Original Article

Genetic Mapping of Disposition Index and Acute Insulin Response Loci on Chromosome 11q

The Insulin Resistance Atherosclerosis Study (IRAS) Family Study

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Glucose homeostasis, a defining characteristic of physiological glucose metabolism, is the result of complex feedback relationships with both genetic and environmental determinants that influence insulin sensitivity and β-cell function. Relatively little is known about the genetic basis of glucose homeostasis phenotypes or their relationship to risk of diabetes. Our group previously published a genome scan for glucose homeostasis traits in 284 African-American subjects from 21 pedigrees in the Insulin Resistance Atherosclerosis Study Family Study (IRASFS) and presented evidence for linkage to disposition index (DI) on chromosome 11q with a logarithm of odds (LOD) of 3.21 at 81 cM flanked by markers D11S2371 and D11S2002 (support interval from 71 to 96 cM). In this study, genotyping and analysis of an additional 214 African-American subjects in 21 pedigrees from the IRASFS yielded independent evidence of linkage to DI. When these two datasets were combined, a DI linkage peak was observed with an LOD of 3.89 at 78 cM (support interval from 67 to 89 cM). Fine mapping with 15 additional microsatellite markers in this 11q region for the entire 42 pedigrees resulted in an LOD score of 4.80 at 80 cM near marker D11S937 (support interval from 76 to 84 cM). In these 42 pedigrees, there was also suggestive evidence for linkage to acute insulin response (AIR) at two separate locations flanking the DI peak (64 cM, LOD 2.77, flanked by markers D11S4076 and D11S981; and 85 cM, LOD 2.54, flanked by markers D11S4172 and D11S2002). No evidence of linkage to the insulin sensitivity index (S_i) was observed. Nine positional candidate genes were evaluated for association to DI and AIR. Among these candidates, single nucleotide polymorphisms (SNPs) in muscle glycogen phosphorylase showed evidence of association with DI ($P < 0.011$). In addition, SNPs in the pyruvate carboxylase gene showed evidence of association ($P < 0.002$) with AIR. Further analysis of these candidate genes, however, did not provide evidence that these SNPs accounted for the evidence of linkage to either DI or AIR. These detailed genetic analyses provide strong evidence of a DI locus on 11q in African-American pedigrees, with additional suggestive evidence of independent AIR loci in the same region. *Diabetes* 55:911–918, 2006

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AIR, acute insulin response; DI, disposition index; GEE, generalized estimating equation; IBD, identity-by-descent; IRASFS, Insulin Resistance Atherosclerosis Study Family Study; LOD, logarithm of odds; MAF, minor allele frequency; MLS, maximum LOD score; PC, pyruvate carboxylase; PYGM, muscle glycogen phophorylase; QTL, quantitative trait locus; SNP, single nucleotide polymorphism.

It is widely accepted that type 2 diabetes is due to a combination of defects in insulin sensitivity and insulin secretion that result in impaired glucose homeostasis. Disposition index (DI) is a quantitative measure that describes the relationship between β-cell sensitivity and insulin sensitivity (1). In metabolically normal individuals, changes in insulin sensitivity are accompanied by compensatory alterations in the β-cell's sensitivity to glucose. In practice, DI is measured as the product of the insulin sensitivity index (S_i) and β-cell function as measured by the acute insulin response (AIR). Despite being key features of glucose homeostasis, the molecular mechanism(s) by which insulin sensitivity and β-cell function are coregulated to create a homeostatic environment are not well understood (1–3).

Genetic analysis in appropriately phenotyped families is a potentially powerful approach for gaining insights into complex biological traits such as DI. To date, few mapping studies have incorporated the analysis of DI as a quantitative trait (4,5). Our group recently reported evidence for a DI locus between D11S2371 and D11S2002 on chromosome 11q at 81 cM with a logarithm of odds (LOD) score of 3.21 (support interval 71–96 cM) in 284 African-American subjects from 21 pedigrees in the Insulin Resistance Ath-

erosclerosis Study Family Study (IRASFS) (6). In addition to DI, other measures of glucose homeostasis have been previously reported to be linked to this region (7–10).

The purpose of the current study is threefold. First, we report confirmatory evidence that DI and other measures of glucose homeostasis link to chromosome 11q in an independent and similarly phenotyped and ascertained sample of African-American pedigrees from the IRASFS. Second, we report fine-mapping quantitative trait locus (QTL) linkage analysis of glucose homeostasis measures for this region on 11q. Third, we report an evaluation of selected candidate genes within 11q and explore their contribution to the variation in measures of glucose homeostasis.

RESEARCH DESIGN AND METHODS

The study design, recruitment, and phenotyping in the IRASFS have been described in detail (11). Briefly, the IRASFS is a multicenter study designed to identify the genetic determinants of insulin resistance through quantitative, intermediate measures of glucose homeostasis. For this component of the study, members of large families of self-reported African-American descent from the Los Angeles, California, area were recruited and examined. Ascertainment of families was not based on phenotypes but instead on large family size. A clinical examination was performed that included interviews, a frequently sampled intravenous glucose tolerance test, anthropometric measurements, abdominal computed tomography scanning, resting blood pressure, blood collection, and spot urine. Specific to this report, measures of glucose homeostasis included those from the frequently sampled intravenous glucose tolerance test: S_i, AIR, and DI.

Genotyping

Genome-wide scan and fine mapping. The genotyping procedure has been previously described (6). Briefly, 383 microsatellite markers (average one marker/9.3 cM) were genotyped at the National Heart, Lung, and Blood Institute Mammalian Genotyping Service (Marshfield, WI; Marshfield set 10) in two independent sets of pedigrees, denoted as set 1 and set 2. Based on the evidence of linkage to 11q in set 1 (6) and the replication of the linkage in set 2 reported herein, an additional 15 microsatellite markers (fine-mapping markers) were genotyped on all African-American pedigrees (set 1 and set 2) within a 62-cM region centered on the linkage peak. Genotyping data from these 15 microsatellite markers were merged with the genome scan data for the fine-mapping analysis.

Candidate genes. Nine positional candidate genes were chosen within the linked region based on their hypothesized functional role in glucose homeostasis. Initially, an average of five approximately equally spaced single nucleotide polymorphisms (SNPs) per positional candidate gene were selected from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). SNPs with available frequency information were preferentially selected from the database. Following preliminary analysis, additional SNPs were chosen in positional candidate genes that showed association to the glucose homeostasis phenotypes DI, AIR, or S_i. These additional SNPs were chosen to provide greater coverage of the linkage disequilibrium block and generally had a minor allele frequency (MAF) >0.20 in the IRASFS African-American pedigrees. Genotyping was performed on the Sequenom MassArray Genotyping System using methods previously described (12). Twenty-two individuals from two families served as blind duplicates (duplicated samples given new unique identifiers) to evaluate genotyping accuracy and exhibited a 0.48% discordance percentage.

Statistical analysis. The statistical analyses reported are for the three primary purposes of this study: *1*) confirmatory test that DI links to 11q in an independent and similarly phenotyped and ascertained replication sample of African-American pedigrees from the IRASFS, *2*) fine mapping of both African-American samples on 11q, and *3*) association analysis of selected candidate genes within the 11q region.

Genome-wide scan QTL linkage analysis. The IRASFS genome scan was completed in two phases, denoted set 1 (284 African-American subjects from 21 pedigrees) and set 2 (214 African-American subjects from 21 pedigrees). The analysis of the first set of 21 African-American pedigrees showed evidence of linkage to 11q and has been previously reported (6). Since that publication, the locations of markers D11S2365 and ATA9B04 on chromosome 11 that were reported at 58 cM on the Marshfield map were changed to 55.96 and 56.27 cM, respectively, in the recent release of the human genome physical map (NCBI build 35, hg assembly 17). These shifts in location resulted in changes in marker order within the region, requiring these data to be reanalyzed due to the sensitivity of linkage analyses to marker order. The reanalysis for linkage of the first set of 21 pedigrees and the linkage analysis of the second set of 21 pedigrees estimated the identity-by-descent (IBD) statistics using maximum likelihood estimates of allele frequencies from the African-American pedigrees and the Monte Carlo approach implemented in the computer program LOKI (13,14).

For the analysis of set 1, set 2, and the combined dataset, quantitative traits were transformed to best approximate the distributional assumptions of the variance-component QTL linkage analysis (i.e., conditional normality after adjusting for covariates, homogeneity of variance). Thus, the results reported represent analyses on the square root of DI and AIR and the natural logarithm of S_i after adding 1. For individuals with a self-reported diagnosis of diabetes or fasting glucose >126 mg/dl, the glucose homeostasis parameters of insulin sensitivity (S_i), DI, and AIR were converted to missing. However, all available genotypic data were used to compute the IBD statistics using the Bayesian Markov Chain Monte Carlo approach implemented in the software LOKI (13,14). Previously, self-reported familial relationships were examined and modified using the genome scan data from both set 1 and set 2 pedigrees and the software PREST (15). Testing for evidence of linkage to a QTL was done using the variance-component approach implemented in the SOLAR software package (16). All LOD scores reported herein are empirical LOD scores determined within SOLAR by simulation (lodadj procedure). The entropy-based measure of linkage information content was computed using Merlin (17).

Fine mapping. Map order and genetic distance for each of the fine-mapping markers were taken from the deCODE genetic map (18). Markers for fine mapping were analyzed using PedCheck (19) to identify any Mendelian inconsistencies. Ninety-nine total genotypes (from 79 individuals across 26 families) were converted to missing. The genotyping data from these 15 microsatellite markers were then merged with the combined genome scan genotyping data from set 1 and set 2, and QTL linkage analyses were computed as described above.

Candidate genes. The genetic location of each positional candidate gene was calculated by extracting all markers from the deCODE genetic map (18) centered around the linkage region (D11S1357:D11S4104) and mapped to the chromosomal sex-averaged genetic positions to the latest human genome physical map positions (NCBI build 35, hg assembly 17). Using R version 1.9.1 (http://www.r-project.org/index.html), a smoothing spline was fit through these points and the spline used to interpolate genetic map positions corresponding to physical positions of gene loci. For each gene, we computed a single representative physical map position as the mean of the 5′ end of the most telomeric exon in any known annotated gene splice variant and the 3′ end of the most centromeric, assuming the genetic interval of the entire locus was small relative to that of the linkage region.

Initially, each of the SNPs evaluated in this work were examined for Mendelian inconsistencies in their genotypes using PedCheck (19). Any genotypes inconsistent with Mendelian inheritance were converted to missing (0.07%). Subsequently, maximum likelihood estimates of allele frequencies were computed using the largest set of unrelated individuals from the combined dataset and then tested for departures from Hardy-Weinberg proportions. To test for an association between individual SNPs and each quantitative phenotype (S_i, AIR, and DI), a series of generalized estimating equations (GEEs; GEE1) was computed (20). The correlation between subjects within a pedigree was adjusted in the analyses by assuming an exchangeable correlation structure among siblings within a pedigree and computing the sandwich estimator of the variance (21). The sandwich estimator is also denoted the robust or empirical estimator of the variance, as it is robust to misspecification of the correlation matrix because it estimates the within-pedigree correlation matrix from moments of the data. For each phenotype (S_i, AIR, and DI) and each SNP, the 2-df (degrees of freedom) test of genotypic association was performed. In addition, three individual contrasts defined by the a priori genetic models (dominant, additive, and recessive) were computed. The contrasts are defined by the genetic models (i.e., dominant model contrasts those with versus without the polymorphism, additive model tests for a dose effect in the number of alleles, and recessive model contrasts individuals homozygous for the polymorphisms versus those who are not) and are tested using a Wald test. If the overall genotypic association is significant, the a priori contrasts are examined directly. If the overall genotypic association is not significant, the a priori contrasts are examined after adjusting for the three comparisons using a Bonferroni adjustment. Tests reported here were computed adjusting for age, sex, and BMI. Subjects with type 2 diabetes were excluded from the analysis of glucose homeostasis traits.

Statistical significance for all tests was evaluated by a simulation study to obtain empiric P values. Specifically, for each SNP and each trait, the gene-dropping algorithm implemented in Mendel (22) was used and the analysis repeated 10,000 times. The empiric P value is the proportion of time that the simulated data had a P value greater than or equal to the observed P

TABLE 1
Descriptive characteristics of participants

	Set 1	Set 2	Combined sample
Pedigrees (*n*)	21	21	42
Individuals (*n*)	337	266	603
Sex (% female)	56.7	63.5	59.7
Age (years)	44.0 ± 14.8	41.5 ± 12.9	42.9 ± 14.0
Diabetes (%)	11.4	13.4	12.3
BMI (kg/m^2)	29.1 ± 6.6 (27.8)	31.1 ± 6.9 (29.9)	30.0 ± 6.8 (29.0)
AIR (pmol/l)	919 ± 790 (669)	1,111 ± 860 (907)	1,003 ± 826 (769)
S_i ($\times 10^{-5}$/min per pmol/l)	1.76 ± 1.21 (1.49)	1.47 ± 1.09 (1.31)	1.63 ± 1.17 (1.41)
DI ($\times 10^{-5}$/min)	1,441 ± 1,317 (1,065)	1,401 ± 1,207 (1,213)	1,423 ± 1,269 (1,150)
Fasting glucose (mg/dl)	95.1 ± 9.8 (93.0)	94.1 ± 9.5 (93.0)	94.6 ± 9.7 (93.0)

Data are means ± SD or means ± SD (median) unless otherwise indicated.

value from the real data. This approach allows the retention of the original correlation among pedigree members in the phenotypic and genotypic data.

The pairwise linkage disequilibrium (LD) statistics D′ and r^2 were computed, and a block of LD was defined based on marker-marker D′ > 0.70 for all markers in the maximal set of unrelated individuals. Haplotypes were constructed within the LD block from SNPs with an MAF >10% in positional candidate genes that showed evidence of association to glucose homeostasis phenotypes. Analysis of these haplotypes was completed using a GEE1 analysis as described above, except the quasi-likelihood was weighted by the probability for each possible haplo-genotype for an individual. Specifically, we computed the expectation-maximization algorithm estimates for haplotype frequencies from the family data using the software ZAPLO (23). To reduce the complexity of the problem, ZAPLO assumes zero recombination between markers and generates all possible haplo-genotypes (i.e., haplotype combinations) for each individual. These haplo-genotypes are used as input into PROFILER (24) to compute the joint probability distribution for the haplotypes of individuals within the pedigree. This joint probability distribution is then used to estimate the probability of each haplotype pair combination (haplo-genotype) possible for each individual, conditional on the family data. Each individual enters into the GEE1 analysis once for each haplo-genotype possibility, weighted by the haplo-genotype probability. Thus, the weight for each individual sums to 1. The weighted GEE1 analyses were completed as above using the same transformations and sandwich estimator of the variance to account for the within-cluster correlation for each of the haplotypes observed with >5% frequency.

To test whether a particular SNP, either directly or through LD with the QTL, contributes to the evidence for linkage, each SNP under a dominant model was entered into the QTL linkage analysis and the change in the magnitude of the LOD score calculated. If the polymorphism directly or indirectly contributes to the evidence for linkage, the initial LOD score will be reduced in a model that includes the polymorphism as a covariate.

RESULTS

The descriptive characteristics of set 1 and set 2 participants are shown in Table 1. Diabetes-affected subjects (12.3%) were excluded from analysis of glucose homeostasis phenotypes. On average, the pedigrees in set 2 had fewer participants than pedigrees in set 1. In addition, set 2 was younger (41.5 ± 12.9), had a greater mean BMI (31.1 ± 6.9 kg/m^2), and included more women (63.5%) than men.

Mapping of DI. A summary of the linkage results for measures of glucose homeostasis on chromosome 11 is presented in online appendix Table 1 (available at http://diabetes.diabetesjournals.org). The variance-component linkage analysis for DI from SOLAR in set 1 was reanalyzed using the new marker order from the latest release of the human genome (NCBI build 35, hg assembly 17) and the LOKI (13,14) IBD calculation software. This reanalysis yielded a maximum LOD score (MLS) of 3.10 at 84 cM, flanked by markers D11S2371 and D11S2002, and a support interval from 72 to 93 cM. In set 2, the MLS was 1.26 at 102 cM, flanked by markers D11S2000 and D11S1986, and a support interval from 80 to 120 cM. In the combined set (set 1 + set 2), the MLS was 3.89 at 78 cM, flanked by markers D11S2371 and D11S2002, and a support interval from 67 to 89 cM.

With evidence of linkage of DI to 11q, fine mapping of the region was carried out by genotyping an additional 15 microsatellite markers across the region to give an average density of one marker/3 cM, with the largest gap being 6.94 cM. A QTL linkage analysis combining the genome scan markers and the 15 additional fine-mapping markers (within the 63.8-cM interval centered at the linkage peak) was completed in the entire African-American sample (Fig. 1*A* and online appendix Table 1). In set 1, the MLS for DI decreased slightly to 3.03 at 81 cM, flanked by markers D11S937 and D11S4172, and a support interval from 75 to 86 cM. In set 2, the MLS increased to 2.29 at 79 cM, flanked by markers D11S2371 and D11S937, and a support interval from 69 to 84 cM. Thus, the MLS maximized within 2 cM in these two independent samples, with set 2 replicating the QTL linkage effect identified in set 1. In the combined set, the MLS was increased to 4.80 at 80 cM at marker D11S937 with a support interval from 76 to 84 cM. Information content of the markers in this region was assessed using the program Merlin (17). Multipoint information content ranged from 0.83 to 0.95 across the fine-mapping region (59.2–123.0 cM) (data not shown).

Mapping of AIR. Variance-component linkage analyses of AIR and S_i, components of DI, were also carried out with the entire marker set including the fine-mapping markers (Fig. 1*B* and online appendix Table 1). Linkage analysis of AIR showed suggestive evidence of linkage with two peaks flanking the DI peak in the combined data (set 1, set 2, and fine-mapping markers) analysis. The most significant linkage evidence for AIR was an MLS of 2.77 at 64 cM, flanked by markers D11S4076 and D11S981, and a support interval from 60 to 73 cM. In addition, a second MLS of 2.54 at 85 cM, flanked by markers D11S4172 and D11S2002, and a support interval from 59 to 91 cM was also observed. S_i showed no significant evidence of linkage to this region.

Positional candidate gene analysis. A total of nine positional candidate genes were evaluated within the linked region for association with glucose homeostasis traits. The location and possible function of these genes in modulation of glucose homeostasis is shown in Table 2. In the association analysis of SNPs genotyped within these genes (online appendix Table 2), the most significant evidence of association was observed with muscle glycogen phophorylase (PYGM) and pyruvate carboxylase (PC) precursor. SNPs in PYGM and PC were consistent with

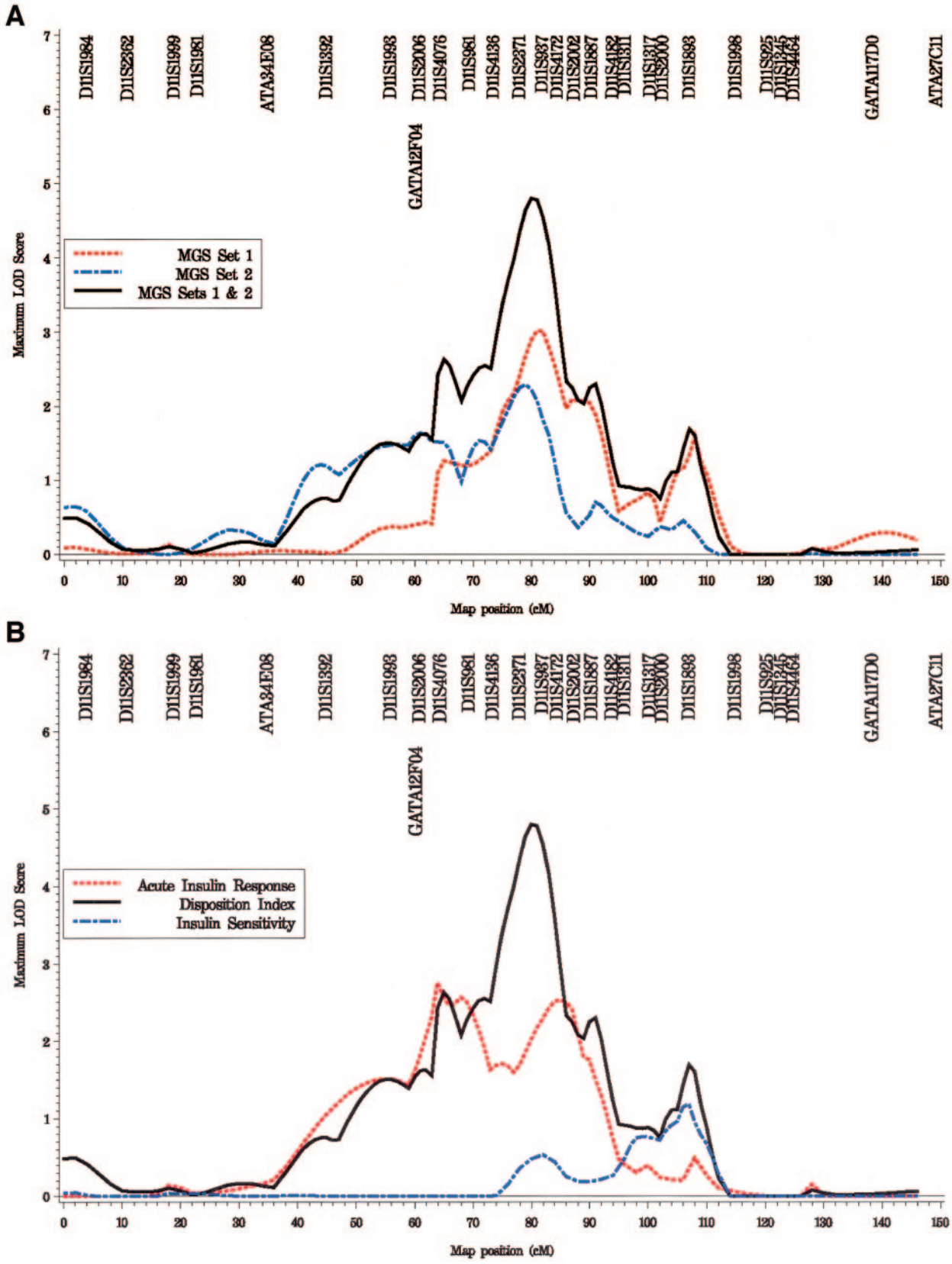

FIG. 1. *A*: **DI linkage analysis results on chromosome 11 after fine mapping for Mammalian Genotyping Service (MGS) set 1 (21 families), Mammalian Genotyping Service set 2 (21 families), and the combined set (42 families).** *B*: **Linkage analysis of glucose homeostasis traits (DI, S_I, and AIR on chromosome 11 for the combined set (42 families).**

TABLE 2
Positional candidate genes on chromosome 11

Name	Gene symbol	Location		Role
		Genetic (cM)	Physical (bp)	
Muscle glycogen phosphorylase	PYGM	70.4	64,270,606	Catalyzes and regulates the breakdown of glycogen to glucose-1-phosphate
Calpain 1	CAPN1	70.8	64,705,919	Calcium-activated cysteine protease; proteolytic cleavage of PTP-1B (27,28), a type 2 diabetes candidate gene
PC precursor	PC	71.8	66,372,572	Catalyzes the carboxylation of pyruvate to oxaloacetate; involved in gluconeogenesis, lipogenesis, insulin secretion, and synthesis of the neurotransmitter glutamate
Galanin preproprotein	GAL	72.9	68,208,611	Inhibits basal insulin and somatostatin secretion and stimulates basal glucagons secretion from the pancreas (29)
Inositol polyphosphate phosphatase-like 1	INPPL1	78.3	71,613,473	Negative regulator of insulin signaling and insulin sensitivity (30)
Uncoupling protein 2	UCP2	80.5	73,363,364	Inhibition of glucose-stimulated insulin secretion (31)
Uncoupling protein 3	UCP3	80.5	73,388,985	
Calpain 5	CAPN5	82.9	76,455,640	Member of the same family of cysteine proteases as type 2 diabetes–susceptibility gene calpain 10
Thyroid hormone responsive	THRSP	83.7	77,452,572	Expressed in the liver and adipocytes; implicated in lipogenesis

PTP-1B, protein tyrosine phosphatase 1B.

Hardy-Weinberg proportions with the exception of two SNPs in PYGM (rs565688, $P = 0.009$; rs477549, $P = 0.025$).

As seen in Table 3, multiple PYGM SNPs were significantly associated with DI from rs625172 through rs2073798 (all $P < 0.032$), with the exception of SNP rs686171, which trended toward association ($P = 0.097$). Simulations were carried out to calculate empiric P values for the individual SNPs. SNPs with an MAF >10% remained significantly associated with DI (empiric $P < 0.041$), with the exception of SNP rs565688 (empiric $P = 0.068$). Examination of LD in this region suggests one LD block encompassing SNPs rs625172 through rs2073798 (inter-SNP $D' \geq 0.96$ over 10.7 kb) (online appendix Table 3), which is consistent with the association results. Results of the haplotypic association analysis of PYGM are summarized in Table 4. Three common haplotypes (>5%) were estimated within the LD block. Of these haplotypes, haplotype TAA, with a frequency of 29%, was significantly associated with DI ($P = 0.020$) following a dominant model of inheritance.

The association analysis results for PC with AIR are presented in Table 5. PC was significantly associated with AIR for SNPs rs896438 through rs7926787 ($P < 0.002$), with the exception of rs7932018 ($P = 0.602$) and rs2077432 ($P = 0.235$). Simulations provided evidence of association with AIR (empiric $P < 0.014$). Examination of LD in this region revealed one LD block encompassing SNPs rs7119426 through rs6591223 (inter-SNP $D' \geq 0.74$ over 40.3 kb) (online appendix Table 4) and containing the trait-associated SNPs. Results of the haplotypic association analysis of PC are presented in Table 6. Five haplotypes with frequencies >5% were estimated within the LD block. Of these haplotypes, haplotype CACAGA, with a frequency of 20%, was significantly associated with AIR in all three genetic models, with the recessive model providing the strongest evidence of association ($P < 0.001$).

To assess the contribution to linkage, individual SNPs from all positional candidate genes were used as covariates in variance-component linkage analysis. For the 17 SNPs analyzed in PC and PYGM, the largest change in MLS occurred with SNP rs2167457 in PC with AIR. This SNP had an MAF of 0.24 and was significantly associated with AIR (empiric $P = 0.004$). Initially, the peak for AIR had an MLS of 2.76 at 64 cM. In our subset of participants

TABLE 3
Genotypic association analysis of PYGM SNPs with DI

SNP	Alleles	MAF	1/1	1/2	2/2	P	Empiric P
rs483962	T/C	0.32	1,423 ± 1,148	1,399 ± 1,286	1,490 ± 1,675	0.937	0.897
rs477549	A/G	0.34	1,515 ± 1,232	1,393 ± 1,271	1,261 ± 1,545	0.296	0.412
rs625172	C/T	0.47	1,539 ± 1,124	1,362 ± 1,320	1,400 ± 1,329	**0.011**	**0.021**
rs565688	T/A	0.30	1,453 ± 1,182	1,384 ± 1,420	1,388 ± 1,170	**0.018**	0.068
rs686171	C/T	0.02	1,442 ± 1,292	885 ± 1,014	—	0.097	0.218
rs569602	T/C	0.06	1,475 ± 1,297	954 ± 870	—	**<0.001**	**0.003**
rs2073798	C/A	0.48	1,498 ± 1,119	1,359 ± 1,261	1,435 ± 1,390	**0.032**	**0.041**

Data are phenotypic mean ± SD. Only nondiabetic subjects were included in this analysis. SNPs are order relative to the gene. Alleles are on the antisense strand. P values determined from a genotypic 2-df test. Empiric P values determined from simulation tests using 10,000 replications. Boldface indicates statistical significance.

TABLE 4
Haplotypic association analysis of PYGM SNPs (rs625172, rs565688, and rs2073798) with DI

Haplotype	Frequency	Disposition	*P* values for each model			Haplo-genotype mean DI ± SD		
			Dominant	Additive	Recessive	+/+*	+/−†	−/−‡
CTC	0.54	Neutral	0.679	0.534	0.089	1,501 ± 1,118	1,377 ± 1,247	1,432 ± 1,312
TAA	0.29	—	**0.020**	0.135	0.459	1,452 ± 1,126	1,395 ± 1,373	1,440 ± 1,137
TTA	0.15	Neutral	0.599	0.828	0.274	1,572 ± 839	1,266 ± 1,318	1,471 ± 1,211

*DI ± SD for individuals whose haplo-genotype has two copies of the haplotype of interest; †DI ± SD for individuals whose haplo-genotype has one copy of the haplotype of interest and any other haplotype; ‡DI ± SD for individuals whose haplo-genotype has any other haplotype combination. Boldface indicates statistical significance.

genotyped for this SNP, the peak for AIR had an MLS of 2.29 at 64 cM. After adjustment for SNP rs216457, the largest LOD score change was a decrease to an MLS of 1.90 following a recessive model (data not shown). While this decrease in LOD suggests modest contribution of this SNP to the evidence of linkage, it does not reach statistical significance. Overall, when used as covariates in the QTL linkage analysis, single SNPs in the positional candidate genes did not significantly alter evidence of linkage.

DISCUSSION

We have carried out a detailed genetic analysis of glucose homeostasis traits on chromosome 11q in African-American families from the IRASFS. These QTL linkage analyses included genetic mapping in an independent and identically phenotyped and ascertained replication sample of pedigrees, fine mapping, and analysis of a set of relevant candidate genes for association with glucose homeostasis traits and their influence on the evidence for linkage. In two independent samples (set 1 and set 2), we observed evidence for linkage to DI maximizing within 2 cM near 80 cM in both samples. QTL linkage analyses of the entire 42 IRASFS African-American sample, including both the genome scan markers and 15 additional microsatellite markers, further delineated this region. QTL linkage analyses provide evidence of a DI locus on chromosome 11q at 80 cM (LOD 4.80) and suggestive evidence of linkage to AIR with peaks that appear to flank the DI linkage signal. The results raise the possibility that multiple loci within the region may be contributing to evidence of linkage with measures of glucose homeostasis.

The biologic and mathematical relationship among DI, AIR, and S_i (DI = AIR × S_i) and the coincident linkages of DI and AIR in this region suggested that we explore the possibility that pleiotrophy may exist within this region. We performed a series of bivariate QTL linkage analyses (AIR × S_i, AIR × DI, and DI × S_i) to test this hypothesis and did not observe either a meaningful increase or decrease in the respective LOD scores. It is important to recognize that the bivariate linkage and pleiotrophy tests do not account for multiple loci in linkage with each other. Thus, the results of the linkage test are inconclusive relative to pleiotrophy, and a more definitive test will be performed through detailed SNP mapping and association tests.

These results represent the first study to identify strong evidence of a QTL for DI. This region of chromosome 11 has, however, been linked to alternative measures of glucose homeostasis not measured in this study. Pratley et al. (10) reported evidence of linkage to 2-h postload insulin with an MLS of 1.31 at marker D11S2371 (82.6 cM) in Pima Indians. This marker is one that flanks our linkage peak for DI. In addition, the FUSION (Finland-United States Investigation of NIDDM Genetics) study reported preliminary findings of linkage to type 2 diabetes (MLS of 1.75 at 84 cM near D11S1314), fasting insulin (MLS of 2.07 at 89.5 cM), and fasting insulin-to-glucose ratio (MLS of 2.32 at 91.0 cM) in diabetic subjects. Evidence for replication of this linkage to type 2 diabetes with an MLS of 2.98 at 82 cM near D11S4172 was subsequently reported by these investigators (7). Most recently, the Framingham Offspring Study reported evidence of linkage to this region for fasting insulin (MLS of 2.43 at 85 cM near D11S2002) and homeostasis model assessment (MLS 2.01 at 92 cM near D11S2002 and D11S2000) (8).

Nine positional candidate genes were evaluated for contributions to the evidence of linkage. Analysis of PYGM and PC resulted in the most significant consistent evidence of association with DI and AIR, respectively. PYGM catalyzes and regulates the breakdown of glycogen to glucose-

TABLE 5
Genotypic association analysis of PC SNPs with AIR

SNP	Alleles	MAF	1/1	1/2	2/2	*P* value	Empiric *P*
rs746018	G/A	0.46	955 ± 780	1,079 ± 937	1,169 ± 565	0.647	0.732
rs4244815	C/T	0.42	1,035 ± 816	992 ± 831	935 ± 783	0.544	0.463
rs896438	G/A	0.06	997 ± 809	1,081 ± 972	665 ± 0	**<0.001**	0.547
rs7119426	T/C	0.23	1,077 ± 883	906 ± 713	721 ± 501	**<0.001**	**0.014**
rs2167457	G/A	0.24	1,104 ± 909	894 ± 694	698 ± 464	**<0.001**	**0.004**
rs7932018	C/T	0.16	995 ± 819	991 ± 816	1,037 ± 866	0.602	0.623
rs3741194	A/G	0.06	1,031 ± 846	763 ± 534	1,024 ± 0	**0.002**	0.554
rs2077432	G/A	0.31	1,032 ± 856	1,000 ± 827	825 ± 575	0.235	0.317
rs7926787	C/G	0.25	1,053 ± 874	971 ± 769	701 ± 487	**<0.001**	**0.011**
rs6591223	A/G	0.39	1,031 ± 839	1,002 ± 802	861 ± 736	0.346	0.305

Data are phenotypic mean ± SD. Only nondiabetic subjects were included in this analysis. SNPs are order relative to the gene. Alleles are on the antisense strand. *P* values determined from a genotypic 2-df test. Empiric *P* values determined from simulation tests using 10,000 replications. Boldface indicates statistical significance.

TABLE 6
Haplotypic association analysis of PC SNPs (rs6591223, rs7926787, rs2077432, rs7932018, rs2167457, and rs7119426) with AIR

Haplotype	Frequency	Disposition	*P* values for each model			Haplo-genotype mean AIR ± SD		
			Dominant	Additive	Recessive	+/+*	+/−†	−/−‡
TGCGCG	0.43	Neutral	0.231	0.191	0.428	928 ± 757	1,010 ± 784	1,051 ± 805
CACAGA	0.20	Lower AIR	**0.011**	**0.002**	**<0.001**	721 ± 501	888 ± 659	1,107 ± 851
TGTGCA	0.15	Neutral	0.339	0.264	0.304	1,181 ± 542	1,132 ± 913	968 ± 743
TGCGCA	0.15	Neutral	0.117	0.100	0.473	1,032 ± 574	1,100 ± 830	986 ± 779
TGCACA	0.05	—	**0.001**	**0.002**	0.467	718 ± 300	1,390 ± 901	970 ± 762

*AIR ± SD for individuals whose haplo-genotype has two copies of the haplotype of interest; †AIR ± SD for individuals whose haplo-genotype has one copy of the haplotype of interest and any other haplotype; ‡AIR ± SD for individuals whose haplo-genotype has any other haplotype combination. Boldface indicates statistical significance.

1-phosphate by removing the glucose residues from α-(1,4)-linkages within glycogen molecules. PYGM was significantly associated with DI within a single LD block. Additionally, SNPs rs565688 and rs625172, which were significantly associated with DI, were also significantly associated with S_i (rs565688, $P = 0.001$; rs625172, $P = 0.017$) (online appendix Table 2). Therefore, the evidence of association seen with DI could be driven by S_i, as the two measures are mathematically and metabolically related (DI = $S_i \times$ AIR). This may also explain the absence of a contribution to the DI linkage peak when these SNPs were used as covariates in the variance-component linkage analysis. Haplotype analysis of PYGM SNPs revealed a 29% frequency haplotype (TAA) significantly associated with DI ($P = 0.020$). This result is nominally significant, but there appears to be no apparent trend in the genotypic trait means associated with this haplotype.

PC is a nuclear-encoded mitochondrial enzyme that catalyzes the conversion of pyruvate to oxaloacetate in a tissue-specific manner; the initial reaction of glucose (liver, kidney) and lipid (adipose tissue, liver, brain) synthesis from pyruvate. PC was significantly associated with AIR in a single LD block. In haplotype analysis within the LD block, a 20% frequency haplotype (CACAGA) was significantly associated with AIR ($P < 0.001$). The trend in the genotypic trait means associated with this haplotype would suggest that its presence is associated with "risk" (i.e., lower AIR). A second less frequent (5%) haplotype (TGCACA) was also significantly associated with AIR ($P = 0.001$). It is interesting to note that Antinozzi and Rothman (25) have recently observed evidence that RNAi inhibition of PC gene expression substantially suppresses secretion of an insulin surrogate in an in vivo cell culture system, a role that is consistent with our evidence of genetic association with AIR. Unfortunately, as with PYGM, there appears to be no consistent trend in the genotypic trait means associated with these haplotypes.

To examine their contribution to the evidence of linkage, each SNP in the candidate genes was analyzed as a covariate in variance-component linkage analysis for traits S_i, AIR, and DI. Individually, no single SNP significantly altered the linkage peaks. This result would preclude a direct contribution of these individual SNPs to the observed evidence of linkage. However, from these results alone, we cannot confidently rule out the possibility that these two positional candidate genes (PYGM and PC) are contributors to the overall evidence of linkage to this region on chromosome 11. Causal variants associated with the variance in DI and AIR could be located within the PYGM and PC gene regions, respectively. If the allele frequencies of the SNPs tested in these genes and the actual (unknown) causal variants differ substantially or are not in LD (e.g., low inter-SNP r^2 values between the loci examined), it may not be possible to detect the effects of these loci (26). Additionally, the results from this study are consistent with the possibility that multiple genes, each with a potentially limited contribution to the trait, result in the observed linkage peak. It is important to also note that ~100 known and hypothetical genes are located in the support interval (76–84 cM) for DI. Further insights into the genes contributing to DI and AIR in this region may result from detailed SNP fine mapping currently in progress for this region.

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