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Research Article

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Glycogen Synthase: A Putative Locus for Diet-induced Hyperglycemia

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Abstract

Inbred mouse strains fed a diabetogenic diet have different propensities to develop features analogous to type 2 diabetes mellitus. To define chromosomal locations that control these characteristics, recombinant inbred strains from diabetes-prone C57BL/6J (B/6J) and diabetes-resistant A/J strains were studied. Insulin levels and hyperglycemia correlated with two different regions of mouse chromosome 7 (two point LOD scores > 3.0). For insulin levels, 15 of 16 recombinant inbred strains were concordant with a region that contains the tubby mutation that results in hyperinsulinemia. For hyperglycemia, 19 of 23 strains were concordant with the *D7Mit25* marker and 20 of 23 strains with the *Gpi-1* locus on proximal mouse chromosome 7. Using more stringent criteria for hyperglycemia, 10 of 11 strains characterized as A/J or B/6J like were concordant with *D7Mit25*. This putative susceptibility locus is consistent with that of the glycogen synthase gene (*Gys*) recently suggested as a candidate locus by analyses of type 2 diabetes patients. Fractional glycogen synthase activity in isolated muscle was significantly lower in normal B/6J diabetic-prone mice compared with normal diabetic-resistant A/J mice, a finding similar to that reported in relatives of human patients with type 2 diabetes. These data, taken together, raise the possibility that defects in the *Gys* gene may in part be responsible for the propensity to develop type 2 diabetes. (*J. Clin. Invest.* 1994. 94:269–276.) Key words: genetics • mouse • non-insulin dependent diabetes mellitus • glycogen synthase • diet

Introduction

Mouse models of type 2, or non-insulin dependent diabetes mellitus, are attractive for genetic analysis because large numbers of identical individuals can be generated. The C57BL/6J (B/6J)[1] mouse is the background strain on which the *ob/ob* mutation is most commonly placed. The mutant spontaneously develops a syndrome that includes hyperglycemia, hyperinsulinemia, and obesity and is therefore often used as a model of type 2 diabetes (1). We have shown that the B/6J mouse will also develop hyperglycemia, hyperinsulinemia, insulin resistance, and obesity when weaned onto a high fat, high simple carbohydrate diet (2), suggesting that the gene(s) for diabetes are contained in the background. When maintained on a low fat diet, the B/6J mouse does not develop this syndrome. However, the lean B/6J has a preexisting defect in glucose-stimulated insulin secretion (3). More recently, we have found that hypertension accompanies the development of diabetes in these animals (4). Thus, the B/6J mouse models many of the features of human type 2 diabetes. Preliminary genetic analysis of crosses between diabetes-prone B/6J and diabetes-resistant A/J suggest that diet-induced hyperglycemia is largely determined by relatively few recessive gene(s) (5). Because a large number of recombinant inbred strains have been developed from crosses of the B/6J and A/J lines (6, 7), we attempted to locate genes that determine hyperglycemia, insulin level, and body weight by mapping the strain distribution pattern (SDP) of the diabetic phenotypes. Linkage was suggested for hyperglycemia and insulin levels located on two different regions of mouse chromosome 7 and none elsewhere in the genome. The best position for the hyperglycemia locus is concordant with the location of a gene for glycogen synthase (*Gys*), the product of which exhibits a physiologic difference in the parental mice.

Methods

Mice. Male mice from B/6J and A/J parental strains and AXB, and BXA recombinant inbred strains were purchased from the Jackson Laboratory (Bar Harbor, ME). The animals were weaned onto the high fat, high sucrose diabetogenic diet (2, 4, 5) before shipment to Duke. On arrival the mice were housed in the vivarium at Duke University in group cages with five animals in each cage and were maintained on this diet. C3H/HeJ-*gld* and *Mus spretus* (Spain) mice and [(C3H/HeJ-*gld* × *Mus spretus*)F_1 × C3H/HeJ-*gld*] interspecific backcross mice were bred and maintained as previously described (8). We chose *Mus spretus* as the second parent in this cross because of the relative ease of detection of informative restriction fragment length variants (RFLVs) in comparison with crosses using conventional inbred laboratory strains.

Phenotypic characterization of AXB and BXA RI strains on a diabetic diet. On a diabetogenic diet, inbred B/6J mice and A/J mice differ in their propensity to develop characteristics of type 2 diabetes. Since previous studies of F_1 and reciprocal backcross mice suggested that relatively few genes may be responsible for this phenomenon (5), recombinant inbred (RI) strains generated from B/6J and A/J progenitors were examined to allow further genetic analysis. RI strains were derived from crosses between two inbred progenitor strains in which the mice from the F_2 generation and each subsequent generation are mated in accordance with a strict inbreeding program (9). After 20 generations, each of the resultant RI strains has a unique contribution from each original progenitor where the alleles from either progenitor strain have become fixed at each locus. RI strains were chosen for this study for several reasons: (*a*) Multiple genetically identical mice could be ana-

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1. *Abbreviations used in this paper:* B/6J, C57BL/6J; cM, centi-Morgan; G69, glucose-6-phosphate; Gys, glycogen synthase; RFLV, restriction fragment length variant; RI, recombinant inbred; UDPG, uridine diphosphoglucose.

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Table I. Glucose, Insulin, and Body Weight Measurements of AXB and BXA RI Strains

Strain	Glucose	SD	SEM	Designation	Insulin	SD	SEM	Designation	Weight	SD	SEM	Designation
A/J	145	17	3		103	70	11		38.8	7.3	1.3	
C57BL/6	221	25	4		255	141	22		52.7	3.3	0.6	
AXB1	183	30	8	B	503	334	89	B	44.4	3.3	0.9	A
AXB2	168	26	9	A	117	79	26	A	49.7	9.2	3.1	B
AXB5	172	15	5	A	116	31	11	A	43.2	5.4	1.9	A
AXB6	160	30	11	A	73	49	18	A	36.8	2.4	0.9	A
AXB8	288	145	39	B	877	628	157	B	45.1	5.7	1.4	
AXB10	186	59	18	B	256	140	42	B	50.5	2.1	0.6	B
AXB14	159	24	11	A	66	28	13	A	38.2	2.2	1.0	A
AXB18	185	22	6	B	117	78	21	A	40.6	4.6	1.2	A
AXB19	181	35	7	B	311	181	34	B	53.8	6.5	1.2	B
AXB20	196	47	13	B	267	295	85	B	53.4	6.4	1.8	B
AXB24	172	30	8	A	175	127	35		44.5	2.2	0.7	A
AXB25	173	22	9	A	164	73	30		47.3	5.1	2.1	
BXA1	202	23	9	B	492	381	144	B	48.3	1.8	0.7	
BXA2	164	17	3	A	144	84	17		51.5	5.1	1.0	B
BXA4	135	21	5	A	63	44	11	A	33.7	3.6	0.8	A
BXA7	161	27	8	A	141	54	15		42.3	4.4	1.3	A
BXA8	174	23	7	A	230	132	38		40.6	2.5	0.7	A
BXA11	145	17	8	A	118	53	24	A	50.3	3.3	1.5	B
BXA12	177	26	5	A	177	120	25		37.9	3.6	0.7	A
BXA14	163	19	6	A	131	109	33	A	42.5	6.2	1.9	A
BXA24	218	59	13	B	297	157	36	B	52.3	3.5	0.8	B
BXA25	161	41	11	A	540	275	74	B	45.2	2.9	0.8	

lyzed to minimize misclassification; (*b*) At each locus mice would be homozygous for one of the parental genotypes; and (*c*) The RI strains have been typed for many loci throughout the mouse genome.

Mice were weaned at 1 mo of age onto a high fat, high sucrose diet previously shown to induce phenotypic characteristics of type 2 diabetes in B/6J mice, at 1 mo of age (2, 4, 5). The diet contained 20.5% protein, 35.8% fat, 36.8% carbohydrate (primarily sucrose), and 0.4% fiber. A minimum of five mice for each strain was examined after 4 mo on the diabetogenic diet. Plasma glucose values were assayed by a glucose analyzer (Beckman Instruments, Inc., Palo Alto, CA) after the mice were fasted for 8 h. Insulin levels were determined by radioimmunoassay (Cambridge Medical Technology, Billerica, MA and Linco Research, St. Louis, MO) as previously described (5).

Criteria were established for each parameter to minimize the number of misclassified strains and still allow designating most of the RI strains tested as either B/6J- or A/J-like. We have already shown that many of the metabolic parameters of the diabetic phenotype can be greatly influenced by subtle environmental variables, such as handling and other nonspecific stressors (10). Therefore, for each cohort examined, A/J and B/6J parental controls were included. Values were then normalized for the parental values and each of the normalized values used to determine the mean and SD for each strain tested. Fasting plasma glucose, insulin, and weight were determined at 4 mo (Table I).

For hyperglycemia, RI strains were designated as A/J-like if the mean glucose value was less than the mean A/J value + 2 SDs and were designated B/6J-like if this value was greater than the mean A/J value + 2 SDs. Considerable variability in the individual mice, as indicated by the relatively high SDs, were observed for many of the RI strains. Parental controls, included in each cohort, indicated that for hyperglycemia only 4 of 85 of parental mice would be misclassified using the less stringent criteria. However, the variability in some of the RI strains was considerably greater than in the parental controls (e.g., AXB8, AXB10, AXB20, BXA24, and BXA25 in Table I). Therefore, RI strains were also classified by a second more stringent set of criteria: A/J-like if $> 3/4$ of the mice in each strain had glucose values lower than the mean A/J value plus 1 SD performed on parental mice; B/6J-like if $> 3/4$ of the mice in each strain had glucose values greater than the mean B/6J value -1 SD. However, only 11 strains could be typed using these criteria.

Insulin levels in the parental mice also had a large variation. Therefore, criteria were set that would minimize the number of parental mice that would be misclassified. Those mice with insulin levels < 135 μU/ml were designated as A/J-like and those with insulin levels > 200 μU/ml as B/6J-like. Using these criteria, of 84 parental mice, 8 would have been misclassified and 13 would have remained unclassified.

For body weight, RI strains were classified as A/J-like if their body weight was less than the mean A/J weight + 1 SD and were classified as B/6J-like if their body was greater than the mean B/6J weight − 1 SD. Using these criteria, none of the parental mice would have been misclassified and 8 of 66 mice would have been unclassified. With the exception of AXB2, the SDs in the RI strains were similar to those observed in the parental controls. In addition, none of the parental mice would have been misclassified, albeit 8 of 66 mice would have remained unclassified.

Typing for genetic markers. DNA was isolated from mouse organs by standard techniques (11) or kindly provided by Dr. Beverly Paigen (Jackson Laboratories). For Southern blot analysis, DNA was digested with restriction endonucleases and 10-μg samples were electrophoresed in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher & Schuell, Inc., Keene, NH), hybridized at 65°C, and washed under stringent conditions, all as previously described (12). Simple sequence length polymorphisms were detected by polymerase chain reaction amplification and size separation on polyacrylamide gels as previously described (13). For selected markers, the clones or sequences used in the current study are provided in Table II. For Gys, a human probe was generated by polymerase chain reaction amplification of a human muscle cDNA library using the published sequence (forward primer base pairs 2101–2119 and reverse primers base pairs 2456–2474) (18). Over 70 different clones or microsatellites were typed as

Table II. List of Selected Genetic Markers

Locus	Name	Clone or sequence	Size of restriction fragment (restriction endonuclease) or simple sequence length (bp)			
			A/J	B/6J	C3H-*gld*	*M. spretus*
D7Mit25	DNA segment, Chr 7 MIT 25	F5′-AGGGGCACATGTTCAACTATG R5′-GGTTGTTTCCAGCTTTGGG (13)	102	110	96	78
D7Mit30	DNA segment, Chr 7 MIT 30	F5′-CCAGGCTATTCTGTGATGTTTG R5′-GGATGACTCCTAAGGAATGGC (14)	242	234	ND	ND
D7Mit70	DNA segment, Chr 7 MIT 70	F5′-CACTTGGGGAACGTCAAGAC R5′-TGTAGACCATAGCCCATAAGCC (14)	136	138	136	146
*D7Nds5**	DNA segment, Chr 7 Nuffield Dept. of Surgery 5	F5′-CTCCACATGTGTATGTGTATG R5′-ATGGAGGCCGAAGAAAGAATC (15)	114	127	ND	ND
Gys	Glycogen synthase	PCR-amplified product; see Methods	Not variant	Not variant	24.0 kb (BamHI)	18.0 kb (BamHI)
Hmg14-rs7a	High mobility group protein 14–related sequence 7a	pM14c (16)	None	7.3 kb (TaqI)	ND	ND
Hmg14-rs7b	High mobility group protein 14—related sequence 7b	pM14c (16)	5.5 kb (TaqI)	None*	ND	ND
Ngfg	Nerve growth factor, gamma	pSM676 (17)	ND	ND	Multiple bands (TaqI)	2.5 kb (TaqI)

* D7Nds5 is a microsatellite sequence associated with the *Ngfg* gene.

part of the current study, which included analysis of over 350 loci. This information has been provided to the genome data base of the mouse, GBASE, and is available to interested investigators.

Data analysis. Gene linkage in the interspecific backcross mice was determined by segregation analysis (19). Gene order was determined by analyzing all haplotypes and minimizing cross-over frequency between all genes that were determined to be within a linkage group. This method resulted in determination of the most likely gene order (20). This gene order was also verified by use of the MAPMAKER multipoint linkage program (21).

The Map Manager program (kindly provided by Dr. Kenneth Manly, Roswell Park Memorial Institute, Buffalo, NY) with the updated SDP was used to determine possible linkages with the phenotypic traits in the RI strains. Several features of this program were used, including "Find Best Location," which allows searches at various confidence limits. The statistical considerations are described in detail in Manly (22) and are based on both conventional statistics (23) and Bayesian analysis (24, 25). To identify possible chromosomal locations for disease modifying genes, the SDP of the phenotyped RI strains was compared with those determined for over 350 genetic markers throughout the mouse genome in the AXB, BXA RI strains. These included both markers previously typed and available in the Map Manager format by R. W. Elliot and over 70 new markers typed as part of the current study. These markers cover ~ 90% of the mouse genome at a resolution of 15 centi-Morgan (cM) when comparisons are made to well-established linkage maps of each mouse chromosome (M. F. Seldin, unpublished data). When possible, the order of markers on each chromosome was established based on backcross mouse studies that are more suitable for determining gene order in small intervals (12).

Measurement of glycogen synthase activity. Mice raised on either diabetogenic or control (16% calories from protein, 73% calories from starch, 11% calories from fat) diets were killed 30 min after an intraperitoneal injection (1 IU/kg) of insulin. Insulin stimulation of glycogen synthase (Gys) activity in the soleus muscle has been demonstrated using a similar method (26, 27). The soleus muscle was dissected, blotted to remove blood, and snap frozen in liquid nitrogen. The method of Nuttal et al. (28) was modified for homogenization of muscle using a Virtishear (Virtus; Gardiner, NY). The homogenate was later defrosted and centrifuged at 9,000 *g* for 20 min, and aliquots of the supernatant were stored at −20°C for protein assay or used immediately for determination of Gys activity. The active form of Gys was assayed at low glucose-6-phosphate (G6P) concentration (0.17 mmol/liter) in the presence of 0.14 mmol/liter uridine diphosphoglucose (UDPG) (29). Total activity was measured at high G6P (7.2 mmol/liter), also in the presence of 0.14 mmol/liter UDPG. Activity was measured using UDP [^{14}C]glucose and is expressed as nanomoles of glucose incorporated into glycogen per minute per milligram of protein. The percent Gys active is calculated as (activity at low G6P concentration/total activity) × 100.

Results

Plasma glucose values correlate with insulin levels but not with body weight. As shown by linear regression (Fig. 1), the plasma

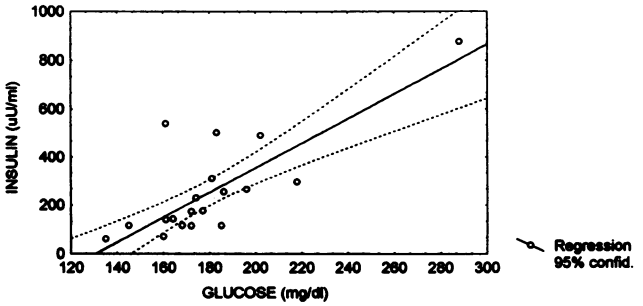

Figure 1. Correlation of glucose levels and insulin levels in AXB and BXA RI strains. Insulin = −666.4 + 5.1074 glucose. Correlation: r = 0.78339.

Table III. Strain Distribution Patterns of Insulin Level and Hyperglycemia Phenotypes

Chr 7	AXB																					*	BXA																					
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	7	8	9	0	1	3	4	5		1	2	4	7	8	9	1	2	3	4	6	7	8	0	2	3	4	5	6
Pmv-15‡	B	A	A	A	A	A	A	B	A	B	A	A	A	A	A	A	A	A	A	A	B	B	U		B	B	A	A	A	A	A	A	B	B	B	B	A	B	A	A	A	B
			X															X	X	X			?			X									X									X
Gpi-1‡	B	A	A	B	A	A	A	B	A	B	A	A	A	A	A	A	B	B	B	A	B	B	A		B	A	A	A	A	A	A	A	B	B	A	B	A	B	A	A	A	A
				?		?											?			?		X	?						?			X			?		?		X				
Hyperglycemia	**B**	**A**	**U**	**U**	**A**	**A**	**U**	**B**	**U**	**B**	**U**	**U**	**U**	**U**	**A**	**U**	**U**	**B**	**B**	**B**	**U**	**U**	**A**	**A**	**B**	**A**	**A**	**A**	**A**	**U**	**A**	**A**	**U**	**A**	**U**	**A**	**U**	**A**	**U**	**U**	**U**	**U**	**B**	**A**	**U**
		X		?		?											?			?		X				X		?										?		?		X		
D7Mit25	B	B	U	A	A	A	U	B	U	B	A	U	A	A	A	U	B	B	B	U	B	B	A		B	A	A	B	A	B	A	A	A	B	A	U	U	U	U	A	A	U
						?										?				?									X				?		?							
D7Nds5	B	B	U	A	A	A	U	B	U	B	A	U	A	A	A	U	B	B	B	U	B	B	A		B	A	A	B	A	B	A	A	B	A	U	U	U	U	A	A	U	
			X			?										?				?							X	X	X				X	?		?						
D7Mit70	B	B	U	B	A	A	U	B	U	B	A	U	A	A	A	U	B	B	B	U	B	B	A		B	A	A	A	B	A	B	B	A	A	B	B	U	U	U	U	A	A	U
						?										?				?			?									X	X	?		?						
D7Mit30	B	B	U	B	A	A	U	B	U	B	A	U	A	A	A	U	B	B	B	U	B	B	U		B	A	A	A	B	A	B	A	A	A	U	U	U	U	A	A	U	
		X			X		?							X	?					?			?						X			X	?		?			X			
c‡	B	A	A	B	B	A	B	B	A	B	A	A	A	A	A	B	B	B	B	B	B	B	U		B	A	A	A	B	A	A	B	A	A	A	B	A	A	A	A	A	B	U
														X									?						X			?										
Pmv-31‡	B	A	A	B	B	A	B	B	A	B	A	A	A	B	B	B	B	B	B	B	B	B	U		B	A	A	A	B	A	B	B	A	A	U	B	A	A	A	A	A	B	A
				X							X		X										?		X						?							X			
Hmg14-rs7b	B	A	U	B	A	A	U	B	U	B	B	U	A	A	B	U	B	B	B	U	B	B	B		B	B	A	A	B	A	B	B	A	A	B	B	U	U	U	U	B	B	U
																X				X			?						X														
Insulin level	**B**	**A**	**U**	**U**	**A**	**A**	**U**	**B**	**U**	**B**	**U**	**U**	**U**	**A**	**U**	**U**	**A**	**B**	**B**	**U**	**U**	**U**	**U**		**B**	**U**	**A**	**U**	**U**	**U**	**A**	**U**	**U**	**A**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**B**	**B**	**U**
Hmg14-rs7a	B	A	U	B	A	A	U	B	U	B	B	U	A	A	B	U	B	B	B	U	A	B	B		B	B	A	A	B	A	B	A	A	B	B	U	U	U	U	B	B	U
Hbb‡	B	A	A	B	A	A	B	B	A	B	B	A	A	A	B	B	B	B	B	B	A	B	B		B	B	A	A	B	A	A	B	A	A	B	B	A	A	A	A	B	B	A
		X	X		X		X			X			X	X	X	X	X	X				?		X		X		X	X	X	X	X				X			X		X	X	X
D7Nds4‡	B	B	B	B	B	A	A	B	B	B	B	B	A	B	A	A	A	A	A	B	A	B	U		A	B	A	B	B	B	B	A	B	A	A	B	A	A	B	A	A	A	B

* The AXB25 strain has recently been deleted from this series of RI strains due to a genetic contamination that occurred several years ago (7). However, it is included here since at each of every 45 loci tested this strain was homozygous for the AA genotype (20 loci) or the BB genotype (25 loci). Only the current typings were included in the analysis because the recent analyses of this strain indicated that 14 of the 56 loci typed differently than original typings (7). ‡ The typings for these loci have been previously determined [*Pmv-16* and *Pmv-31* (7, 30); *Gpi-1*, *c*, and *Hbb* (31, 32); and *D7Nds4* (33)]. Note the loci shown in boxes met the second more stringent criteria for classification (see text).

glucose values correlate significantly with the insulin levels ($r = 0.78$, $P < 0.05$). Certain individual strains, particularly BXA 25, are exceptions to this phenomenon and may explain our previous failure to observe this phenomenon with an analysis of fewer RI strains (5). In contrast, there is no significant correlation between either glucose and body weight ($r = 0.31$, $P > 0.05$) or between insulin and body weight ($r = 0.27$, $P > 0.05$).

Comparison of the SDPs suggest chromosomal locations for genes that control glucose and insulin levels. At the most stringent confidence level (99.99) using conventional statistics, no linkage is suggested, nor is linkage suggested using stringent Bayesian analyses options. However, at a high confidence level (99.9) using conventional statistics, linkage is suggested for both insulin levels and hyperglycemia for two regions of mouse chromosome 7 and none elsewhere in the genome. The best positions are indicated in Table III and the LOD scores with various mouse chromosome 7 loci are shown in Fig. 2. The maximum LOD scores were 3.2 and 3.1 for the insulin level and hyperglycemia phenotypes, respectively. In addition, when comparisons were made using a more stringent phenotype assignment for hyperglycemia, 10 of 11 strains were concordant with the SDP of *D7Mit25*. Together, these results provide suggestive data that a predominant genetic factor determining hyperglycemia is located in this region of mouse chromosome 7 and that a predominant genetic factor for hyperinsulinemia is located in a more distal position of the same chromosome.

In contrast, there is no strong linkage when body weight phenotypic assignments are subjected to a similar analysis. Possible chromosomal locations can only be suggested when the confidence level is reduced to 95%, a level at which there will be as many apparent linkages among nonlinked loci as among linked loci.

Chromosomal mapping of the Gys gene to mouse chromosome 7. Because the SDP of hyperglycemia suggests that a region of mouse chromosome 7 might contain a disease-modifying gene, we were intrigued by the observation that a locus defined by the *Gys* gene was associated with the development of type 2 diabetes in a patient population study (37). This is particularly of interest because this gene maps to human chromosome 19, a segment of which is homologous with linkage groups on mouse chromosome 7 (34). Therefore, mapping

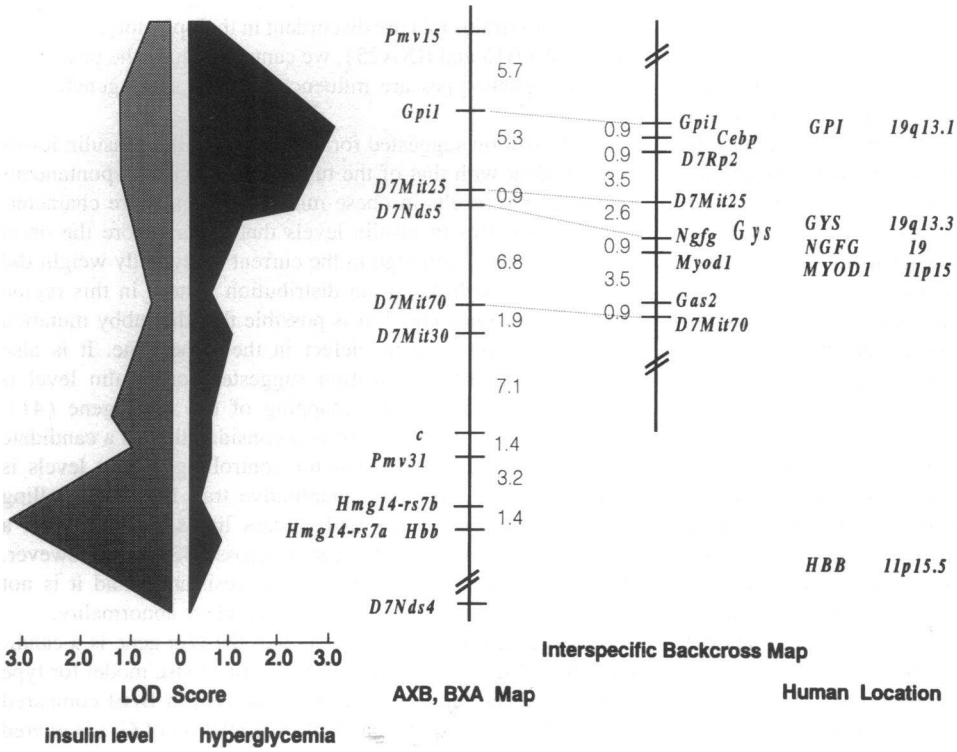

Figure 2. The LOD score distribution of insulin level and hyperglycemia phenotypes in relationship to mouse chromosome 7 markers. Two point binomial LOD scores calculated by Map Manager, corresponding to the map position of genetic markers, are depicted. The AXB, BXA map shows the position of genetic markers from proximal to distal on mouse chromosome 7. The map distance between markers is indicated in centi-Morgans as calculated by the Map Manager program. No map distance is shown between the *Hbb* and the most distal marker *D7Nds4* because these markers were not linked. The interspecific map shows the position of markers and the distance between markers as determined by recombination frequency in 114 [(C3H/HeJ-*gld* × *Mus spretus*)F_1 × C3H/HeJ-*gld*] mice. Haplotypes for three of these markers is shown in Fig. 3 and the haplotype distribution of the other markers has been published previously (34). The corresponding human symbol and mapped location is shown on the far right (35, 36).

of this gene in the mouse was undertaken. To determine the chromosomal location of the *Gys* gene, we analyzed a panel of DNA samples from an interspecific cross that has been characterized for > 600 genetic markers throughout the genome. The genetic markers included in this map span between 50 and 80 cM on each mouse autosome and the X chromosome (for examples see references 34 and 38). Initially, DNA from the two parental mice [C3H/HeJ-*gld* and (C3H/HeJ-*gld* × *Mus spretus*) F_1] were digested with various restriction endonucleases and hybridized with *Gys* cDNA probe to determine RFLVs to allow haplotype analyses. Informative RFLVs were detected with BamHI-restricted DNAs: C3H/HeJ-*gld,* 24.0 kb and *Mus spretus,* 18.0 kb. Each of 114 BamHI-restricted DNAs from the [(C3H/HeJ-*gld* × *Mus spretus*)F_1 × C3H/HeJ-*gld*] interspecific backcross mice displayed either the homozygous (CC) or heterozygous F_1 pattern (SC) when hybridized with the *Gys* probe.

Comparison of the haplotype distribution of the *Gys* indicated that in all of the 114 meiotic events examined, the *Gys* locus co-segregated with the nerve growth factor gamma chain (*Ngfg*) (Fig. 3), a locus previously mapped to mouse chromosome 7 (34). The best gene order (20) ± the SD (19) indicates that *Gys* is located 2.6±1.5 cM distal to *D7Mit25* and 2.6±1.5 cM proximal to *D7Mit70.* A map showing relationships between loci in the AXB, BXA RI strains and those determined in the interspecific backcross mice is shown in Fig. 2.

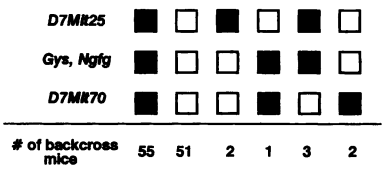

Figure 3. Segregation of *Gys* among mouse chromosome 7 loci in [(C3H/HeJ-*gld* × *Mus spretus*)F_1 × C3H/HeJ-*gld*] interspecific backcross mice. The number of backcross mice with each haplotype is shown below each column. Closed squares represent the homozygous C3H pattern and open squares the F_1 pattern.

Gys levels are different in A/J and B/6J mice. The results in Table IV show the Gys activity for A/J and B/6J mice on diabetogenic and control diets. When fed the control diet, B/6J mice compared with A/J mice have a reduced percent (fractional) Gys activity. Interestingly, total Gys activity (under maximal G6P stimulation) is elevated in these B/6J mice. A similar elevation is observed using saturating concentrations of both G6P and UDPG (data not shown). The diabetogenic diet produces increased percent Gys activity along with hyperglycemia in the B/6J mice.

*Table IV. Muscle Gys Activity in Mice Fed Diabetogenic and Control Diets**

	Control diet		Diabetogenic diet	
	A/J	B/6J	A/J	B/6J
Total (nmol/min·mg)	4.9±0.4	6.5±0.6‡	5.1±0.4	6.0±0.5
Percent active	62.0±7.0	46.0±3.0§	52.0±5.0	57.0±2.0
Plasma glucose (mg/dl)	138.0±8.0	148.0±6.0	145.0±4.0	200.0±6.0‖
Plasma insulin (μU/ml)	35.0±5.0	38.0±6.0	40.0±10.0	145.0±18.0‖

* Values are means±SEM. ‡ $P < 0.05$, B/6J vs. A/J. § $P = 0.06$ B/6J vs. A/J. ‖ $P < 0.05$ low fat vs. high fat in B/6J and in B/6J vs. A/J.

Discussion

Susceptibility to type 2 diabetes is almost certainly due to a complex interaction of multiple genetic and environmental factors. In the current study, the genetic analysis of our mouse model, in which a high fat, high simple carbohydrate diet induces features of type 2 diabetes, imply a candidate susceptibility gene. Correlation of fasting plasma glucose and the SDP patterns of genetic markers suggests linkage to a segment of mouse chromosome 7. These results are particularly provocative because this region of mouse chromosome 7 is homologous with the region of human chromosome 19 that has been implicated in a study of multiplex families with type 2 diabetes (37).

It is notable that the analytic methods applied in this study should only be applicable if a single genetic locus has a large effect on the manifestation of a phenotypic trait. That is, for a single locus to be implicated in the expression of a trait, it must be necessary and sufficient or be "complemented" by several other genes, none of which is necessary or sufficient alone to allow the trait to be expressed. It is likely from our previous studies of this model that other genetic factors are important (5). In addition, the range of plasma glucose values in the RI strains (78–472 mg/dl) was substantially greater than that observed in the parental mice (117–266 mg/dl), suggesting complex genetic interactions. The small number of RI strains precludes any meaningful analysis using methods designed to identify quantitative trait loci. Also, the large variability seen in some of the RI strains suggests that either these strains are not completely inbred or that genetic factors contribute to the lability of fasting glucose measurements. Because extensive testing of these RI strains has not indicated genetic heterogeneity (7), the latter explanation is more likely. We have previously postulated that a defect in autonomic regulation of glucose metabolism may be involved in the development of type 2 diabetes in these animals and in humans (39). Such a defect would render the metabolic parameters very sensitive to environmental factors and may account for this large intrastrain variability.

It is also worth noting that two double cross-overs were observed between our phenotypic assignments of plasma glucose values and each of the markers in this region of mouse chromosome 7 (AXB24 and BXA24). This phenomenon can be potentially the result of (*a*) misclassification, (*b*) detection of previously undefined cross-overs, or (*c*) evidence that other genetic loci can exert a larger effect on the phenotype than the candidate genetic locus. Although it is not possible to formally distinguish between these possibilities, these results may also provide additional suggestive evidence that the susceptibility to developing hyperglycemia is a complex genetic phenomenon.

In the current study we observed that plasma glucose values correlated with insulin levels. While this correlation can result simply from the physiologic effects of glucose on insulin secretion, recent data from our laboratory using perifused isolated islets suggest that B/6J mice have a defect in glucose-stimulated insulin release as well as previously documented insulin resistance (unpublished data). Therefore the B/6J mouse has two defects that may or may not be controlled by a single gene alteration. Alternatively, the correlation may be explained by the linkage between the putative susceptibility loci that contain the predominant genetic factors controlling the phenotypic expression of these traits. That is, both loci were mapped to regions of mouse chromosome 7, with the maximum LOD scores separated by ~ 28 cM (0.28 recombination frequency). However, because these results are heavily influenced by the typing of only two strains that are discordant in their phenotypic assignments (AXB18 and BXA25), we cannot exclude the possibility that both phenotypes are influenced by the same genetic factor(s).

The position suggested for a locus controlling insulin levels is coincident with that of the tubby (*tub*) locus, a spontaneous mutation that results in obese mice. These mice are characterized by increases in insulin levels that occur before the onset of obesity (40). Although in the current study body weight did not correlate with the strain distribution pattern in this region of mouse chromosome 7, it is possible that the tubby mutation represents a more severe defect in the same gene. It is also worth noting that the position suggested for insulin level is ~ 20 cM proximal to the mapping of the *Ins-2* gene (41), nearly excluding this locus from a consideration as a candidate gene. The position suggested for controlling insulin levels is coincident with the major quantitative trait locus, controlling plasma cholesterol levels and carcass lipids, implicated in a recent study using an interspecific cross (42, 43). However, these mice have only modest insulin resistance, and it is not clear whether this is a primary or secondary abnormality.

The genetic analysis suggests that the *Gys* gene is a candidate for a disease-modifying gene in our mouse model for type 2 diabetes. The reduced percent active Gys in B/6J compared with A/J mice suggests that insulin regulation of Gys is altered in these mice. This abnormality in percent activity after insulin administration is similar to observations in insulin-resistant humans during a euglycemic, hyperinsulinemic clamp (44). In human muscle biopsies obtained 10 min after insulin infusion, the fractional Gys activity was 29% less in insulin-resistant subjects. The B/6J mice on the low fat diet can apparently compensate for a reduced percent active Gys by increasing the total Gys activity. An estimate of the Gys activity available in the muscle tissue at physiologic G6P concentrations (0.17 mmol/liter) can be obtained by multiplying the total activity by the percent active. Both strains of mice have similar physiologic synthase activity available on the control diet (2.9 vs. 3.0 nmol/min·mg for A/J vs. B/6J). This observation is compatible with the apparently similar insulin sensitivity of these mouse strains on the low fat, low sucrose control diet, as indicated by their similar plasma glucose and insulin concentrations. It is likely that the increase in percent Gys activity in the B/6J, but not the A/J mice, as a result of the high fat, high sucrose diet, is explained by a response of the synthase activity not only to hyperinsulinemia, but also to the elevated plasma glucose that develops in the B/6J strain. Based on a report that hyperglycemia normalizes insulin-stimulated glucose storage rates in subjects with non-insulin dependent diabetes mellitus (45), it has been proposed that the plasma glucose increases to a concentration required to normalize glucose disposal rates. Mechanisms proposed to explain this action of glucose include the stimulation of glycogen synthase phosphatase by an increase in G6P secondary to hyperglycemia (46, 47).

The results suggest that the B/6J mouse has a defect in a mechanism for insulin activation of Gys activity as measured at the level of percent activity. This observation implies a possible defect in phosphorylation/dephosphorylation mechanisms (47), which alter the percent activity. These observations are also compatible with a possible defect in either the coding or regulatory sequence of the *Gys* gene. The mechanism for increased resistance to insulin action on glucose disposal (as sug-

gested by the elevated plasma glucose and insulin) in the B/6J mouse on the diabetogenic diet (and to a lesser extent in the A/J mouse) is currently unknown. It is possible that this increased insulin resistance selectively produces diabetes in the B/6J mouse compared with the A/J mouse because of the apparent reduced capability of the B/6J mouse to increase the percent Gys activity after insulin administration.

There is a growing body of evidence that suggests alterations in Gys activity in type 2 diabetes. Thorburn et al. (48) showed that insulin-stimulated Gys activity was significantly impaired in patients with type 2 diabetes, with a corresponding decrease in muscle glycogen content. Vestergaard et al. (49) found that decreased Gys activity in type 2 patients corresponded to decreased mRNA and suggested that a pretranslational defect was present. These investigators did not find any evidence for *Gys* coding sequence differences in an analysis of eight type 2 diabetic patients. However, this study used single-stranded polymorphism confirmation analysis, a good screening tool but a technique that may not identify all single base pair mutations and which therefore cannot be interpreted as excluding all possible single base pair mutations. Both Vaag et al. (50) and Schalin-Jantti et al. (51) found decreased insulin-stimulated Gys activity in first degree relatives of individuals with type 2 diabetes. Thus, as in our mouse model, the impairment of Gys activity is unlikely to occur simply as a consequence of diabetes and may be an early defect in the metabolic cascade leading to diabetes. More recently, an association was noted between *Gys* and type 2 diabetes (37) in a Caucasian population. In addition, patients with the diabetic allele were insulin resistant and more likely to have hypertension, a finding similar to those described in our mouse model. A defect in *Gys* could play a central role in defective insulin release in type 2 diabetes. A defect in the activity of the Gys enzyme that would result in impaired insulin sensitivity might produce impaired glucose tolerance and subclinical hyperglycemia. Over time, even mild hyperglycemia may desensitize the islet response to glucose, further worsening glucose tolerance in peripheral tissues (52). Together these data suggest that further investigation of the role of Gys in susceptibility to type 2 diabetes is warranted.

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