# Genome-Wide Scans Reveal Quantitative Trait Loci on 8p and 13q Related to Insulin Action and Glucose Metabolism

## The San Antonio Family Heart Study

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**Type 2 diabetes is a complex disease that arises from physiological disruptions of the body's sensitivity to insulin and ability to metabolize glucose. Multipoint linkage analyses for insulin sensitivity phenotypes were conducted in 1,280 Mexican Americans from 41 families who participated in the San Antonio Family Heart Study. A significant linkage signal (logarithm of odds [LOD] = 2.98) affecting corrected insulin response to glucose was detected on chromosome 13q between *D13787* and *D13S252*, in the region where the *MODY-4* gene has previously been mapped. Another signal on chromosome 13 was observed at *D13S285* (LOD = 1.86), where the insulin receptor substrate 2 gene resides. Significant linkage (LOD = 3.09) for insulin response to glucose was found on chromosome 8 between *D8S1130* and *D8S1106*, near the lipoprotein lipase and macrophage scavenger receptor genes. Multipoint analysis of abdominal skinfold with an LOD of 2.68 showed signals in the same region. There was also suggestive evidence for linkage of quantitative insulin sensitivity check index and fasting glucose to a previously reported location at *D9S301* (LOD = 2.19). These results indicate that chromosomal locations on 8p and 13q might harbor genes that affect a variety of insulin- and glucose-related phenotypes that contribute to the observed variations in these important risk factors for diabetes in Mexican Americans. *Diabetes* 53: 1369–1374, 2004**

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CIR, corrected insulin response; HOMA-IR, homeostasis model assessment of insulin resistance index; IBD, identity-by-descent; IPF-1, insulin promotor factor-1; IRG, insulin response to glucose; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; NPL, nonparametric linkage; QTL, quantitative trait locus; QUICKI, quantitative insulin sensitivity check index.



Type 2 diabetes/insulin resistance is a major component of the metabolic syndrome, which also includes hyperlipidemia, central obesity, hypertension, and cardiovascular diseases. Dramatic changes in environment and lifestyle have led to an epidemic increase in the number of diabetes patients, with the current 160 million expected to reach 220 million in 2010 and 300 million in 2025 (1,2). Its familial nature and differential prevalence in diverse populations suggest that type 2 diabetes is the consequence of the interactions of genetic and environmental factors (3). According to Permutt and Hattersley (4) and Rich (5), the best-fit genetic model, inferred from most of the previous genetic studies for type 2 diabetes, involves a few genes with moderate effect, superimposed on a polygenic background.

Linkage studies have implicated many regions on a number of chromosomes as being related to type 2 diabetes phenotypes. Among them, regions on chromosomes 1q, 2q, 3q, 9q, 10q, and 11q have been replicated (6). In addition, there is evidence of a quantitative trait locus (QTL) on chromosome 3, which may be influencing the expression of the metabolic syndrome (7,8). However, more genetic investigations in different populations are necessary to further our understanding of this complex condition.

In the present study, genome-wide scans for insulin resistance—a major risk factor for type 2 diabetes—were conducted in Mexican Americans from the San Antonio Family Heart Study (SAFHS) (9). The insulin resistance traits examined by multipoint analysis included fasting and 2-h glucose, fasting and 2-h insulin, and the calculated insulin resistance indexes (homeostasis model assessment of insulin resistance index [HOMA-IR], quantitative insulin sensitivity check index [QUICKI], corrected insulin response [CIR], and insulin response to glucose [IRG]).

### RESEARCH DESIGN AND METHODS

**Subjects and data.** Mexican Americans who are from San Antonio, Texas, and have been participating in the SAFHS (9) are the subjects of this study. Probands were selected randomly from a census tract in San Antonio of >90% Mexican-American residency, without regard to any preexisting medical conditions. Probands who were selected were 40–60 years of age, had a spouse who was willing to participate, and had at least six offspring who were

≥16 years of age. All relatives (≥16 years of age) of the proband and his or her spouse and women who were not pregnant were invited to participate.

At a clinic visit, abdominal skinfolds were measured. Blood was collected after an overnight fast and again 2 h after a 75-g glucose load (Orangedex; Custom Laboratories, Baltimore, MD). Plasma was prepared and stored at −80°C until analyzed. DNA was isolated from lymphocytes for genotyping. All procedures were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

Criteria for exclusion of 212 subjects from this study were *1*) use of diabetic medication and *2*) diagnosis of diabetes based on a fasting glucose ≥126 mg/dl and 2-h glucose of ≥200 mg/dl (10). Data from 1,302 (532 male; 770 female) individuals were available. The age ranged from 16 to 94 years, with a mean of 38. There are 41 pedigrees in this sample, ranging in size from 3 to 95 members, with an average of 25.

For abdominal skinfold measurement, the subjects were asked to relax the abdominal wall muscle as much as possible. The subjects stood straightly with body weight evenly distributed on both feet. The sites for abdominal skinfold measurements were selected at the locations 3 cm lateral and 1 cm inferior to the midpoint of the umbilicus. The average of three repeated measurements from the left of the umbilicus was obtained as the phenotype for later genetic analysis. Plasma glucose concentrations were determined using the glucose oxidase method with an Abbott V/P Analyzer (Abbott Laboratories, Abbott Park, IL). Insulin levels were measured by radioimmunoassay using a commercially available kit (Diagnostic Products, Los Angeles, CA). HOMA-IR, QUICKI, CIR, and IRG were calculated according to standard methods:

$$\text{HOMA-IR} = \text{fasting insulin (mU/ml)} \times \text{fasting glucose (mmol/l)}/22.5 \quad (11,12)$$

$$\text{QUICKI} = 1/[\log \text{fasting insulin (mU/ml)} + \log \text{fasting glucose (mg/dl)}] \quad (13)$$

$$\text{CIR} = \text{2-h insulin (mU/ml)}/[\text{2-h glucose} \times (\text{2 h glucose} - 70\ \text{mg/dl})] \quad (14,15)$$

$$\text{IRG} = [\text{2-h insulin} - \text{fasting insulin}](\text{mU/ml})/[\text{2-h glucose} - \text{fasting glucose}]\ (\text{mmol/l}) \quad (11)$$

**Genotyping.** Genotyping data for the linkage analyses were obtained from 1,280 individuals according to standard protocols, as previously described (16,17). All family members were genotyped for 417 markers. That included seven candidate genes: *INSR*, *INS*, *GLUT2*, *GLUT4*, *GCK*, *FABP2*, and *B3AR*. These markers are spaced at an average interval of 10 cM. Briefly, PCRs were performed with fluorescently labeled primers from the MapPairs 6 and 8 Linkage Screening Sets (Research Genetics, Huntsville, AL). Reaction products were pooled and typed with an automated DNA sequencer and GeneScan and Genotyper software (Applied Biosystems, Foster City, CA).

**Multipoint linkage analysis.** To locate a specific QTL influencing a phenotype, we first estimated the proportion of the total phenotypic variance attributable to the genetic effects (18). The total phenotypic variance ($\sigma^2_p$) can be decomposed into the genetic and environmental components (19) as $\sigma^2_p = \sigma^2_g + \sigma^2_e$, where $\sigma^2_g$ is the variance contributed by the genetic components and $\sigma^2_e$ is the variance contributed by the environmental components. The environment factor includes diet, physical activity, and any measurement errors. The $\sigma^2_g$ can be separated further into additive genetic variance as a result of specific QTLs ($\sigma^2_a$) and residual genetic (non-QTL) variance. The additive genetic heritability ($h^2$) is the proportion of the total phenotypic variance attributable to additive genetic components.

A multipoint variance component linkage method was used to test for linkage between marker loci and the traits under study. If a locus on a chromosome is linked to the quantitative trait (e.g., fasting glucose), then the expected genetic covariances between family members can be expressed as a function of the identity-by-descent (IBD) relationships at that locus. The overall expected IBD relationship between relative pairs is twice the kinship ($\phi$). Kinship is defined as the probability that two homologous genes drawn at random, one from each individual, will be IBD. IBD at the specific QTL locus ($\hat{\Pi}$) is estimated using genetic marker data. The covariance matrix takes the following form:

$$\Omega = \hat{\Pi}\sigma^2_q + 2\Phi\sigma^2_a + I\sigma^2_e$$

where omega ($\Omega$) is the covariance matrix of the entire family. The pi ($\hat{\Pi}$) is a matrix of the proportions of the specific QTL that the relative pairs share as IBD. $\sigma^2_q$ is the additive genetic effect of the specific QTL, $\phi$ is the kinship matrix, $\sigma^2_a$ is the residual (non-QTL) genetic effect, I is an identity matrix, and $\sigma^2_e$ represents the random environmental effect.

The null hypothesis is that the additive genetic variance of the specific QTL ($\sigma^2_q$) for the trait equals zero. The likelihood of the null hypothesis ($H_0$), where $\sigma^2_q$ is constrained to zero, will be compared with the likelihood of the alternative hypothesis ($H_a$), where the $\sigma^2_q$ is estimated. Twice the difference between the log likelihood of the two models yields a test statistic. This test statistic is asymptotically distributed as a ½:½ mixture of a $\chi^2$ distribution with 1 df (degree of freedom) (20). The logarithm of odds (LOD) score is used to demonstrate the significance of the test. It is calculated as the following: LOD = log 10(likelihood of $H_a$) − log 10(likelihood of $H_0$). The calculations of locus-specific IBDs for relative pairs and the multipoint linkage analyses were performed using the SOLAR program (21).

TABLE 1
Relative pairs in this study

| Relative relationship | Size |
|---|---|
| Parent-offspring | 403 |
| Siblings | 450 |
| Grandparent-grandchild | 308 |
| Avuncular | 717 |
| Half-siblings | 104 |
| Great grandparent–grandchild | 92 |
| Grand avuncular | 231 |
| Half avuncular | 114 |
| First cousins | 882 |
| First cousins, once removed | 634 |
| First cousins, twice removed | 65 |
| Half first cousins | 62 |
| Second cousins | 190 |
| Total | 4,252 |

In the population used in the present study, the prevalence of diabetes varied considerably, from 2% in subjects <30 years to 40% in those >70 years of age (22). Also, age and sex significantly affected plasma levels of glucose, insulin, lipids, and lipoproteins (22). Thus, the age, sex, and age$^2$ and their interactions were included in the analyses above as covariates.

For meeting the assumption of normal distribution of the variance component linkage method, the distributions of all phenotypes were examined and fasting and 2-h insulin, HOMA-IR, IRG, and CIR were logarithm transformed. Subjects with 2-h glucose less than or equal to fasting glucose, 2-h insulin less than or equal to fasting insulin, and 2-h glucose ≤70 mg/dl (which would be undefined when log transformed) were removed from the analyses of CIR and IRG. Data ≥±4 standard deviations from the means were blanked out. The kurtosis of all phenotypes were reexamined and additional outliers were removed so that the kurtosis for each of the traits was ≤1.9, thereby avoiding an inflation of type 1 error (23). The relative pairs used in quantitative genetic and linkage analyses are shown in Table 1.

## RESULTS

The means, heritabilities, and sample sizes for fasting and 2-h glucose, fasting and 2-h insulin, average abdominal skin fold, QUICKI, IRG, CIR, and HOMA-IR are shown in Table 2. The fasting plasma glucose levels were >126 mg/ml in 12% of the subjects in this study. Approximately 33% of the subjects had HOMA-IR above 3, which suggests insulin resistance.

Table 3 lists the chromosome regions with LOD scores >1.85, or signals found in the same regions, using different traits for multipoint analyses. In all of the genome scans, sex, age, and age$^2$ and the interactions between them were

TABLE 2
Characteristics of study population

| Trait | Mean | Size | $h^2$* |
|---|---|---|---|
| Fasting glucose (mg/dl) | 85.76 ± 10.11 | 818 | 0.24 ± 0.05 |
| 2-h glucose (mg/dl) | 97.5 ± 29.99 | 765 | 0.27 ± 0.06 |
| Fasting insulin (units/ml)† | 11.8 ± 7.4 | 767 | 0.32 ± 0.07 |
| 2-h insulin (units/ml)† | 68.78 ± 56.53 | 771 | 0.34 ± 0.07 |
| QUICKI × 100 | 16.85 ± 0.93 | 809 | 0.40 ± 0.06 |
| IRG† | 132.45 ± 649.45 | 819 | 0.13 ± 0.08 |
| CIR† | 0.0165 ± 0.4208 | 631 | 0.30 ± 0.07 |
| HOMA-IR† | 2.84 ± 2.16 | 789 | 0.39 ± 0.06 |
| ABDOM (mm) | 41.2 ± 13.9 | 961 | 0.38 ± 0.07 |

Data are means ± SD. *$P < 0.05$; †logarithm transformed. ABDOM, abdominal skinfold average.

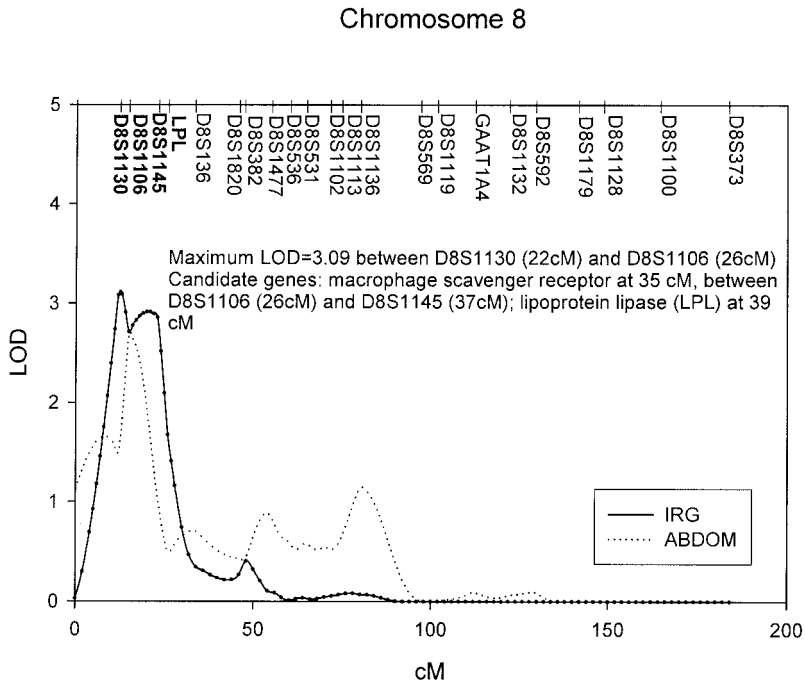


**FIG. 1. The multipoint analyses of insulin response to glucose index (IRG) and the abdominal skinfold average (ABDOM) on chromosome 8.**

retained in the models as covariates at $P < 0.10$. The largest LOD score for IRG was 3.09 on chromosome 8 between marker *D8S1130* and *D8S1106*. In the same region, multipoint linkage analysis of abdominal skinfold measurement showed an LOD score of 2.68. IRG was also linked to chromosome 12 near marker *D12S1090*, with an LOD score of 2.11. The second largest LOD score detected was with CIR and localized on chromosome 13 between markers *D13S787* and *D13S252*. CIR also was linked to chromosome 12 between marker *D12S395* and *D12S1045*. Multipoint analysis of fasting glucose resulted in an LOD score of 2.19 on chromosome 9 at marker *D9S301*. Both indexes of insulin sensitivity, QUICKI and HOMA-IR, were mapped to chromosome 13 near marker *D13S285*. A region on chromosome 18 near marker *D18S858* was suggested to be associated with variations in the measurement of 2-h insulin.

Only the genome wide scans using CIR and IRG as traits showed significant linkage results (LOD ≥3) in our study. We also conducted linkage analysis using abdominal skinfold average as the phenotype. It is interesting that the curves of the multipoint linkage analyses of abdominal skinfold measurement and IRG present peaks at the same location on chromosome 8 (Fig. 1). Figure 2 shows the significant linkage of CIR on chromosome 13. The positions of candidate genes and the exact cytogenic locations of the peaks are also given in both figures. All of the genetic locations reported in centimorgans refer to Marshfield map. The two figures are graphed based on our genetic map.

TABLE 3
Genome scan results with LOD >1.86 or repeat signal (LOD >1.30)

| Chromosome nearest marker | | Trait | LOD score |
|---|---|---|---|
| 8 | D8S1130-D8S1106 (22–26 cM) | IRG* | 3.09 |
| | D8S1106 (26 cM) | ABDOM | 2.68 |
| 9 | D9S301 (66 cM) | Fasting glucose | 2.19 |
| 12 | D12S1090 (56 cM) | IRG* | 1.9 |
| | D12S395-D12S1045 (137–161 cM) | CIR* | 2.24 |
| 13 | D13S787-D13S252 (9–16 cM) | CIR* | 2.98 |
| | D13S285 (111 cM) | HOMA IR* | 1.35 |
| | D13S285 (111 cM) | QUICKI | 1.86 |
| 18 | D18S858 (80 cM) | 2-h insulin* | 1.87 |

*Logarithm transformed.

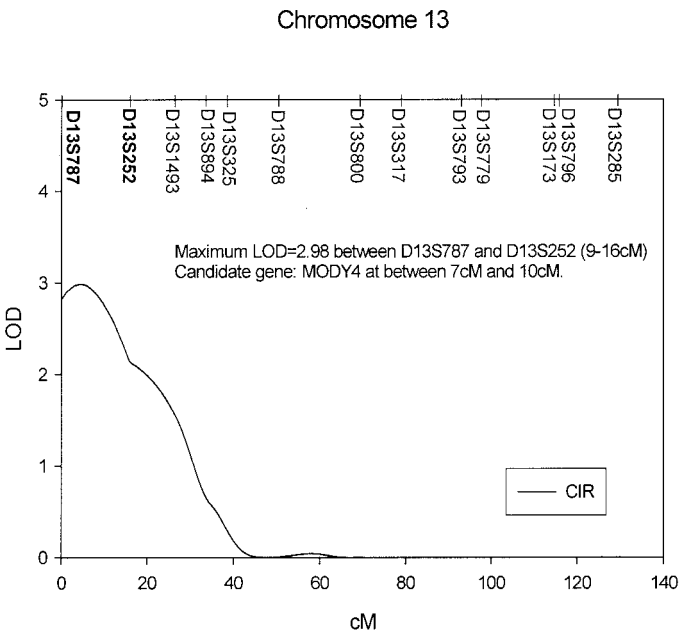


**FIG. 2. The multipoint analysis of correct insulin response to glucose index (CIR) on chromosome 13.**

## DISCUSSION

The strongest linkage signal detected in this study of insulin resistance was that for IRG on chromosome 8p between marker *D8S1130* (22 cM) and *D8S1106* (26 cM). This region is near the location of the macrophage scavenger receptor gene (35 cM) and lipoprotein lipase gene (39 cM). The genome-wide scan of abdominal skinfold also showed a peak near marker *D8S1106*, with an LOD score of 2.68. However, a multipoint linkage analysis on IRG adjusted for abdominal skinfold measurement lowered the LOD score from 3.09 to 2.95.

Several previous studies have found signals in the same region on chromosome 8p (23–28). A genome scan for type 2 diabetes–susceptibility genes in indigenous Australians suggested a linkage to a nearby marker *D8S549* (32 cM), with an LOD score of 1.77 (24). In Japanese subjects, Iwasaki et al. (25) showed a nominally significant linkage (LOD score = 0.73) with type 2 diabetes on chromosome 8 at 15 cM. Quantitative sib-pair linkage analysis in 48 Chinese families with type 2 diabetes indicated a significant linkage of systolic blood pressure with the marker *D8S261* (37 cM) in nondiabetic family members at high risk for insulin resistance (26). Diastolic blood pressure showed suggestive evidence for linkage to the lipoprotein lipase gene locus with an LOD score of 1.8 in Dutch dyslipidemic families (27). In German twin pairs (28), a significant linkage was detected for HDL cholesterol and triglycerides with macrophage scavenger receptor gene locus but not with the lipoprotein lipase gene. Wilson et al. (29) showed that diabetic patients with dysfunctional lipoprotein lipase alleles were prone to have severe lipemia, and the lipoprotein lipase and diabetic defects might be additive or synergistic. Considering that abdominal obesity, hypertension, dyslipidemia, and insulin resistance constitute the metabolic syndrome, this study replicates the above findings that a common genetic locus around the lipoprotein lipase or macrophage scavenger receptor gene may contribute to the different progressions typical of the metabolic syndrome.

The second strongest signal linked to CIR was found on chromosome 13 between marker *D13S787* and *D13S252* (9–16 cM), where the *MODY-4* gene is located. This is particularly interesting given an earlier proposal that mild variations in *MODY-4* might affect an individual's risk for type 2 diabetes (30). In Finnish diabetic patients, BMI mapped to chromosome 13 at 5 cM (multipoint LOD = 3.28) (31). In familial type 2 diabetes, HDL exhibited evidence of linkage to an area between *D13S171* and *D13S263* (25–38 cM) (32). However, to our knowledge, this is the first linkage study demonstrating suggestive or significant signal in this region using glucose, insulin, or type 2 diabetes as phenotypes.

*MODY-4* is also referred to as insulin promotor factor-1 (*IPF-1*) and codes for the major transcription factor controlling the development of the pancreas islet and regulation of β cell insulin and somatostatin expression in response to glucose influx. A homozygous frame shift mutation in *IPF-1* results in pancreatic agenesis, whereas a heterozygous mutation leads to maturity-onset diabetes of the young (MODY) (33). Milder mutations might predispose one to type 2 diabetes instead of causing MODY by influencing the gene expressions of insulin (33), GLUT-2 (34), glucokinase (35), and islet amyloid polypeptide (36) in β-cells. For instance, the prevalence of the D76N and Q59L substitution mutations and an in-frame proline insertion (InsCCG243) in non-MODY white individuals with type 2 diabetes is 6% in a French study (37), whereas the prevalence of the D76N, C18R, and R197H missense mutations is 1% of a population in the U.K. (33). However, variants in the exon-intron region of *IPF1* were not detected in 61 Japanese subjects with type 2 diabetes (38). The prevalence of *IPF1* variants (G212R, P239Q, and D76N) were 3.5, 2.7, and 1.1% in early-onset type 2 diabetes, late-onset type 2 diabetes, and healthy control subjects, respectively, in Scandinavians (39). Thus, further investigation is necessary to determine the contribution of variants of *MODY-4*, a nearby locus, or its promoter region to insulin resistance in different populations.

The signal on chromosome 13 at marker *D13S285* linked to both QUICKI and HOMA-IR is in the region where the *IRS-2* gene resides. After insulin is secreted into the blood stream and transported to the target cells, it binds to the insulin receptor. The insulin receptor is a tyrosine kinase, which initiates a cascade of phosphorylation and dephosphorylation of several substrates. *IRS-2* is one of these substrates. This protein is expressed universally and is the major form of IRSs in β-cells. Although mice with defects in the *IRS-2* gene present with phenotypes similar to human type 2 diabetes (40), polymorphisms in *IRS-2* in white individuals do not seem to be associated to this common disease (41). *IRS-2* also was investigated and not considered as a major gene for early-onset type 2 diabetes (42). However, the mutations in the promoter region of *IRS-2* were not investigated in that study. To our knowledge, the current research is the first genome-wide scan to report its gene region as being linked with type 2 diabetes phenotypes. Because the peak is between the first two markers, fine mapping in this region may be useful to narrow down the exact location of this linkage in future work.

Multipoint analysis of fasting glucose resulted in an LOD score of 2.19 (66 cM) on chromosome 9 at marker *D9S301*. In the same genetic region, a previous study in another group of Mexican Americans (43) showed linkage to HDL cholesterol levels with an LOD score of 2.4. In patients with type 2 diabetes (diagnosed by fasting glucose levels), a low HDL cholesterol level is common. Therefore, we conducted a bivariate multipoint analysis of fasting glucose and HDL cholesterol levels but did not improve the significance of the linkage signal. Also, using HDL cholesterol levels as a covariate does not change the signal in this region (unpublished results). Marker *D9S301* showed suggestive evidence of linkage with a nonparametric linkage (NPL) score of 3.9 in a Finnish study when using type 2 diabetes as the trait (44). A genome scan for type 2 diabetes susceptibility genes in a Chinese population revealed a suggestive locus near *D9S175* (70 cM, NPL = 2.94) (45). In addition, a study in Pima Indians (46) showed an LOD score of 1.64 at *D9S1123* (78 cM) linked with fasting insulin levels. Therefore, a locus around *D9S301* might harbor a gene influencing insulin sensitivity.

The signals found on chromosomes 12 and 18 also replicate previous findings. The Botnia study (44) mapped type 2 diabetes to chromosome 12 near marker *D12S304*,

whose location is in the same region as the signal for insulin resistance traits reported here. Shaw et al. (47) observed a susceptibility locus for late-onset type 2 diabetes linked to marker *D12S321* (65 cM) on chromosome 12, with an LOD score 3.65. In early-onset diabetic Scandinavians (48), an NPL score of 1.9 was documented on chromosome 18 near marker *D18S535* (64 cM). This marker is 16 cM upstream of our signal for 2-h insulin concentrations.

In this study, HOMA-IR and QUICKI were used as indicators for insulin resistance, whereas IRG and CIR were used as indicators for insulin secretion in response to glucose ingestion. It is not surprising that HOMA-IR and QUICKI were associated with the same marker on chromosome 13, considering that both are calculated from fasting glucose and fasting insulin levels and that the correlation coefficient between log(HOMA-IR) and QUICKI is $-0.96$ ($P < 0.01$). The different magnitude of the LOD scores might be due to slightly different distribution from the inverse transformation in QUICKI calculation and the different sample size resulting from trimming outliers. In addition to 2-h glucose and 2-h insulin levels, which were used to estimate CIR, fasting glucose and fasting insulin levels were used to obtain IRG. These two indexes mapped to different genetic locations, although they are significantly correlated (both log transformed; $r = 0.67$, $P < 0.01$). Modest correlations were also found between QUICKI and log(IRG) ($r = -0.21$, $P < 0.01$), between QUICKI and log(CIR) ($r = -0.11$, $P < 0.01$), and between log(HOMA-IR) and log(CIR) ($r = 0.10$, $P < 0.01$). However, log(IRG) and log(HOMA-IR) are not significantly correlated ($r = 0.03$, $P = 0.4$). Given the different chromosomal regions to which these indexes map suggest that insulin resistance may be regulated by multiple genes with each index giving a different aspect of insulin resistance.

In summary, our findings suggest that chromosomal regions around the macrophage scavenger receptor or *LPL* gene on chromosome 8, *MODY-4* on chromosome 13, and *D9S301* are linked with insulin sensitivity in nondiabetic Mexican Americans. Further information on these genes or gene regions in different populations is needed to understand their role in genetic predisposition for type 2 diabetes.

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