Rapid Publication

Evidence for Linkage Between a Region on Chromosome 1p and the Acute Insulin Response in Pima Indians

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A low acute insulin response (AIR) is a predictor of non-insulin-dependent diabetes mellitus (NIDDM) in insulin-resistant Pima Indians. We have initiated a search for regions of the genome linked with the AIR using sib-pair linkage analysis as a first step in identifying genes that are determinants of this phenotype. Eighteen short tandem-repeat polymorphisms from chromosome 1 were genotyped in over 900 Pima Indians and tested for linkage with NIDDM and in a subset of Pima Indians for linkage with AIR. The anonymous DNA marker D1S198 on chromosome 1p was linked with AIR ($P = 0.000056$) in 175 sib pairs from 60 families, all with normal glucose tolerance, but no linkage was observed between D1S198 and NIDDM ($P = 0.44$, 996 sib pairs). Additional markers genotyped on chromosome 1 did not show linkage with AIR or NIDDM. This study indicates that a locus on chromosome 1p may be a determinant of the phenotypic variation seen in the AIR. *Diabetes* 44:478–481, 1995

Insulin resistance, obesity, and an insulin secretory dysfunction, along with increased hepatic glucose production, are the major metabolic alterations that are characteristic of people with non-insulin-dependent diabetes mellitus (NIDDM) (1). Specifically, in Pima Indians the development of NIDDM is predicted by insulin resistance, obesity, and a low acute insulin response (AIR) (2). Concordance rates of NIDDM among monozygotic twins range from 45 to 96%, while the corresponding concordance rate among dizygotic twins is only 3–37%, suggesting the importance of genetic determinants in NIDDM (3). To elucidate the genetic basis of a complex disease such as NIDDM, we are actively searching the entire genome for genes that contribute to these three major predictors of NIDDM.

Segregation analysis has failed to uncover clear patterns of inheritance or modes of transmission for the major forms of NIDDM (4,5), except for maturity-onset diabetes of the young, a rare form of NIDDM, which is inherited in an autosomal dominant fashion (6). Linkage strategies have relied on segregation-based methods of linkage analysis, such as logarithm of odds (LOD) score analyses or association analyses using candidate genes (7–9). Linkage analyses using the candidate gene approach have proven successful, leading to the identification of mutations in the insulin receptor and glucokinase genes that are responsible for some rare forms of NIDDM (10,11), but the potential list of additional candidate genes for NIDDM has become prohibitively long (12). The LOD score method of linkage analysis, however, requires specification of disease allele frequencies as well as modes of inheritance. Since formal segregation analyses of NIDDM fail to provide consistent estimates of these parameters, which is not completely unexpected because NIDDM is most likely a multifactorial disease, several different genetic models have been specified and tested (7,8). To circumvent these problems associated with candidate gene approaches or model-dependent linkage analyses, we are examining short tandem-repeat polymorphisms (STRPs) spaced at intervals throughout the genome and phenotypes that are predictors of NIDDM, such as insulin resistance and AIR, coupled with sib-pair linkage analysis (13,14). Presumably, it should be easier to identify the fewer genetic determinants of these component phenotypes than to identify the genes that in combination and by interaction result in the full syndrome of NIDDM. The major advantage of sib-pair linkage analysis is the model independence, although a disadvantage of this linkage method is that it does not allow for the estimation of genetic distances (13). However, since the density of known STRPs is increasing rapidly, estimates of genetic distance are less important. Recently, using this approach, a region on chromosome 4q was linked with maximal insulin action in Pima Indians (15).

Insulin secretion in response to an intravenous glucose challenge occurs in two phases. In the first phase of AIR, plasma insulin concentrations rapidly increase, peaking within 2–5 min. This is followed by a slower second phase of insulin release if the glucose infusion is continued (16). First, the AIR aggregates in families. The variance in AIR within a

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AIR, acute insulin response; CEPH, Centre d'Etude du Polymorphisme Humain; CHLC, Cooperative Human Linkage Center; dNTP, deoxynucleotide triphosphate; NIDDM, non-insulin-dependent diabetes mellitus; LOD, logarithm of odds; PGM, phosphoglucomutase; PCR, polymerase chain reaction; RFLP, restriction fragment-length polymorphism; STRP, short tandem-repeat polymorphism.

TABLE 1
Characteristics of the markers genotyped on chromosome 1

Marker STRP	Distance	CEPH/Pima Indian alleles (*n*/*n*)	Heterozygosity CEPH/Pima Indian	*P* for linkage with NIDDM (*n*)	*P* for linkage with AIR (*n*)
D1S199	0	11/10	0.84/0.73	0.412 (864)	0.061 (151)
D1S220	44	9/11	0.83/0.69	0.765 (1070)	0.058 (178)
D1S203	48	4/4	0.64/0.56	0.445 (973)	0.169 (146)
D1S473	50	9/9	0.82/0.81	0.888 (996)	0.172 (151)
D1S230	52	7/7	0.79/0.68	0.893 (960)	0.429 (146)
D1S246	52	10/9	0.72/0.72	0.962 (858)	0.618 (138)
D1S438	53	9/8	0.88/0.79	0.855 (982)	0.146 (153)
D1S515	54	7/7	0.63/0.62	0.926 (990)	0.088 (142)
D1S198	58	8/10	0.81/0.75	0.442 (996)	0.000056 (175)
D1S219	61	9/10	0.83/0.81	0.903 (1030)	0.125 (181)
D1S224	62	6/7	0.66/0.61	0.863 (1054)	0.021 (179)
D1S464	64	9/9	0.83/0.81	0.500 (986)	0.557 (154)
D1S216	65	16/15	0.90/0.76	0.583 (932)	0.441 (151)
D1S500	68	8/6	0.63/0.45	0.061 (947)	0.014 (143)
D1S305	121	9/10	0.84/0.72	0.553 (864)	0.776 (158)
APOA2	135	6/8	0.70/0.66	0.491 (426)	0.709 (107)
D1S238	156	12/12	0.87/0.74	0.213 (460)	0.024 (72)
D1S213	196	11/10	0.87/0.77	0.044 (740)	0.762 (149)

STRPs are listed in their relative genetic order spanning ~200 cM of chromosome 1. Distance is sex-averaged percent recombination. Heterozygosities in a sample of CEPH individuals were obtained from the Genome Data Base (19); heterozygosities for each marker in the Pima Indian were calculated according to Nei (29) from 207 individuals, none of whom were first-degree relatives. For *P* for linkage with NIDDM and AIR, *n*, number of sib pairs in the analysis.

family is smaller than the variance in AIR seen between families, suggesting that the phenotypic variation in this trait is under genetic control (17). Secondly, the AIR phenotype is reproducible within individuals. Replicated measurements of the AIR in 10 individuals resulted in variances of <10%, while five- to sixfold differences in the maximal AIR are observed between individuals (18).

Previously, we have evaluated two candidate genes for evidence of their participation in the control of AIR. STRPs at the glucokinase and GLUT2 loci were evaluated for linkage with AIR and NIDDM. Although no evidence was obtained for the participation of either of these genes in the susceptibility to NIDDM in Pima Indians, weak evidence of linkage was observed between GLUT2 and AIR (17). As an extension of the work on the genetic control of AIR, we have evaluated 18 STRPs located along chromosome 1. The anonymous DNA marker, D1S198, was linked with AIR in normal glucose-tolerant Pima Indians.

RESEARCH DESIGN AND METHODS

Highly polymorphic STRPs were chosen either from the Généthon maps or from Cooperative Human Linkage Center (CHLC) genetic maps. Primer information and Centre d'Etude du Polymorphisme Humain (CEPH) allele frequency information were obtained from the Genome Data Base (19). The markers chosen consist of primer pairs developed by Généthon and CHLC (20,21). Primer pairs for each marker were purchased from Research Genetics (Huntsville, AL) or synthesized using an Applied Biosystems oligonucleotide synthesizer (model 391, Foster, CA). DNA for genotyping was extracted from Epstein-Barr virus–transformed lymphocytes or from blood samples drawn in conjunction with other studies (1). Individuals included in the analysis have participated in longitudinal studies of NIDDM in the Pima Indian (2,22). AIR (the log of the mean plasma insulin concentration above basal over 3, 4, and 5 min after the injection of 25 g of intravenous glucose) was determined in normal glucose-tolerant individuals as described by Janssen et al. (17).

Generally, triplex polymerase chain reaction (PCR) was used to amplify three different STRPs in a single 5-μl PCR. Coamplified STRPs were pooled on the basis of size and tested, individually and pooled, in a sample of 10 test subjects. Of each forward primer, 20 pmol was end-labeled with 10 μCi γ^{32}P-ATP using 10 U T4 polynucleotide kinase (New England Biolabs, Beverly, MA) in a total volume of 20 μl, providing labeled primer for 100 PCRs. The labeling reaction was incubated at 37°C for 30 min and terminated by incubation at 65°C for 10 min. Since PCRs were routinely carried out using microtiter plate formats, a 500-μl master mix of PCR reagents was prepared for 100 reactions. The master mix consisted of 120 pmol of each reverse primer mixed with the 20 pmol of each labeled forward primer, and 10× PCR buffer II (Perkin-Elmer/Cetus, Norwalk, CT) was added to a final 1× concentration. All four deoxynucleotide triphosphates (dNTPs), were added to a final concentration of 200 μmol/l of each dNTP. To this, 1.5 mmol $MgCl_2$ and 15 U amplitaq polymerase were added, and the final volume was adjusted to 500 μl. Of this mix, 4.5 μl was added to 50 ng of genomic DNA (0.5 μl of a 100 ng/μl stock). The mixture was heated to 94°C for 3 min in a Gene Amp PCR system 9600 (Perkin-Elmer/Cetus). The amplification conditions consisted of 25 cycles of 94°C for 30 s, 55°C for 60 s, and 72°C for 45 s, followed by a final extension of 72°C for 5 min. Samples were heat denatured and the amplified products were resolved on a 6% acrylamide sequencing gel in 1× TBE (0.0089 M Tris, 0.0089 M boric acid, 0.002 M EDTA, pH 7.5) at 75-W constant current. Gels were dried on filter paper and exposed to X-OMAT film (Kodak, New Haven, CT) overnight at room temperature. PCR products were sized with a pUC 18 sequencing ladder, and one CEPH individual, 1347–02, was included for comparative purposes. Autoradiograms were routinely scored by two independent readers, and differences between readers were resolved by a third independent reader. Sib-pair linkage analysis was carried out using the computer program package SAGE, Statistical Analysis for Genetic Epidemiology (23). Statistical analyses were performed using the programs of the SAS Institute (Cary, NC).

RESULTS

STRPs were genotyped in over 900 Pima Indians representing 226 nuclear families. The characteristics of the markers genotyped on chromosome 1 are shown in Table 1. The average heterozygosity for the 18 markers was 0.79 ± 0.09 (mean ± SD) in Caucasians and 0.69 ± 0.10 in a sample of Pima Indians. While the numbers of alleles observed were generally similar in both groups, several markers exhibited allele size classes not reported in Caucasians (data not shown). None of the 18 STRPs genotyped in the Pima Indians deviated significantly from Hardy-Weinberg expectations. Allele frequencies at each STRP locus were estimated from a sample of 207 individuals, none of whom were first-degree relatives. The results of sib-pair linkage analyses using markers on chromosome 1 and AIR are shown in Fig. 1.

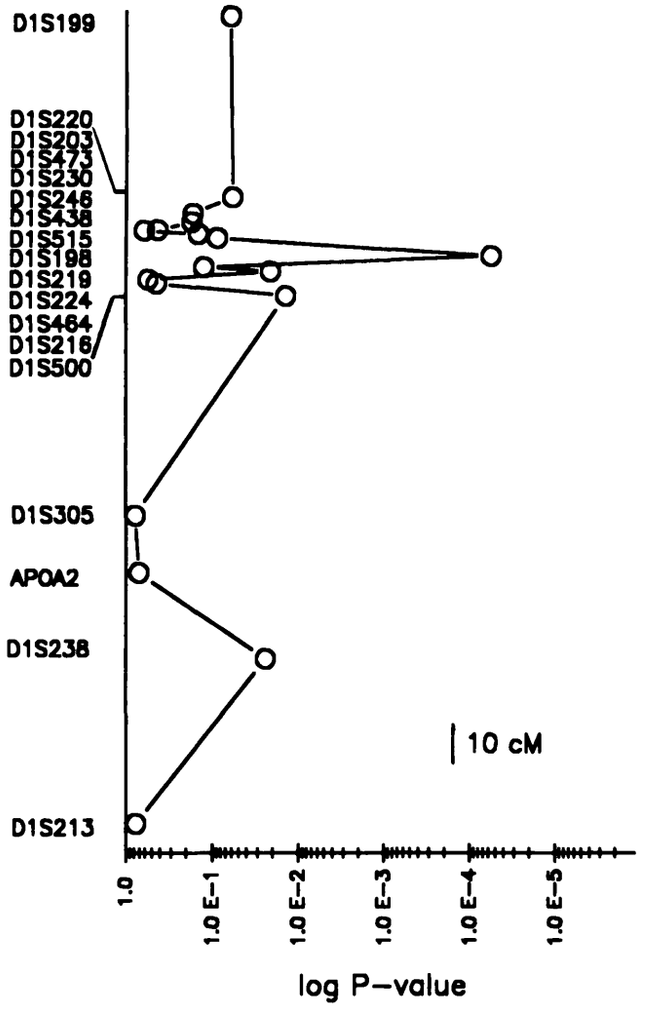

FIG. 1. Plot of linkage with AIR vs. 18 STRP markers on chromosome 1. The markers are ordered genetically with the telomere of the short arm toward the top and the telomere of the long arm toward the bottom (20). The markers span ~70% of the genetic length of chromosome 1 (21).

Genotype data and measures of AIR were available on a maximum of 181 sib pairs, constituting 60 nuclear families, 52 of which had genotype data on one or both parents. The minimum P value, indicating the strongest linkage with AIR, is observed at D1S198 ($P < 1 \times 10^{-5}$, 175 sib pairs), but there was no evidence of linkage with NIDDM ($P = 0.442$). Approximately 4 cM telomeric to D1S198 lies D1S515. Sib-pair linkage analysis using D1S515 was only suggestive of linkage with AIR ($P = 0.088$, 142 sib pairs). Centromeric to D1S198, at an estimated distance of 3 cM, is the marker D1S219, and 1 cM beyond is D1S224. The strength of the linkage also decreases in a centromeric direction toward D1S219 ($P = 0.125$, 181 sib pairs) and D1S224 ($P = 0.021$, 179 sib pairs). Markers more distant in either direction from D1S198 showed no linkage with AIR or NIDDM. D1S198 was not linked with two measures of obesity, percent body fat and body mass index, or with insulin resistance (data not shown). No association was observed between any allelic class at D1S198 and AIR. Version 1 of the CHLC genetic map places D1S198 equidistant in the interval between D1S21, close to the GLUT1 locus (telomere), and D1S188, close to the amylase locus (centromere), in a region of ~50 cM (21).

DISCUSSION

The results indicate that a genetic element close to D1S198 controls phenotypic variation in the AIR. Because NIDDM is complex and most likely multifactorial, loci with small effects on the disease might not be detected and may explain why no linkage was observed between D1S198 and NIDDM (Table 1). Instead, by examining a component phenotype of NIDDM such as AIR, with presumably less complicated genetic control, loci that are important in the development of NIDDM but do not individually exert a major effect on the disease can be detected.

In sib-pair linkage analysis, the number of alleles at a specific locus that are identical by descent between two siblings is regressed on the degree of phenotypic similarity between the siblings. The strength of the regression is a measure of linkage between the marker under study and the trait analyzed. P values <0.001 are considered indicative of linkage (23). The strong linkage between AIR and D1S198 suggests that a locus in this interval controls some of the phenotypic variation in AIR. Since no other highly polymorphic markers are currently available within the 7-cM region surrounding D1S198, it is not clear whether the maximal linkage is at D1S198 or close by. Unfortunately, other markers available in this region either are restriction fragment-length polymorphisms (RFLPs) or are of low heterozygosity (24). D1S198 is currently positioned in the interval between GLUT1 and AMY2B on the genetic maps of chromosome 1 (21,25). GLUT1 lies telomeric to D1S220, while AMY2B lies centromeric to D1S216 (Fig. 1), encompassing a region of ~50 cM. Additionally, both GLUT1 and AMY2B have been positioned on the cytogenetic map of chromosome 1. GLUT1 maps to the interval between 1p35 and 1p31, while AMY2B maps to 1p21 (26). Fine-structure genetic mapping with additional anonymous markers followed by physical mapping will be required to precisely localize and identify the region that contains genetic elements that regulate AIR in the cytogenetic interval between 1p35 and 1p21.

The amount of insulin released in the AIR phase could be controlled in a variety of ways. Because the signal for insulin release is glucose dependent, glucose transporters are good candidate genes for AIR or NIDDM. Both GLUT1 and GLUT5, the constitutive and the fetal skeletal-muscle/kidney glucose transporters, respectively, map to chromosome 1p. Although the tissue distribution of these glucose transporters does not include β-cells, they could indirectly effect AIR by altering the ambient plasma glucose concentration at the time of testing, thereby altering AIR. Previous analyses of GLUT1 in American whites failed to support linkage with NIDDM (8), which is consistent with our observations. Another study demonstrated associations between RFLP variants at GLUT1 and NIDDM in three populations (9), and thus, GLUT1 remains a viable candidate for control of the AIR unless the reasons for the associations can be resolved. Other enzymes involved in carbohydrate metabolism also map to chromosome 1p, including phosphoglucomutase 1 (PGM1) and amylo-1,6-glucosidase (25). Both PGM1 and amylo-1,6-glucosidase are central in glycogen metabolism. PGM1 catalyzes the interconversion of glucose-1-phosphate with glucose-6-phosphate, while amylo-1,6-glucosidase is a glycogen debranching enzyme. Although not a normal site of glycogen storage, the pancreas does contain small amounts of glycogen, and chronic hyperglycemia raises pancreatic glycogen stores (27). A reduction in extracellular glucose levels leads

to the mobilization of this glycogen (28), suggesting that all the enzymes required for glycogen metabolism, including PGM and amylo-1,6-glucosidase, are expressed in the pancreas. Differential mobilization of glycogen in the pancreases of hyperglycemic individuals by these enzymes could lead to altered intercellular levels of glucose and thus alter the AIR. Lastly, other loci mapped to chromosome 1 that are of interest include the amylase gene cluster, Janus kinase (JAK1), the leukocyte antigen–related tyrosine phosphatase (LAR), the medium chain acyl coenzyme A dehydrogenase (ACADM), dihydrolipoamide branched chain transacylase (DBT), and the oncogene *jun* (25). Physical mapping and evaluation of these genes will be required, as will the development of additional polymorphic markers to refine these linkage observations and localize the gene or genes on chromosome 1p that determine variation in AIR in Pima Indians.

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