Brief Genetics Report

Linkage of Serum Insulin Concentrations to Chromosome 3p in Mexican Americans

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Hyperinsulinemia predicts the development of type 2 diabetes, and family studies suggest that insulin levels are regulated in part by genes. We conducted a genome-wide scan to detect genes influencing variation in fasting serum insulin concentrations in 391 nondiabetic individuals from 10 large multigenerational families. Approximately 380 microsatellite markers with an average spacing of 10 cM were genotyped in all study subjects. Insulin concentrations measured by radioimmunoassay were transformed by their natural logarithms before analysis. In multipoint analysis, peak evidence for linkage occurred on chromosome 3p ~109 cM from pter in the region of 3p14.2-p14.1. The multipoint logarithm of odds (LOD) score was 3.07, occurring in the region flanked by markers D3S1600 and D3S1285 (P value by simulation <0.0001). In a two-point analysis, LOD scores ranged from 0.75 to 2.52 for the nine markers typed in the region spanning 88–143 cM from pter. The fasting insulin resistance index was highly correlated with fasting insulin concentrations in this sample and also provided strong evidence for linkage to this region (LOD = 2.99). There was no evidence in our genome-wide scan for linkage of insulin levels to any other chromosome. These results provide evidence that a gene-influencing variation in insulin concentrations exists on chromosome 3p. Possible candidate genes in this region include GBE1 and ACOX2, which encode enzymes involved in glycogen and fatty acid metabolism, respectively. *Diabetes* 49:513–516, 2000

I nsulin is a polypeptide hormone that plays a vital role in glucose homeostasis by stimulating the uptake of glucose into the cell. Serum insulin concentrations are correlated with tissue sensitivity to insulin, and elevated insulin levels are associated with insulin resistance, dyslipidemia, obesity, hypertension, and an increased risk of type 2 diabetes and cardiovascular disease. Within popula-

tions, the degree of insulin sensitivity varies widely among individuals, and in fact, the range observed in normal individuals can equal that seen in diabetic individuals (1). Moreover, while insulin resistance is a consistent predictor of type 2 diabetes, mild or even severe insulin resistance may often occur in individuals who will never develop diabetes.

Although the causes of insulin resistance (and hyperinsulinemia) are not well understood, family and twin studies have consistently shown that genes play a substantial role in determining variation in serum insulin concentrations (2–5). Genes modulating serum insulin levels could operate at several different levels. For example, at the level of the target cells, there are a host of genes that regulate peripheral insulin action, such as those that encode the molecules that mediate insulin signaling and those that catalyze the uptake and metabolism of glucose. Genes influencing insulin sensitivity could also act on tissues extrinsic to the target cell as, for example, on tissues involved in any of the pathways regulating energy homeostasis and obesity. And on a third level, circulating insulin levels could be influenced by genes that regulate the insulin secretory response. To date, mutations leading to marked elevations in serum insulin have been identified in a few genes, but the frequency of these mutations in the population is low. The genes that account for the bulk of the population variance in serum insulin concentrations have not yet been identified.

In an effort to localize these genes, we conducted a genome-wide scan in a population-based sample of 10 large families from San Antonio, Texas. Families were of Mexican-American ancestry, a population characterized by relative hyperinsulinemia and a disproportionately high burden of diabetes (6). A total of 471 adult members of these families participated in this study. Individuals with diabetes, representing ~16% of family members, were excluded from the analyses presented in this article. Among the 391 nondiabetic subjects, mean age was 35–36 years, and the mean BMI was 28.2 and 29.6 kg/m^2 in men and women, respectively (Table 1). Impaired glucose tolerance was present in 9.2% of men and 14.6% of women. The median insulin concentration was ~10–11 µU/l, and the median value of the fasting insulin resistance index was 2.04 in men and 2.22 in women. The distribution of the natural logarithm transformed fasting insulin values was normal, as reflected by a skewness coefficient (g_1) of 0.169 and a kurtosis coefficient (g_2) of 0.123.

The heritability of fasting insulin in our study was 0.53 ± 0.09, consistent with estimates reported from other popula-

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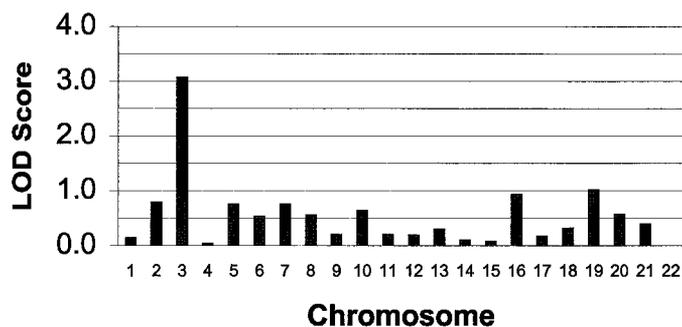
FIG. 1. Genome scan of insulin concentrations: peak multipoint LOD score by chromosome.

TABLE 1
Characteristics of nondiabetic study subjects from 10 extended pedigrees according to sex

	Men	Women
n	170	221
Percentage with impaired glucose tolerance	9.2	14.6
Age (years)	36.2 ± 15.9	35.1 ± 14.8
BMI (kg/m^2)	28.2 ± 5.6	29.6 ± 7.2
Fasting insulin (µU/ml)	9.95 (6.30, 18.10)	10.60 (7.05, 3.97)
FIRI	2.04 (1.37, 3.96)	2.22 (1.46, 3.73)

Data are means ± SD or median (25th and 75th percentile), unless otherwise indicated.

tions (2,3). We partitioned the total genetic variance into components due to a quantitative trait locus (QTL) and residual heritability, and tested for linkage to 380 markers spaced at 10 cM average density throughout the genome (Fig. 1). We detected only two chromosomal regions with multipoint logarithm of odds (LOD) scores >1.0: one occurring on chromosome 3p (multipoint LOD = 3.07) and the other occurring on chromosome 19q (multipoint LOD = 1.01).

A detailed summary of the multipoint linkage analysis of fasting insulin and chromosome 3 markers is shown in Fig. 2. Peak evidence for linkage occurred ~109 cM from pter. The position corresponding to the maximum LOD score was flanked by markers D3S1600 and D3S1285, a region that maps to 3p14.2-p14.1. The one LOD unit CI corresponding to the region of peak linkage extended from 3p21.1-p12.2.

The correlation between insulin resistance, as assessed by the fasting insulin resistance index (FIRI), and fasting insulin was extremely high in our sample (r = 0.988). Linkage analysis with FIRI revealed nearly identical results as with fasting insulin. The peak LOD score for FIRI was 2.99, occurring at position 107 cM on chromosome 3.

To evaluate the potential for obtaining false evidence for linkage with fasting insulin, we derived the distribution of nominal LOD scores under the null hypothesis of no linkage by simulation. We simulated an unlinked marker with five equifrequent alleles, assigned genotypes to each founder,
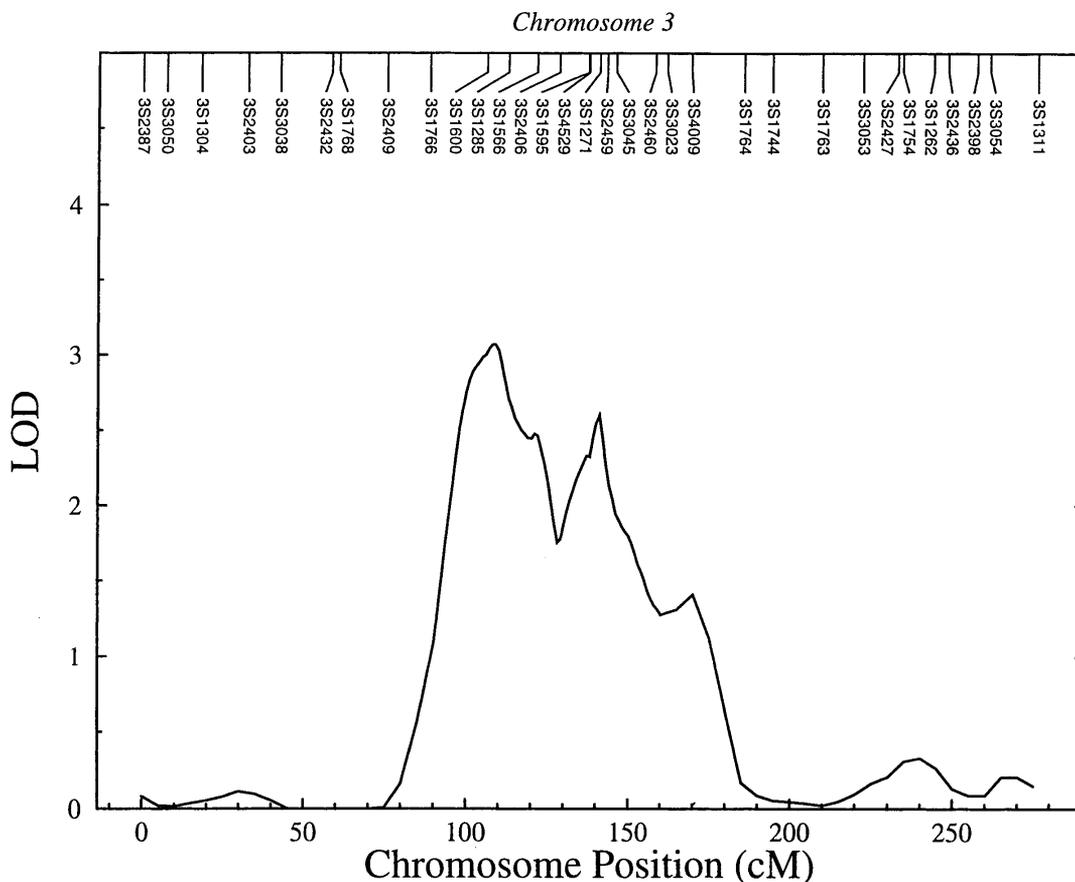

FIG. 2. Multipoint linkage analysis of insulin levels with chromosome 3 markers.

and then dropped genotypes down through the pedigree based on Mendelian expectations and the founder genotypes. We then performed linkage analysis with the simulated unlinked marker to generate an expected LOD score under the null hypothesis. We then repeated this procedure 10,000 times and estimated the probability of observing a false positive as the number of times we obtained a LOD score of

3.09. The highest LOD score we obtained for fasting insulin in the simulation was 2.94, and thus the empirical P value associated with our observed LOD score was <0.0001.

Results from two-point linkage analyses provided further support for linkage. The two-point LOD scores associated with markers D3S1600 and D3S1285 were 2.52 and 1.45, respectively. In the 54-cM region beginning at position 88.6 cM from pter, all seven additional markers that were typed provided LOD scores of 0.75. These markers and their corresponding LOD scores included the following: D3S1766 (LOD = 0.82); D3S1566 (LOD = 1.89); D3S2406 (LOD = 0.87); D3S1595 (LOD = 1.91); D3S4529 (LOD = 0.75); D3S1271 (LOD = 1.51); and D3S2459 (LOD = 1.45).

The results of our genome scan suggest that a QTL influencing variation in serum insulin levels maps to the short arm of chromosome 3, with peak evidence for linkage occurring in the region of 3p14.2-p14.1. To our knowledge, markers in this region have not previously been linked to measures of insulin sensitivity or to susceptibility to type 2 diabetes. This region does, however, contain at least two possible candidate genes for insulin resistance. ACOX2 encodes peroxisomal branched-chain acyl-CoA oxidase, an enzyme involved in the degradation of long-branched fatty acids and bile acid intermediates (7). This gene has been mapped to the interval flanked by markers D3S1600 and D3S1285, i.e., the region of peak linkage observed in our study. A second gene potentially influencing insulin sensitivity is GBE1, which encodes the glycogen branching enzyme. This enzyme, along with the enzyme glycogen synthase, is responsible for the synthesis of glycogen. GBE1 has been mapped to 3p12, to an interval on our map flanked by markers D3S1566 and D3S2406 (8).

Results from several other genome-wide scans of insulin levels and related traits have been published, although none have reported strong evidence for linkage. Part of the difficulty may be that insulin sensitivity is also influenced by aging and numerous lifestyle factors, including dietary fat intake (9,10) and physical inactivity (11–14). In Pima Indians, there was no strong evidence for linkage to a variety of insulin sensitivity and secretion measures anywhere in the genome. However, modest evidence for linkage (multipoint LOD scores <2) was observed between markers in the region of 3q21–24 and both fasting insulin levels and a measure of in vivo insulin action (15). There was also mild evidence for linkage of fasting insulin to two other regions: marker D3S3038 in the region of 3p25 and several markers in the regions of 4p15-q12 (multipoint LOD scores <1.5). Linkage analyses of fasting insulin concentrations have also been carried out in the Quebec Family Study, where evidence was obtained for linkage with markers on chromosome 20q (16) and with markers in the region of 1p32-p22 (17). In none of these regions did we detect any evidence for linkage in our study. It is likely that the effects of the major genes influencing variation in serum insulin concentrations are small and the power of the available studies to detect them is low. It is also possible that the relative impact of specific genes influencing variation in insulin levels varies among populations of different ethnic backgrounds.

In conclusion, our results suggest that a gene influencing serum insulin concentrations in this population may be located on chromosome 3p. Significant evidence for linkage was also observed with fasting insulin resistance index, a measure highly correlated with fasting insulin concentrations in this sample. Because hyperinsulinemia predicts the development of diabetes and cardiovascular disease, an increased understanding of the factors associated with variation in insulin levels could provide important insights into strategies for preventing these diseases. Such efforts may be especially relevant for Mexican Americans, who experience a disproportionately high burden of diabetes and an adverse cardiovascular risk profile.

RESEARCH DESIGN AND METHODS

The San Antonio Family Heart Study (SAFHS) is a population-based family study designed to identify the genetic determinants of atherosclerosis and its risk factors (18). Using a house-to-house recruitment procedure, we identified 40- to 60-year-old residents with large families from low-income neighborhoods in San Antonio, Texas, and invited them and their first-, second-, and third-degree relatives to participate. The invitation to participate was extended regardless of the probands' (or relatives') medical history. This report is based on our initial set of 10 large families (n = 471), for whom extensive genotyping has been completed.

Participating subjects received a medical examination in our clinic in the morning after a 12-h fast. Blood samples were obtained using a standard venipuncture technique for DNA processing and measurement of blood chemistries. Serum was separated from clotted blood by centrifugation and then stored at –80°C until assayed. Serum concentrations of insulin were measured by commercial radioimmunoassay (Diagnostic Products, Los Angeles, CA). The coefficient of variation between duplicate aliquots, measured in a single laboratory run, was 6.5% for fasting insulin.

DNA was isolated from lymphocytes for polymerase chain reaction (PCR) and automated genotyping. The DNA was amplified with fluorescently labeled primer pairs from MapPairs Human Screening Set Version 6 (Research Genetics, Huntsville, AL) that detect highly polymorphic microsatellite markers. PCRs were performed according to the manufacturer's protocol. Aliquots of the PCRs were pooled into multiplexed panels for genotyping with Applied Biosystems (Perkin Elmer, Foster City, CA) Model 377 DNA Sequencers and Genescan and Genotyper DNA Fragment Analysis software.

A total of 380 microsatellite markers from 22 autosomes were included in the analysis. The distances between markers were computed from our data using the CRI-MAP software program (19) and verified for consistency with the genetic maps available from the Marshfield Medical Research Foundation (Marshfield, WI) (www.mfldclin.edu/genetics) and University of Southampton (Southampton, U.K.) (http://cedar.genetics.soton.ac.uk/public_html/gene.html/). The average spacing between markers was 10.0 cM, and the largest spacing was 27 cM (on chromosome 9).

All subjects with diabetes (n = 74) were excluded from analysis. Insulin concentrations were transformed by their natural logarithms to remove skewness. To reduce the possible influence of extreme observations on the linkage analysis, we excluded from analysis four additional individuals whose transformed values differed from the mean level by >3 SDs. We also created a variable corresponding to the FIRI, defined as fasting glucose (in mmol/l) × fasting insulin (in mU/l)/22.5, a measure that correlates nearly perfectly with insulin resistance as measured by the HOMO-R parameter of the homeostasis model (20).

We tested for linkage with the genetic markers using a multipoint linkage analysis procedure implemented in the SOLAR software package (21). This program uses variance component methodology to model the expected genetic covariances between relatives as a function of the probability that the relatives have inherited the same allele at the marker locus from a common ancestor. The total phenotypic variance (σ^2_P) is then partitioned into components attributable to covariate effects, effects of a specific locus (σ^2_{QTL}), and the residual additive genetic effects ($\sigma^2_{h^2r}$). The variance attributable to the QTL is parameterized by the probability of allele-sharing (i.e., the identity-by-descent matrix) at a specific locus, while the variance attributable to the residual genetic effects is parameterized as a function of the relationship among individuals (i.e., the kinship matrix). Genotypic information from all available relatives was used to estimate allele-sharing probabilities, and multipoint analysis was conducted using a multipoint approximation approach. The hypothesis of linkage is evaluated by testing whether the variance attributable to the QTL is significantly greater than

zero. Maximum likelihood estimates of the model parameters were obtained and the likelihood of the pedigree data was computed under each model using the SOLAR software package.

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