens.

Based on the phenotypes of the recombinant congenic strains, we hypothesized that the 129/Sv strain would contribute susceptibility alleles in this cross. However, only one of the five loci identified is 129/Sv-derived. This finding is not unprecedented. In studies of genetic modifiers of the *Lepr^{fa}* mutation, Chung et al. (29) found that the susceptibility alleles to *Lepr^{fa}*-associated diabetes are not always derived from the susceptible strain, a phenomenon known as "transgression" that has also been seen in studies of QTL controlling body fat and diet (46,47). A possible explanation is that these alleles are silenced by more dominant alleles in the context of the resistant strain but are active in the context of the susceptible strain. In this respect, it should be noted that in our BC1^{IR} mice, average insulin levels are even more elevated than in F₂^{IR} mice, suggesting that some B6-derived alleles can indeed confer insulin resistance. It is likely that with increasing backcross generations, the effect of these alleles is silenced by the protective effect of additional alleles. The derivation of recombinant congenic strains for each of the candidate regions should address this question.

The loci affecting insulin levels in mice of mixed genetic background are unknown. However, one of the lessons of the type 2 diabetes mouse (*ir/irs-1^{+/-}*) is that epistatic interactions are likely to develop among genes affecting the same pathway. Accordingly, in mice heterozygous for the *ir* mutation, the hyperinsulinemia-predisposing alleles could conceivably rep-

resent gene products important for insulin action. Interesting clues as to the potential contributing alleles derive from the analysis of increasingly dense physical genetic maps of candidate intervals. From our analysis, several interesting candidates emerge. The linkage on chromosome 10 overlaps the obesity QTL *weight 1* identified in GK rats (48,49) and with several interesting candidates (Table 4). The main region identified on chromosome 2 in this scan overlaps *Nidd5*, an insulin/obesity QTL identified by Hirayama et al. (50) in the TSOD mouse, a model of insulin resistance/hyperinsulinemia. The other region on chromosome 2 is syntenic to a region on human chromosome 11 that contains the recently cloned obesity modifier *Mahogany* (33,34) (Table 4). The linkage on chromosome 1, albeit less strong (LOD 3.47), is especially intriguing, since it includes two genes that are important in insulin action, IRS-1 and the phosphoinositol phosphatase SHIP. In both cases, there is strong genetic evidence that these gene products affect insulin action (5,6,10,51,52). This linkage also sheds new light on our previous observation that combined heterozygosity for *ir* and *irs-1* null alleles causes diabetes. If a major susceptibility allele is indeed tightly linked to *irs-1* and potentiates the effects of the *ir* mutation, it could explain the increased incidence of diabetes in this cross in addition to heterozygosity for the *irs-1* allele. With the exception of the QTL at 27 cM on chromosome 2, none of the linkages identified in this study overlaps other known insulin resistance/diabetes QTL in mice or rats. Future studies will be directed at examining candidate genes in the regions identified through QTL analysis and at constructing recombinant congenic strains to carry out fine mapping of the susceptibility loci reported here.

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