Genomic Scan of Glucose and Insulin Metabolism Phenotypes: The HERITAGE Family Study

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Genetic factors play a role in the regulation of glucose metabolism-related traits such as insulin sensitivity (S_I), insulin secretion, and glucose effectiveness (S_G). Several genomic scans have been performed to localize genes involved in glucose metabolism-related traits. However, few of these studies have been performed with phenotypes derived from the frequently sampled intravenous glucose tolerance test (IVGTT) using the minimal modeling (MINMOD) approach. Here, we report on such a scan for glucose metabolism-related traits derived from MINMOD analysis of IVGTT data in 322 sibling pairs from 95 sedentary white families and 75 sibling pairs from 49 sedentary black families from the HERITAGE Family Study. In addition to S_I and S_G, we also considered acute insulin response to a glucose challenge ($AIR_{Glucose}$), which is an index for insulin secretion, and disposition index (DI, product of S_I and $AIR_{Glucose}$), which is a measure of the activity of pancreatic β cells corrected for insulin resistance. These traits were adjusted for age, sex, and body mass index (BMI) in each of 8 sex-by-generation-by-race groups, and then standardized residuals were used as the phenotypes in the linkage analyses. Analyses were with the multipoint variance components linkage method, as implemented in the computer program SEGPATH, using 509 markers. Several regions with promising linkages (LOD score $\geq$ 1.75, $P \leq .0023$) were detected. They include five regions (1q41 and 8p23.2 for S_I, 4q32.1 and 10p15.3 for $AIR_{Glucose}$, and 13q32.1 for DI) in whites and 2 regions (9p11.2 for S_G and 10q26.11 for S_I) in blacks. Three of these regions (4q32.1, 9p11.2, 10p15.3) are likely to harbor genes that influence interindividual variation in glucose metabolism-related traits as they replicate findings from other studies. Fine mapping and association studies of candidate genes within these genomic regions are warranted.

TYPE 2 DIABETES MELLITUS (T2DM) is a multifactorial and heterogeneous disease characterized by chronic hyperglycemia due to pancreatic β-cell dysfunction and insulin resistance. The pathogenic mechanisms underlying the development of T2DM and intermediate traits, such as insulin sensitivity (S_I), insulin secretion, or glucose effectiveness (S_G), continue to be investigated from a variety of populations.

It has been strongly suggested that T2DM pathogenesis involves interactions between environmental factors and appreciable genetic components, as evidenced by twin and family studies.[1] The primary mechanism causing T2DM is insulin resistance with or without associated pancreatic β-cell dysfunction. Significant genetic influences were found in studies of surrogate indices for insulin secretion and insulin resistance such as fasting plasma insulin and the homeostasis model assessment (HOMA) insulin resistance index. Those measures, however, are only moderately correlated with those of euglycemic hyperinsulinemic clamps,[2] the standard for S_I measurement, especially in individuals with T2DM or impaired glucose tolerance.[3] In addition, various pancreatic β-cell function indices are inaccurate in the absence of appropriate adjustment for insulin resistance.[4] In contrast, the parameters derived from a frequently sampled intravenous glucose tolerance test (IVGTT) analyzed with the minimal model (MINMOD) alleviate these problems.[5,6]

Modest to moderate heritability estimates (20% to 40%) were reported in the Finland–United States Investigation of Non–Insulin-Dependent Diabetes Mellitus Genetics (FUSION) Study[7] and the HERITAGE Family Study (HERITAGE)[8] for acute insulin response ($AIR_{Glucose}$) to an intravenous glucose challenge (an index for insulin secretion), S_I, disposition index (DI, derived from the product of S_I and $AIR_{Glucose}$, an index of pancreatic β-cell function corrected for insulin sensitivity and a measure of overall glucose homeostasis), and S_G, the effect of glucose on its utilization independent of insulin. More recently, genome scans aimed at localizing genomic regions that harbor quantitative susceptibility genes for T2DM and related metabolic phenotypes have begun to appear. In the present study, we searched for quantitative trait loci influencing MINMOD-dependent parameters such as $AIR_{Glucose}$, DI, S_I, and S_G derived from an IVGTT in sedentary white and black families who participated in the HERITAGE Family Study.

MATERIALS AND METHODS

HERITAGE was designed to investigate the role of the genotype in cardiovascular, metabolic, and hormonal responses to aerobic exercise training, and the contribution of regular exercise to changes in cardio-

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Table 1. Baseline Age (years), BMI (kg/m^2), $AIR_{Glucose}$ (pmol/L × 10 min), DI, S_G (×100 min^{-1}), and S_I (×10^{-4} min^{-1}/μU/mL)

Variables	No.	Means	SE	No.	Means	SE
Whites		Fathers			Mothers	
Age	99	53.5*,†	±0.5	94	51.9*,†	±0.5
BMI	98	28.4†	±0.4	93	27.6†	±0.5
$AIR_{Glucose}$	96	387.3	±35.2	85	310.4†	±20.5
DI	96	1041.7*,†	±95.7	85	1356.3*	±125.3
S_G	96	1.4*,†	±0.1	85	1.8*	±0.1
S_I	96	3.4*,†	±0.3	85	4.9*	±0.4
		Sons			Daughters	
Age	163	25.2†	±0.5	170	25.3†	±0.5
BMI	161	25.6*,†	±0.4	167	23.7*,†	±0.3
$AIR_{Glucose}$	154	464.3*	±29.2	154	385.8*,†	±21.8
DI	152	1540.9†	±85.3	153	1528.9	±77.2
S_G	152	1.6*,†	±0.1	153	1.9*	±0.1
S_I	152	4.3†	±0.2	153	5.0	±0.3
Blacks		Fathers			Mothers	
Age	29	50.0*,†	±1.3	60	46.6*,†	±0.9
BMI	29	27.5	±1.0	59	29.4	±0.7
$AIR_{Glucose}$	22	497.7*,†	±72.4	46	902.1*	±169.1
DI	21	1373.0†	±394.8	45	1769.9†	±245.9
S_G	21	1.5†	±0.2	45	1.9	±0.2
S_I	21	2.9	±0.7	45	2.9	±0.3
		Sons			Daughters	
Age	88	27.0†	±0.8	149	27.6†	±0.6
BMI	86	27.4	±0.6	147	27.9	±0.6
$AIR_{Glucose}$	77	1145.3†	±111.5	114	1058.2	±71.9
DI	77	2579.9†	±327.1	113	2328.9†	±137.4
S_G	77	2.3†	±0.2	113	2.2	±0.1
S_I	77	3.2	±0.5	113	2.7	±0.2

*Significant ($P < .05$) mean differences for father-mother or son-daughter (within-generation) comparisons.
†Significant ($P < .05$) mean differences for father-son or mother-daughter (within-sex) comparisons.

vascular disease and diabetes risk factors. A detailed description of the HERITAGE protocol, population, and inclusion and exclusion criteria has been published elsewhere.[9] First, subjects were required to be in good health in order to complete a 20-week exercise-training program. Second, subjects had to be sedentary at baseline with no evidence that they engaged in regular vigorous exercise for the preceding 6 months. Vigorous activity was defined as any activity lasting 30 minutes or more involving a rate of energy expenditure of 7 METS or more (1 MET equals 3.5 mL oxygen uptake per kg body weight per minute and represents the rate of energy expenditure at rest) in individuals 50 years and older or 8 METS or more for younger individuals. Third, age was required to be 65 years or younger for parents, and 17 years and older for offspring. Moreover, blood pressure had to be less than 159/99 mm Hg, and body mass index (BMI) 40 kg/m^2 or less. Fourth, none of the subjects took antihypertensive or lipid-lowering medications. Several subjects whose BMIs were slightly higher than 40 kg/m^2 were approved for participation by supervising physicians, because they were considered to be in good health and able to complete the required training program. The sample sizes within 8 sex-by-generation-by-race groups are given in Table 1. From the HERITAGE cohort, a total of 322 sibling pairs from 95 nuclear White families and 75 sibling pairs from 49 nuclear black families had complete MINMOD data. The institutional review boards at the five participating centers approved the study protocol. Written informed consent was obtained from each participant.

The IVGTT protocol as proposed by Walton et al[10] was performed in the morning after fasting for 12 hours. Subjects were provided with instructions and details of the procedures before the visit. Upon arrival at each of the clinical centers, weight and height were measured and recorded. A nomogram was used to calculate the dosage of intravenous glucose (20 g/m^2 body surface area) and the total volume to be injected (40 mL/m^2), which was aliquoted in 30-mL syringes. An additional syringe of 10 mL saline was prepared to rinse the vein after glucose injection. A microperfusion butterfly and/or an indwelling catheter (local option) were inserted into an antecubital vein in each arm for injection and sampling, respectively. Blood samples were collected through the venous catheter at −15 and 0 minutes. Then, glucose was injected intravenously via the microperfusion butterfly system or indwelling catheter in the opposite arm over a period of 3 minutes, followed by the injection of saline. Blood samples were taken at 1, 3, 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 minutes after the end of the glucose injection in the opposite arm. At each time point, a 5-mL blood sample was collected in an EDTA tube and kept on ice. After centrifugation of whole blood, plasma samples were obtained, which were kept frozen at −80°C before shipping to the core laboratory at Laval University in Québec City within 1 month.

Plasma insulin was measured by radioimmunoassay after polyethylene glycol separation.[11] Polyclonal antibodies, which cross-react more than 90% with proinsulin and presumably its conversion intermediates, were used.[12] Therefore, insulin refers to immunoreactive insulin defined as the sum of insulin, proinsulin and split-proinsulin. In the present cohort with normal fasting glucose levels and no history of

Table 2. Summary of Promising Linkage Results (LOD ≥ 1.75, $P \leq .0023$) in Whites and Blacks

Chromosome	Marker	Distance (LDB)	Trait	Population	LOD	*P* Value
1q41	D1S2703	231.012	S_I	White	1.89	.00160
4q32.1	D4S2417	167.660	$AIR_{Glucose}$	White	1.79	.00204
8p23.2	D8S277	4.054	S_I	White	2.23	.00068
9p11.2	D9S1817	46.663	S_G	Black	1.90	.00156
10p15.3	D10S1435	1.383	$AIR_{Glucose}$	White	1.78	.00212
10q26.11	D10S1230	124.248	S_I	Black	1.80	.00200
13q32.1	D13S793	95.754	DI	White	2.08	.00098

T2DM, it is estimated that about 10% of the immunoreactive insulin is in the form of proinsulin and its conversion intermediates.[13] The intra- and interassay coefficients of variation for fasting insulin were 7.7% and 10.3%, respectively. Plasma glucose was enzymatically determined using a reagent kit distributed by Diagnostic Chemicals Ltd (San Antonio, TX). In this study, $AIR_{Glucose}$ was computed as the incremental integrated area under the insulin curve for the first 10 minutes of the IVGTT. S_G and S_I were derived from the MINMOD analysis.[14] DI is simply the product of $AIR_{Glucose}$ and S_I.

Since distributions were skewed, $AIR_{Glucose}$, DI, and S_I were transformed using a natural logarithm, whereas S_G was transformed using a square root. These transformed variables were further adjusted for the effects of age, age^2, age^3, and BMI within each of the 8 sex-by-generation-by-race groups in both the mean and the variance (ie, heteroscedasticity) using a stepwise multiple regression procedure. For each of the regressions, only terms that were significant at the 5% level were retained. Each of the adjusted variables used in the linkage analyses was finally standardized to a mean of 0 and a SD of 1.

Polymerase chain reaction (PCR) conditions and genotyping methods were detailed previously.[15] Automatic DNA sequencers from LI-COR were used to detect the PCR products. Genotypes were scored automatically using the computer program SAGA. Incompatibilities of Mendelian inheritance were checked, and markers showing incompatibilities were re-genotyped (<10%). Microsatellite markers were selected from the Marshfield panel version 8a. Map locations in LDB composite units were taken mainly from the Location Database (LDB) of Southampton (http://cedar.genetics.soton.ac.uk), and the Marshfield Institute map (http://www.marshmed.org/genetics) for the remaining markers.

Multipoint linkage analyses were performed using the variance components model as implemented in the computer program SEGPATH.[16,17] Under the variance components model, a phenotype is under the influence of the additive effects of a trait locus (g), a residual familial background modeled as a pseudo-polygenic component (G_R), and a residual nonfamilial component (r). The effects of the trait locus and the pseudo-polygenic component on the genotype are quantified by the heritabilities, h_g^2 and h_r^2, respectively. Allele-sharing probabilities at each marker location for each sibling pair were estimated using the multipoint approach in the computer program MAPMAKER/SIBS[18] and were input to the SEGPATH model. Other parameters in the model include spouse (u) and additional sibling (b) resemblance, and the phenotype mean and variance in the offspring. The linkage hypothesis was tested by restricting $h_g^2 = 0$. A likelihood ratio test contrasting the null versus the alternative hypothesis is asymptotically distributed as a 50:50 mixture of a χ^2 with 1 *df* and a point mass at 0.[19] The LOD score was computed as $\chi^2/(2 \times \log_e 10)$.

RESULTS

A total of 509 microsatellite and single nucleotide polymorphism markers covering all 22 autosomes were typed. The mean heterozygosities were 0.72 and 0.75 in whites and blacks, respectively. The mean intermarker spacing was 7.0 LDB units. Means and SEs for baseline $AIR_{Glucose}$, DI, S_G, and S_I are presented, separately by sex, generation, and race groups, with group differences assessed simply using SE comparisons (Table 1).

Data adjustments were performed within the sex-by-generation-by-race groups. In whites, BMI and age were not significant predictors of $AIR_{Glucose}$ in parents, but they accounted for 14% and 19% of the variance in sons and daughters, respectively. BMI and age accounted for 28%, 10%, 4%, and 0% of the variance for DI, and 40%, 21%, 37%, and 25% of the variance for S_I, in fathers, mothers, sons, and daughters, respectively. BMI accounted for 10% and 3% of the variance in fathers and sons, respectively, whereas BMI and age were not significant predictors of SG in mothers and daughters. In blacks, BMI and age terms accounted for 25%, 9%, 16%, and 4% of the variance for $AIR_{Glucose}$, and 0%, 17%, 10%, and 4% of the variance for DI, in fathers, mothers, sons, and daughters, respectively. Whereas BMI and age terms were not significant predictors of S_G in fathers and offspring, age^3 accounted for 31% of the variance in mothers. BMI accounted for 39%, 25%, 29%, and 15% of the S_I variance in fathers, mothers, sons, and daughters, respectively, whereas age was not a significant predictor of S_I in any of the groups.

In contrast to prior recommendation,[18] Rao and Province proposed a LOD score of 1.75 ($P < .0023$) for flagging a promising linkage.[20] This is equivalent to 1 false positive per scan based on discrete marker density. Promising LOD scores and associated *P* values in this study are summarized in Table 2. The genome-wide linkage scan results for baseline $AIR_{Glucose}$, DI, S_G, and S_I are depicted in Fig 1 for whites and Fig 2 for blacks. The strongest linkage signals were found on chromosomes 1q41 (Fig 3A) and 8p23.2 (Fig 3B) for S_I, 4q32.1 (Fig 3C) and 10p15.3 (Fig 3D) for $AIR_{Glucose}$, 13q32.1 for DI (Fig 3E) in whites, and on chromosomes 9p11.2 for S_G (Fig 4A) and 10q26.11 for S_I (Fig 4B) in blacks.

DISCUSSION

Evidence of an appreciable genetic component for MINMOD intermediate metabolic traits of T2DM has been reported.[7,8] The heritabilities reach about 20% to 30% for $AIR_{Glucose}$, DI, S_I, and S_G in Finnish families of the FUSION Study,[7] but 30% to 40% for $AIR_{Glucose}$, DI, K_G (glucose disappearance index, an overall index of intravenous glucose tolerance), and S_I in sedentary black and white families of HERITAGE.[8] However, genome-wide linkage scans have not been carried out yet for these MINMOD-derived phenotypes in the HERITAGE Family Study. Genome scans for T2DM or

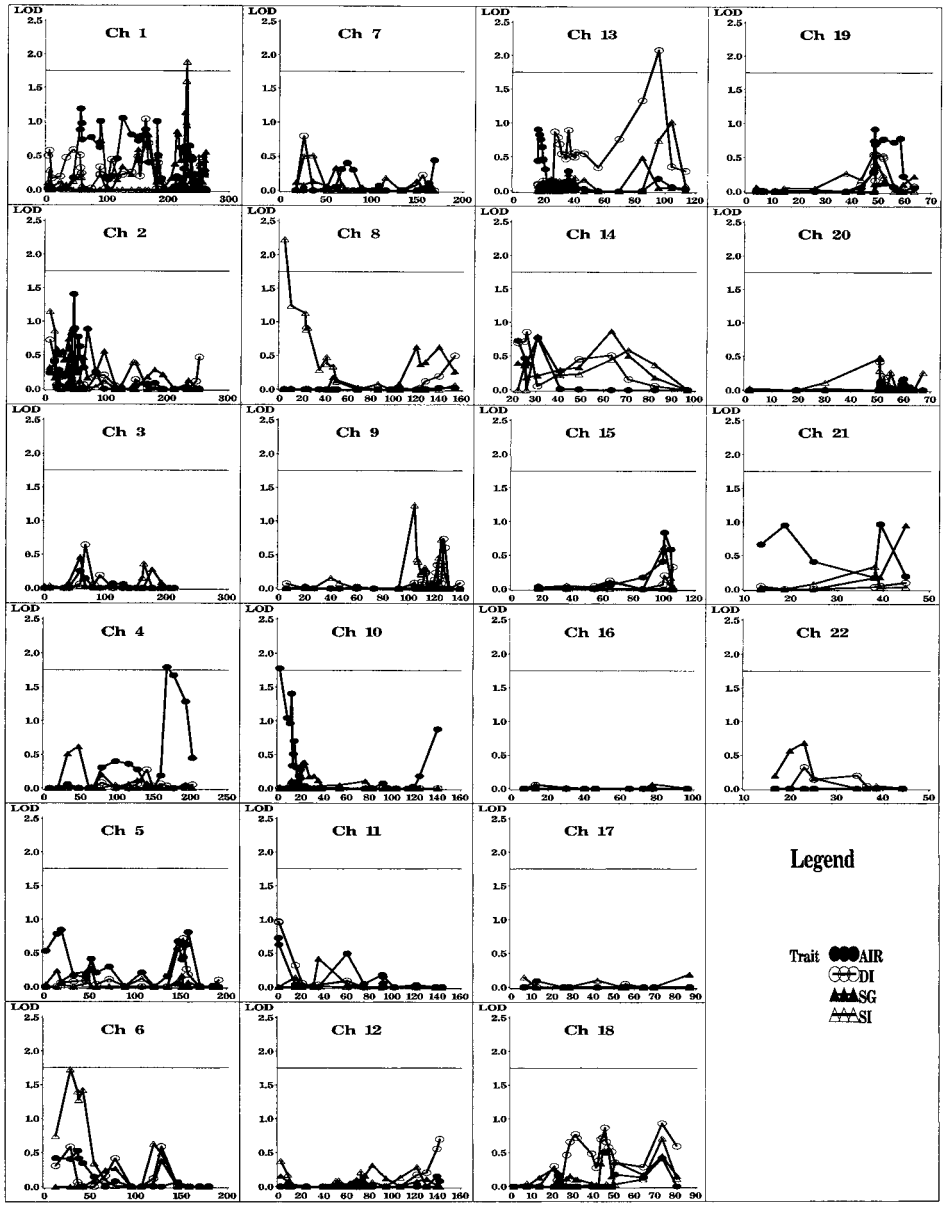

Fig 1. Linkage results for $AIR_{Glucose}$, DI, S_G, and S_I covering the 22 autosomes in whites. LOD score of the horizontal reference line is 1.75 for promising linkages.

T2DM-related quantitative metabolic traits have been performed in a variety of samples. Quantitative trait loci have rarely been replicated, suggesting that T2DM is a disorder with complex etiology and potentially heterogeneous pathophysiological mechanisms across populations. These studies include reports on Mexican Americans with linkage evidence on chromosome 2q37,[21] Pima Indians (1p31, 1q25.2, 7q22.2, 11q24.1),[22-24] Finnish (2p25.3-25.2, 2p11, 3p24.3-22.1, 4q32-33, 6q22.2-22.31, 9p13-q21, 10p14, 10p12.33-12.2, 11q21, 12q24, 13q11-12.11, 13q21.33, 16p12-11, 17p13.3, 17p12-11, 19q13.33, 20p13, 22q13.2),[25-29] indigenous Australians (2q24.3, 3q29 and 8p22),[30] Chinese Hans (1p36.3-36.23, 9p21, 9q13-21, 20q13.3),[31,32] Israeli Jews (4q32.1, 8q11.1, 14q32.12, 20p13, 20q13.11),[33] UK Europeans (1q24-25, 5q13, 5q32, 7p15.3, 8p21-22, 8q24.2, 10q23.3),[34] Utah Caucasians with Northern European ancestry (1q21-23),[35] and Canadian Oji-Cree (6q16.3, 8p23.3, 16p13.3, 22q13.2).[36] In addition, S_I measured by euglycemic clamp has been recently dissected using both path and linkage analyses in hypertensive Hispanic families.[37,38] It was shown no linkages on chromosome 7q, whereas fasting insulin and HOMA phenotype did show linkages at this genomic region.[38]

Striking linkage results are not expected for complex traits like T2DM or T2DM-related metabolic traits, as they are under the influence of multiple genes with complex gene-gene and gene-environmental interactions. This suggests that a few of such studies should be on replications of moderate linkages. Some of the results herein replicate other findings (4q32.1, 9p11.2 and 10p15.3). However, these genomic regions did not replicate across races in the present scan. The latter may be due

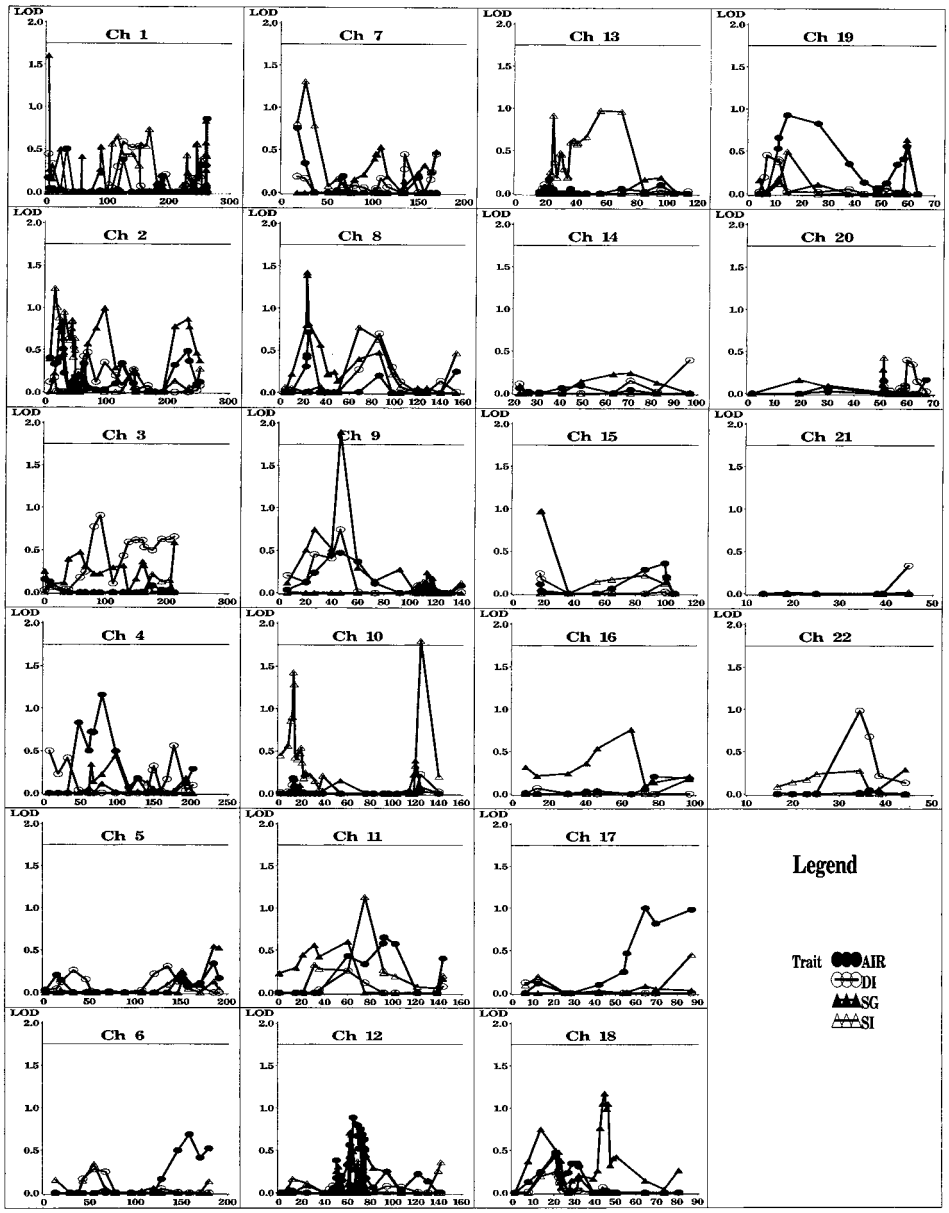

Fig 2. Linkage results for $AIR_{Glucose}$, DI, S_G, and S_I covering the 22 autosomes in blacks. LOD score of the horizontal reference line is 1.75 for promising linkages.

to the small sample size in blacks, different genetic background, or other unidentified complications. In the following, we review the regions that provide the most promising linkages for follow-up based on cross-study replication.

For one region, 4q32.1 (D4S2417, 168 LDB, $AIR_{Glucose}$), we found 3 previous reports of linkage within a 1-LOD interval (4q32.23-q35.2). Lindgren et al[25] searched for T2DM susceptibility loci in Finnish families of the Botnia study, and reported linkages with markers between D4S3015 (4q32.1, 171 LDB) and D4S2951 (4q32.3, 177 LDB), which are 3 LDB and 9 LDB, respectively, from D4S2417. Permutt et al[33] looked for T2DM susceptibility loci in a genetically isolated population of Ashkenazi Jewish descent in Israel, and found the strongest linkage signal at D4S1501 (4q32.1, 158 LDB), which is 10 LDB from D4S2417. A modest linkage with D4S415 (4q32.1, 167 LDB) for $AIR_{Glucose}$ was found in Pima Indians, which also replicated our finding of a linkage at 4q32.1 for $AIR_{Glucose}$, 1 LDB from D4S2417,[39] suggesting the region of particularly implicated for insulin secretion. No candidate genes were found within the 1-LOD interval. However, the carboxypeptidase E gene (CPE, 4q31.1, 151 LDB) and the intestinal fatty acid–binding protein gene (FABP2, 4q26, 128 LDB), which may be considered as candidates, are 17 LDB and 40 LDB, respectively, from D4S2417. FABP2 markers were linked with 2-hour insulin during an oral glucose tolerance test in Mexican American nondiabetic subjects.[40] Together, these results suggest that further investigation involving fine mapping of 4q32.1 region is warranted.

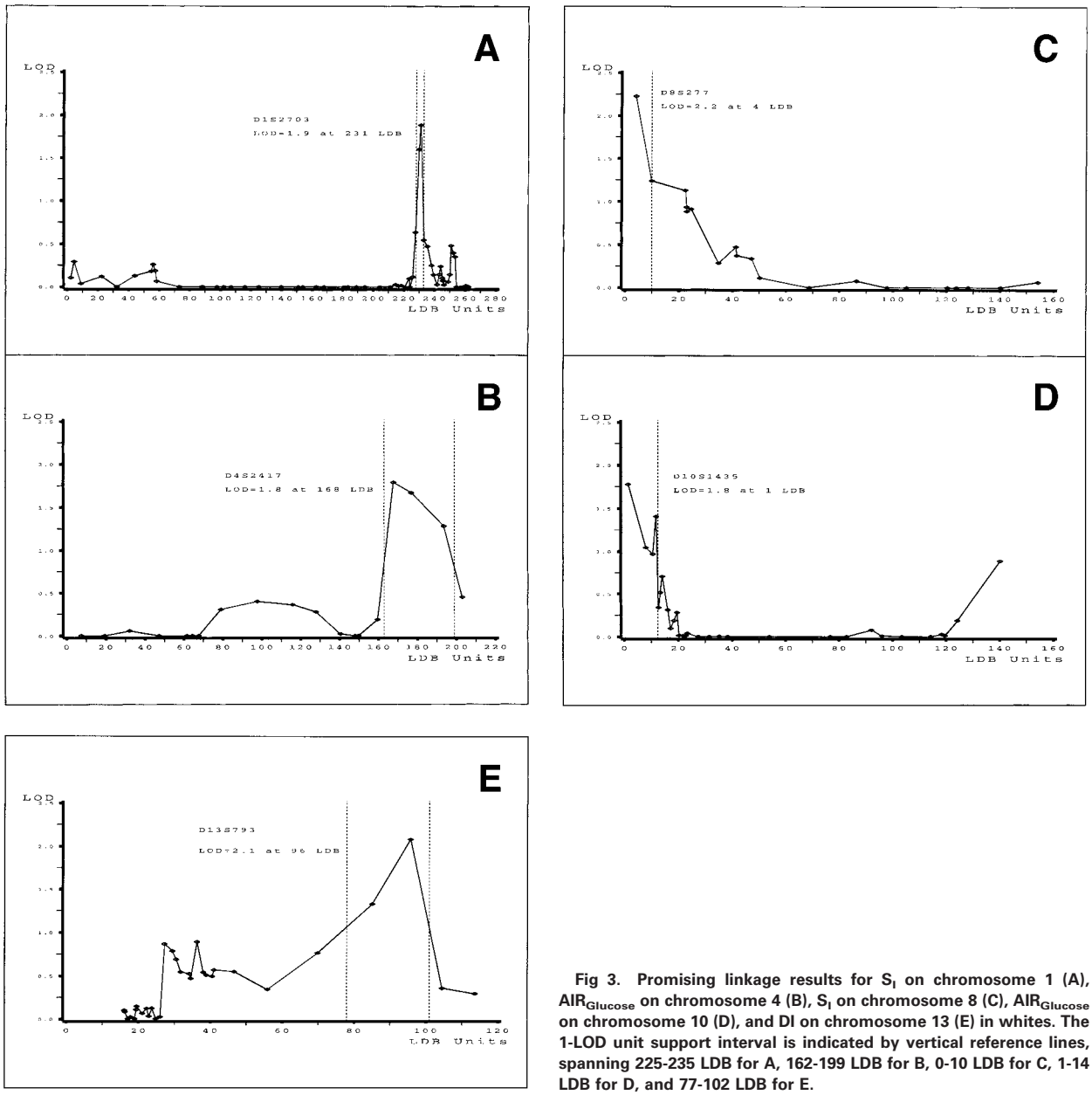

Fig 3. Promising linkage results for S_I on chromosome 1 (A), $AIR_{Glucose}$ on chromosome 4 (B), S_I on chromosome 8 (C), $AIR_{Glucose}$ on chromosome 10 (D), and DI on chromosome 13 (E) in whites. The 1-LOD unit support interval is indicated by vertical reference lines, spanning 225-235 LDB for A, 162-199 LDB for B, 0-10 LDB for C, 1-14 LDB for D, and 77-102 LDB for E.

The 1-LOD interval (9p12-q12) around the region 9p11.2 (D9S1817, 47 LDB) in blacks for S_G replicates the region 9p13-q21 (40-70 LDB) reported for T2DM susceptibility loci in Finnish families of the Botnia study.[25] The study in Chinese Hans has revealed a region (9p22.1, D9S171, 20 LDB; 9q21.11, D9S175, 73 LDB) harboring T2DM susceptibility genes,[32] which is not within our 1-LOD interval but is adjacent to the broader region defined in the Botnia study.[25] Two potentially interesting candidates within the 1-LOD interval were proposed. They are interleukin-11 receptor gene (IL11RA, 9p11.2, 46 LDB) and X25 gene (FRDA, Friedreich's ataxia gene, 9p13-21, 55 LDB), 1 and 8 LDB units, respectively, from our linkage peak of D9S1817. The latter gene encodes frataxin, a mitochondrial protein, which is associated with disturbances of glucose metabolism. Moreover, a trinucleotide repeat polymorphism (10-36 GAA repeats) in FRDA is associated with T2DM.[41] Further dense mapping of this region is clearly warranted.

The linkage at 10p15.3 (D10S1435, 1 LDB, $AIR_{Glucose}$) in whites peaked at the most distal marker measured on the p-terminal, and was thus less convincing. However, the 1-LOD interval 10p14-15.3 overlapped with the quantitative trait loci on 10p14-15.1 (D10S189-D10S223, 8-15 LDB; peak at 10p14, D10S1720, 12 LDB) reported for $AIR_{Glucose}$ in the FUSION study.[28] There are no clear candidate genes encoded within the 1-LOD interval. Further investigations will be necessary to

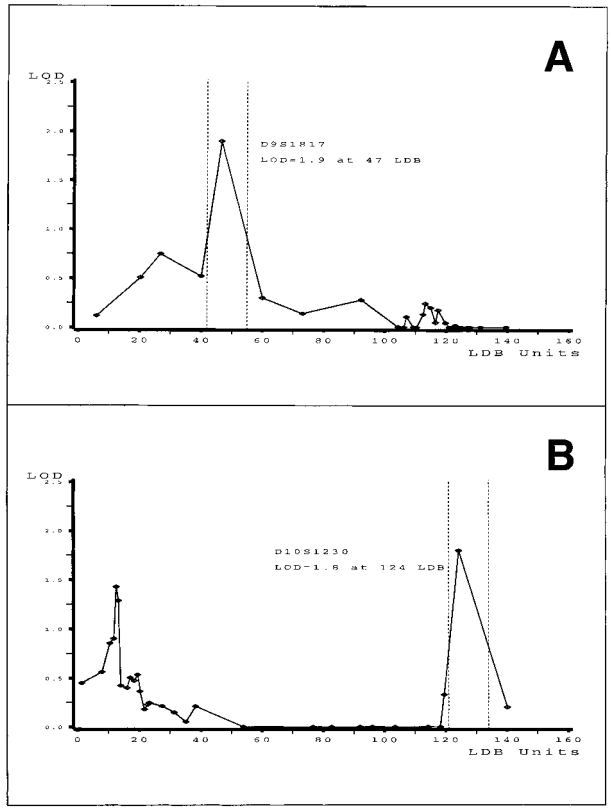

Fig 4. Promising linkage results for S_G on chromosome 9 (A) and S_I on chromosome 10 (B) in blacks. The 1-LOD-unit support interval is indicated by vertical reference lines, spanning 41-55 LDB for A and 121-134 LDB for B.

identify loci possibly involved in insulin secretion before full scale fine mapping effort can be recommended.

Insulin secretion in response to a glucose challenge occurs in 2 phases with the first phase known as $AIR_{Glucose}$, which is characterized by an abrupt rise in plasma insulin levels over 3 to 5 minutes followed by a decline in insulin levels for about 10 minutes, when circulating glucose levels fall. Defective $AIR_{Glucose}$ is usually a predictor of T2DM. The present study revealed promising linkages with genomic regions at 4q32.1 and 10p15.3 for $AIR_{Glucose}$ that replicate findings of other studies. However, we did not replicate the linkage for $AIR_{Glucose}$ on 1p31 in Pima Indians.[23,24] $AIR_{Glucose}$ was similarly derived from an IVGTT procedure in both studies. The ethnic difference is the obvious potential explanation for this discordance. In addition, the HERITAGE subjects are sedentary and relatively healthy, whereas the Pima Indians are known to have a high prevalence of T2DM, suggesting a different genetic architecture. Moreover, the heritability for $AIR_{Glucose}$ is higher in the relatively isolated Pima Indians than in any other populations, possibly due to more constant environmental exposures.[7] As for the linkages in regions 1q41, 8p23.2, 10q26.11, and 13q32.1, no replications with other studies were found.

In summary, promising genomic regions that harbor T2DM-related quantitative metabolic trait loci for MINMOD phenotypes derived from an IVGTT were localized on 1q41 and 8p23.2 for S_I, 4q32.1 and 10p15.3 for $AIR_{Glucose}$ and 13p32.1 for DI in whites, and on 9p11.2 for S_G and 10q26.11 for S_I in blacks. Further fine mapping and association studies in regions 4q32.1, 9p11.2, and 10p15.3 appear warranted due to the cross-study replications reported here.

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