Genome-Wide and Fine-Mapping Linkage Studies of Type 2 Diabetes and Glucose Traits in the Old Order Amish

Evidence for a New Diabetes Locus on Chromosome 14q11 and Confirmation of a Locus on Chromosome 1q21-q24

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We conducted a genome scan using a 10-cM map to search for genes linked to type 2 diabetes in 691 individuals from a founder population, the Old Order Amish. We then saturated two regions on chromosomes 1 and 14 showing promising linkage signals with additional markers to produce a ~2-cM map for fine mapping. Analyses of both discrete traits (type 2 diabetes and the composite trait of type 2 diabetes and/or impaired glucose homeostasis [IGH]), and quantitative traits (glucose levels during a 75-g oral glucose challenge, designated glucose 0–180 and HbA_{1c}) were performed. We obtained significant evidence for linkage to type 2 diabetes in a novel region on chromosome 14q11 (logarithm of odds [LOD] for diabetes = 3.48, $P = 0.00005$). Furthermore, we observed evidence for the existence of a diabetes-related locus on chromosome 1q21-q24 (LOD for type 2 diabetes/IGH = 2.35, $P = 0.0008$), a region shown to be linked to diabetes in several other studies. Suggestive evidence for linkage to glucose traits was observed on three other regions: 14q11-q13 (telomeric to that above with LOD = 1.82–1.85 for glucose 150 and 180), 1p31 (LOD = 1.28–2.30 for type 2 diabetes and glucose 120–180), and 18p (LOD = 3.07, $P = 0.000085$ for HbA_{1c} and LOD = 1.50 for glucose 0). In conclusion, our findings provide evidence that type 2 diabetes susceptibility genes reside on chromosomes 1, 14, and 18.
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Type 2 diabetes is a classic example of a complex disease; environmental variation, genetic influences, and interactions among these factors all contribute to the risk of developing the disease (1–4). Investigations targeting specific candidate genes have yielded relatively little insight into susceptibility genes for the common form of type 2 diabetes (5). More recently, researchers have turned to genome-wide approaches for identifying genes linked to type 2 diabetes and serum glucose levels (4,6–23). Promising linkage signals have been observed in several chromosomal regions in different study populations, with some of them overlapping (24). To date, only one susceptibility gene (calpain-10) has been cloned through genome-wide approaches (25).

The Old Order Amish of Lancaster County, Pennsylvania, are a genetically well-defined Caucasian founder population who live in a relatively homogeneous environment and often have large sibships, thus potentially enhancing our ability to detect linkages to type 2 diabetes and related traits (26). The prevalence of type 2 diabetes in the Amish is ~5%, and the phenotypic characteristics of this disease in the Amish are similar to common type 2 diabetes in other populations (26). Here, we report the results of genome-wide linkage analyses that were carried out in extended families of the Amish Family Diabetes Study (AFDS). Analyses were conducted on both discrete traits (type 2 diabetes and/or impaired glucose homeostasis [IGH]) and quantitatively distributed traits related to diabetes (plasma glucose levels and HbA_{1c}), and we used additional saturation markers placed in two chromosomal regions of particular interest. Our findings provide evidence for susceptibility genes for type 2 diabetes located on chromosomes 1q21-q24 and 18p and in a novel region on chromosome 14q11.

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Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

AFDS, Amish Family Diabetes Study; AUC, area under the curve; IGH, impaired glucose homeostasis; LOD, logarithm of odds; LRT, likelihood ratio test; OGTT, oral glucose tolerance test; STR, short tandem repeat.

RESEARCH DESIGN AND METHODS

Subjects and phenotypes. The AFDS was initiated in 1995 with the goal of identifying susceptibility genes for type 2 diabetes and related traits (26). Individuals with type 2 diabetes were identified by door-to-door interviews with the help of liaisons from the Amish community. Individuals who reported diabetes onset between 35 and 65 years of age were invited to participate. All first- and second-degree family members of the probands who were ≥18 years of age were contacted and invited to participate in the study. If another diabetic individual was identified in the family (e.g., aunt or uncle), then the family was expanded further to include that person's first- and second-degree relatives (≥18 years of age). Examinations were conducted at the Amish Diabetes Research Clinic in Strasburg, Pennsylvania, or in the subjects' homes. A genome scan was performed on the initial set of 691 individuals enrolled and examined in the AFDS between February 1995 and February 1997. Nearly all of these individuals share common ancestors insofar as the entire Amish community of Lancaster County (now numbering >30,000 individuals) are descendents of a small number of Amish families who emigrated to this area in the mid 1700s. These subjects can be connected into a single 14-generation pedigree (27). The study protocol was approved by the Institutional Review Board at the University of Maryland School of Medicine, and informed consent was obtained from each study participant.

A 3-h oral glucose tolerance test (OGTT) was administered to all subjects without a prior history of diabetes. After collection of a fasting blood sample, a 75-g oral glucose challenge was administered (Trutol 100; Casco Nerl Diagnostics, Baltimore, MD), and additional blood samples were obtained at 30-min intervals for analysis of plasma glucose concentrations. Glucose concentrations were assayed with a Beckman glucose analyzer (Beckman Coulter, Fullerton, CA) using the glucose oxidase method (interassay coefficient of variation = 1.52%). Glucose area under the curve (AUC) during the 3-h OGTT was calculated using the trapezoid method. HbA_{1c} was measured by high-pressure liquid chromatography (interassay coefficient of variation = 4.3% for low standard and 2.5% for high standard). BMI was calculated as weight (kg) divided by height squared (m^2).

Criteria for the diagnosis of diabetes were adapted from American Diabetes Association recommendations (28). Diabetes was defined by a single fasting venous plasma glucose level (≥7 mmol/l), a 2-h OGTT venous plasma glucose level (≥11.1 mmol/l), or current treatment with insulin or oral hypoglycemic agents. Diabetic subjects with an age at diagnosis <35 years were reclassified as diabetes status unknown to minimize heterogeneity due to inclusion of subjects with type 1 diabetes. Nondiabetic subjects were classified as having IGH based on age-, sex-, and BMI-adjusted fasting and 2-h OGTT venous plasma glucose levels (fasting plasma glucose level between 6.1 and 7 mmol/l or 2-h OGTT plasma glucose between 7.8 and 11.1 mmol/l). A combined phenotype, type 2 diabetes/IGH, consisted of subjects with type 2 diabetes or IGH as defined above. Normoglycemia was defined as having fasting venous plasma glucose level <6.1 mmol/l and a 2-h OGTT venous plasma glucose level <7.8 mmol/l. Due to missing data, diabetes and IGH statuses could not be determined for 42 and 87 subjects, respectively.

Genotypes. We initially performed linkage analysis using 373 highly polymorphic microsatellite short tandem repeat (STR) markers on 22 autosomes and the X chromosome. These markers were part of the ABI Prism Linkage Mapping Set (Perkin-Elmer) and have been described previously (29). The mean marker heterozygosity was 0.75, with a range from 0.33 to 0.91. The marker order and sex-averaged distances between markers were estimated from our data by maximum likelihood methods using CRI-MAP (30). The average interval between markers was 9.7 cM, and the largest gap between markers was 25.4 cM, occurring on chromosome 7. The genetic maps calculated by CRI-MAP using the Amish data were on average 8.9% longer than the corresponding map reported by Marshfield (http://research.marshfieldclinic.org/genetics/MapMarkers/maps/IndexMapFrames.html). Overall, there was excellent agreement with marker order between the Amish map and the Marshfield map, with only five occasions in which marker order was not concordant.

In two regions where interesting linkage signals were observed in our initial scan, we typed additional STR markers to increase the map density and information content. These included 36 markers on chromosome 1 between 90 and 180 cM (average intermarker distance 1.9 cM) and 18 markers on chromosome 14 between 0 and 51 cM (average intermarker distance 2.3 cM). These markers were integrated into the framework map by use of CRI-MAP and by inferring genetic distance through the use of physical map information when the genetic distance could not be properly estimated (Human Genome Project Working Draft at University of California, Santa Cruz, http://genome.ucsc.edu/). Marker allele frequencies used in all analyses were estimated from the entire set of subjects.

To identify Mendelian and/or pedigree errors, the 14-generation pedigree was broken down into 41 smaller pedigrees that were 6 generations in depth with marriage and inbreeding loops broken (D.M. Nielsen, personal communication). Pedigree errors were identified and corrected by looking for subjects with systematic Mendelian inconsistencies in the scan markers and by use of SibError (31), a program that detects sibs, half-sibs, and monozygotic twins by investigating eight identity-by-state statuses using 50 unlinked markers. Mendelian inconsistencies were identified and genotypes involved in these discrepancies deleted via an algorithm based on Unknown (32), a program that detects, for each marker, the minimal set of subjects whose genotypes, when removed, result in eliminating said Mendel errors (R. Idury, personal communication). Seven pedigree errors were identified and corrected. Three sets of monozygotic twins were identified, and we removed one twin from each of the three sets before analysis. Overall, the completeness of genotype calls for markers used in the linkage analysis was 96.3 ± 2.4% (means ± SD) (range 81.9—99.8%).

Statistical analysis

Qualitative trait linkage analysis. The two qualitative traits, type 2 diabetes and type 2 diabetes/IGH, were analyzed using two-point and multipoint nonparametric allele sharing methods as implemented in Genehunter-Plus (33) with the exponential model and S_{all} function. Multipoint allele sharing was assessed at 1-cM intervals. The single 14-generation pedigree was subdivided into smaller pedigrees fitting the size constraints of the Genehunter program. For the type 2 diabetes phenotype, the single 14-generation pedigree was broken down into 15 pedigrees with 54 affected subjects and 112 subjects who were either unaffected or of unknown diabetes status. For the type 2 diabetes/IGH phenotype, 31 pedigrees were analyzed with 140 affected subjects, 105 unaffected subjects, and 144 subjects with unknown type 2 diabetes/IGH status.

Separate analysis of pedigrees that are indeed related to one another introduces the risk of underestimating the degree with which any two individuals are related. This could result in the overestimation of the difference between expected and observed allele sharing, possibly leading to inflated evidence of linkage. To address this issue, we simulated unlinked markers whose information content was equivalent to those in the observed linkage peaks. These unlinked markers were then dropped down through the entire 14-generation pedigree (D.M. Nielsen, personal communication). This single large pedigree was divided into the smaller analysis pedigrees (see above), and these were then analyzed by Genehunter-Plus. We conducted 20,000 replicates and defined the probability that the observed logarithm of odds (LOD) score is a false positive report as the proportion of replicates exceeding our observed LOD score. The *P* values reported for the observed LOD scores from the type 2 diabetes and type 2 diabetes/IGH analyses were obtained from this simulation.

Conditional analyses were performed for the discrete trait, type 2 diabetes/IGH, to test for interactions between genomic regions (the type 2 diabetes trait was not tested due to its small sample size). To minimize the number of comparisons, we conditioned on regions with LOD scores of ≥1.18 (pointwise $P \leq 0.01$ [34]) and examined the rest of the genome for further evidence of diabetes loci. Using the Genehunter NPL score, two weighting schemes were applied; the first one tests a multiplicative model or epistatic model, and the second tests for heterogeneity among families (35). To determine the empirical significance of LOD scores from the conditional analyses, we randomly assigned weights to the families through permutation, keeping the overall number of families with a weight of 1 for a given weighting scheme constant with the number observed with a weight of 1. These permutations were conducted 5,000 times.

Quantitative trait linkage analysis. Linkage analyses were also performed on the quantitative traits, the plasma glucose levels obtained during a 75-g 3-h OGTT (fasting glucose levels and glucose levels obtained at 30-min intervals, noted as glucose 30, 60, 90, 120, 150, and 180), glucose AUC, and HbA_{1c}. Sample sizes used for each trait-specific analysis ranged from 525 (for glucose AUC) to 661 (HbA_{1c}), as described in Table 1. To eliminate confounding influences of diabetes treatment, subjects taking medications for diabetes were excluded from these analyses. Furthermore, to reduce skewness, extreme outliers were excluded from these analyses and HbA_{1c} values were transformed by their natural logarithm. To reduce the computational complexity of these analyses, we divided the single large pedigree into 28 separate smaller pedigrees, ranging in size from 3 to 69 individuals. Even after splitting the large pedigree, the sample included a very large number of relative pairs, among these 1,384 pairs of siblings, 1,404 avuncular (aunt, uncle/niece, nephew) pairs, 110 grandparent-grandchild pairs, and 1,261 first cousin pairs. These analyses were conducted using a pedigree-based likelihood method to partition the total phenotypic variation into effects due to covariates, effects of a specific locus (or linkage), and residual additive genetic effects (or heritability). Statistical significance was assessed by computing the likelihood of the pedigree data under competing genetic models (linkage versus no linkage) and comparing the likelihoods using the likelihood ratio test (LRT), which

TABLE 1
Clinical characteristics of study population by sex*

Trait†	*N* (M/F)	Male	Female	h^2‡
Age (years)	691 (308/383)	47.2 ± 15.4	46.7 ± 15.9	NA
BMI (kg/m^2)	679 (302/377)	26.3 ± 3.7	28.1 ± 5.6‡	0.41 ± 0.07
Diabetes (%)	649 (294/355)	8.5	12.7	NA
IGH (%)	534 (244/290)	14.3	20.4	NA
Plasma glucose levels (mmol/l)§				
Glucose 0	629 (281/348)	5.26 ± 1.13	5.25 ± 1.12	0.21 ± 0.06
Glucose 30	562 (250/312)	8.74 ± 2.08	8.42 ± 1.75‖	0.34 ± 0.07
Glucose 60	556 (247/309)	8.71 ± 2.45	9.07 ± 2.45	0.38 ± 0.08
Glucose 90	557 (247/310)	7.34 ± 2.60	8.22 ± 2.65‖	0.37 ± 0.07
Glucose 120	559 (248/311)	6.05 ± 2.26	7.36 ± 2.50¶	0.33 ± 0.07
Glucose 150	545 (243/302)	5.02 ± 1.81	6.11 ± 2.24¶	0.29 ± 0.07
Glucose 180	532 (237/295)	4.46 ± 1.40	4.99 ± 1.83‖	0.21 ± 0.06
Glucose AUC (mmol · l^{-1} · h)	525 (236/289)	20.28 ± 5.07	22.05 ± 5.47‖	0.44 ± 0.08
HbA_{1c} (%)§	608 (277/331)	5.05 ± 0.56	4.91 ± 0.59	0.30 ± 0.08

*See reference 26 for additional characteristics of the AFDS; †Age, BMI, glucose traits, and HbA_{1c} reported as mean ± SD; §Due to confounding influences of diabetes treatment on these traits, subjects with previously diagnosed diabetes were not included; ‡Heritability estimates ± SE, all with $P < 0.0001$; ‖$P < 0.05$; ¶$P < 0.0001$. NA, not available.

yields a χ^2 statistic with (in this case) a single degree of freedom. *P* values obtained from the LRT were then converted to LOD scores using the formula: LOD = $\chi^2/[2 \times \ln(10)]$. Linkage analysis for the quantitative traits was carried out using the SOLAR software program (35).

Use of the LRT to evaluate evidence for linkage using variance component methods can be problematic when the multivariate normality assumption is violated (37). We therefore used simulation to empirically estimate the probability of obtaining false evidence for linkage. We derived the distribution of nominal LOD scores under the null hypothesis of no linkage by simulating 10,000 unlinked markers, dropping them through the pedigrees, and conducting linkage analysis with each of the markers for each of the glucose traits and HbA_{1c}. The probability of obtaining a false positive result was defined as the proportion of replicates for which we obtained a specified LOD score or higher. The *P* values obtained from the simulation study were then converted into LOD scores as described above. All LOD scores from quantitative trait locus analyses presented in this report were obtained from this simulation.

RESULTS

Genome-wide linkage analysis. Clinical characteristics of the 691 study subjects are shown in Table 1. The mean age was ~47 years, and the mean BMI was 26.3 and 28.1 kg/m^2 in men and women, respectively ($P < 0.0001$). Diabetes was present in 8.5% of men and 12.7% of women, and IGH was present in 14.3% of men and 20.4% of women. The greater prevalence of type 2 diabetes and IGH in women was likely due to greater BMI. Since participants were ascertained around family members with type 2 diabetes, these prevalence rates do not reflect the prevalences of type 2 diabetes or IGH in the general Amish population, which are estimated to be 5 and 20%, respectively (26).

The sibling relative risk(s) for type 2 diabetes in this population was previously reported to be 3.3 (95% CI 1.58–6.80) (26). The heritabilities for the glucose traits measured during the 3-h OGTT and HbA_{1c} in family members of diabetic subjects not previously known to have diabetes are shown in Table 1. The heritabilities for all glucose traits were significantly greater than zero ($P < 0.0001$), with estimates ranging from 0.21 for glucose 0 (fasting) and glucose 180 to 0.38 for glucose 60. The heritability for the integrated measures, glucose AUC and HbA_{1c}, were 0.44 and 0.30, respectively.

Results from the initial linkage analyses based on the 10-cM screening set of 373 markers are summarized in Table 2. These analyses revealed LOD scores of ≥2.0 for one or more traits in only three chromosomal regions. Between 104 and 110 cM on chromosome 1, we observed linkage peaks for glucose 180 with a LOD of 2.16 and for glucose 150 with a LOD of 1.98. At position 14–33 cM on chromosome 14, we observed LOD = 2.81 for glucose 150 at 14 cM, LOD = 2.06 for glucose 180 at 29 cM, and LOD = 2.41 for glucose AUC at 33 cM. On chromosome 18 at position 9 cM, we observed linkage peaks for HbA_{1c} (LOD = 3.07) and glucose 0 (LOD = 1.50). LOD scores of 1.50–1.99 for one or more traits were observed in three other chromosomal regions, all on chromosome 2: at position 36–38 cM (LOD = 1.77 and 1.75 for glucose 120 and glucose AUC, respectively), at position 150 cM (LOD = 1.59 for glucose 120), and at position 265 cM (LOD = 1.87 for HbA_{1c}). Eight more chromosomal regions (on chromosomes 4, 7, 8, 15–17, and 19) showed linkage signals, with LOD between 1.18 (corresponding to a pointwise $P = 0.01$) (Table 2) and 1.49. The complete genome scan results can be viewed at http://medschool.umaryland.edu/Endocrinology/Amish/amlinkindex.html or in an online appendix at http://diabetes.diabetesjournals.org.

Fine mapping on chromosomes 1 and 14. Based on results from the initial linkage analyses, we genotyped additional STR markers on chromosomes 1 and 14. On chromosome 1, we typed an additional 36 markers within the 90- to 180-cM interval on our framework map (flanked by markers D1S209 and D1S484), and on chromosome 14, we genotyped an additional 18 STR markers falling within the region between 0 and 50 cM on our map (flanked by D14S261 and D14S288). Results of the linkage analyses using the extended set of markers on chromosomes 1 and 14 are shown in Fig. 1 for the discrete traits, and glucose 150 and 180 (results for other traits can be viewed at http://medschool.umaryland.edu/Endocrinology/Amish/amlinkindex.html or http://diabetes.diabetesjournals.org). On chromosome 1p, evidence for linkage to the discrete type 2 diabetes trait increased to 1.78 ($P = 0.0043$), occurring at 116 cM (nearest marker: D1S2766). Results for quantitative trait locus analysis remained similar to those using framework markers only. The peak signal for glucose 180 occurred at 112 cM near D1S551 (LOD = 2.31, $P = 0.00055$). Analyses

TABLE 2
Chromosomal regions showing evidence for linkage, with a LOD ≥ 1.18 ($P \leq 0.01$), to diabetes and related traits using 373 framework STR markers

Chromosome	Location (cM)	Trait	Peak LOD score	Empirical P value	LOD-1 support interval (cM)	Flanking markers
1	106	Type 2 diabetes	1.44	0.0115	90–124	D1S209, D1S216
	104	Glucose 120	1.63	0.0031	90–120	D1S209, D1S216
	104	Glucose 150	1.98	0.0013	92–120	D1S209, D1S216
	110	Glucose 180	2.16	0.0008	95–124	D1S216, D1S207
2	36	Glucose AUC	1.75	0.0023	15–45	D2S312, D2S220
	38	Glucose 120	1.77	0.0022	20–59	D2S312, D2S220
	150	Glucose 120	1.59	0.0034	130–170	D2S347, D2S368
	265	HbA_{1c}	1.87	0.0017	255–275	D2S206, D2S338
4	40	HbA_{1c}	1.23	0.0087	25–65	D4S419, D4S391
	139	Type 2 diabetes/IGH	1.28	0.0141	126–156	D4S1575, D4S424
7	100	Glucose 60	1.45	0.0049	50–120	D7S669, D7S657
8	160	Glucose 180	1.27	0.0078	130–165	D8S284, D8S272
14	11	Glucose 0	1.56	0.0037	0–35	D14S283, D14S80
	14	Glucose 150	2.81	0.00016	0–36	D14S283, D14S80
	23	Glucose 120	1.75	0.0023	10–40	D14S80, D14S70
	29	Glucose 180	2.06	0.0010	11–42	D14S80, D14S70
	33	Glucose AUC	2.41	0.0004	16–53	D14S70, D14S288
15	114	Glucose 120	1.30	0.0072	100–120	D15S130, D15S1014
16	120	Glucose 180	1.48	0.0045	109–120	D16S520
17	109	Type 2 diabetes/IGH	1.48	0.0081	90–128	D17S949, D17S802
18	9	Glucose 0	1.50	0.0043	0–28	D18S59, D18S452
	9	HbA_{1c}	3.07	0.000085	0–31	D18S59, D18S452
19	45	Glucose 120	1.37	0.0060	30–55	D19S226, D19S433

of three other quantitative traits also provided supportive evidence for linkage, including glucose 120 (LOD = 1.28 at 106 cM near D1S216), glucose 150 (LOD = 1.90 at 113 cM near D1S551), and glucose AUC (LOD = 0.71 at 105 cM near D1S216). Since the analyses of glucose traits excluded subjects with previously diagnosed diabetes, linkage of glucose traits and diabetes to the same region of chromosome 1p31 provide complimentary evidence that a gene influencing glucose homeostasis resides in this region. In the region of 1q21–24, the LOD score for type 2 diabetes/IGH increased to 2.35 ($P = 0.0008$) at 166 cM near D1S2858. D1S2715 at 166.4 cM has the largest two-point LOD score in this region (LOD = 2.07). However, dropping this marker from the saturation map resulted in only a slight drop in the multipoint LOD score at 166 cM, from 2.35 to 2.09.

On chromosome 14, we also observed increased evidence supporting linkage using a denser genetic map. For the analysis of diabetes, the peak LOD score increased to 3.48 ($P = 0.00005$) at 7 cM near D14S1023. The information content at 7 cM increased from 0.62 in the scan to 0.76 in the fine-mapping study. With an intermediate 5-cM map the LOD score for type 2 diabetes at 7 cM was 2.66. This 5-cM map did not contain D14S1023, the marker with the largest two-point LOD score (LOD = 1.57) in this region. The peak LOD score for glucose 150 (LOD = 2.16, $P = 0.0008$) occurred at the same position (7 cM). Again, since the analysis of glucose traits excluded subjects with previously diagnosed diabetes, linkage to both diabetes and glucose traits provide complimentary evidence for linkage in this region. Similar to the analysis with the framework markers, the denser map showed a secondary peak for glucose 150 (LOD = 1.82, $P = 0.0019$) that coincided at virtually the same position (at 35 cM near D14S1060) as the peak LOD for two other traits, glucose 180 (LOD = 1.86 at 35 cM), and glucose AUC (LOD = 1.84 at 44 cM).

Conditional analyses for type 2 diabetes/IGH phenotype. We examined the family-wise NPL scores for the type 2 diabetes/IGH trait at three regions that had LOD scores of ≥1.18 (pointwise $P \leq 0.01$) for the conditional analyses. The three regions that were conditioned on were 1q23 near D1S2858 (LOD = 2.35 at 166 cM), 4q28 near D4S1575 (LOD = 1.28 at 139 cM), and 17q24 near D17S949 (LOD = 1.48 at 109 cM). Two regions showed increased evidence of linkage under a positive weighting scheme (epistatic model). At 1q24-q31 (D1S218-D1S238, 204 cM), the LOD score for type 2 diabetes/IGH increased from 0.89 to 2.60 ($P = 0.0022$) when we conditioned on the linkage results at the 4q28 region. After conditioning on linkage results at 1q23, the LOD scores at 5q22 near D5S421 (115 cM) increased from 0.55 to 1.42 ($P = 0.003$). Under a negative weighting scheme (heterogeneity model), only one additional region was identified with a significant increase in LOD score. When conditioning on linkage results at 4q28, the LOD score at 14q11 (D14S1023, 6 cM) increased from 0.95 to 1.75 ($P = 0.0048$). The region at 14q11 is the same region showing evidence for linkage to the trait, diabetes (Fig. 1). Conditioning on 17q24 did not significantly increase evidence for linkage elsewhere in the genome.

DISCUSSION

Type 2 diabetes has an important genetic basis in the Amish, as it does in other populations. The sibling relative risk of type 2 diabetes was estimated to be ~3.3 (26), suggesting a familial aggregation of this disease in this population. Furthermore, in nondiabetic family members of subjects with known diabetes, there are moderate yet

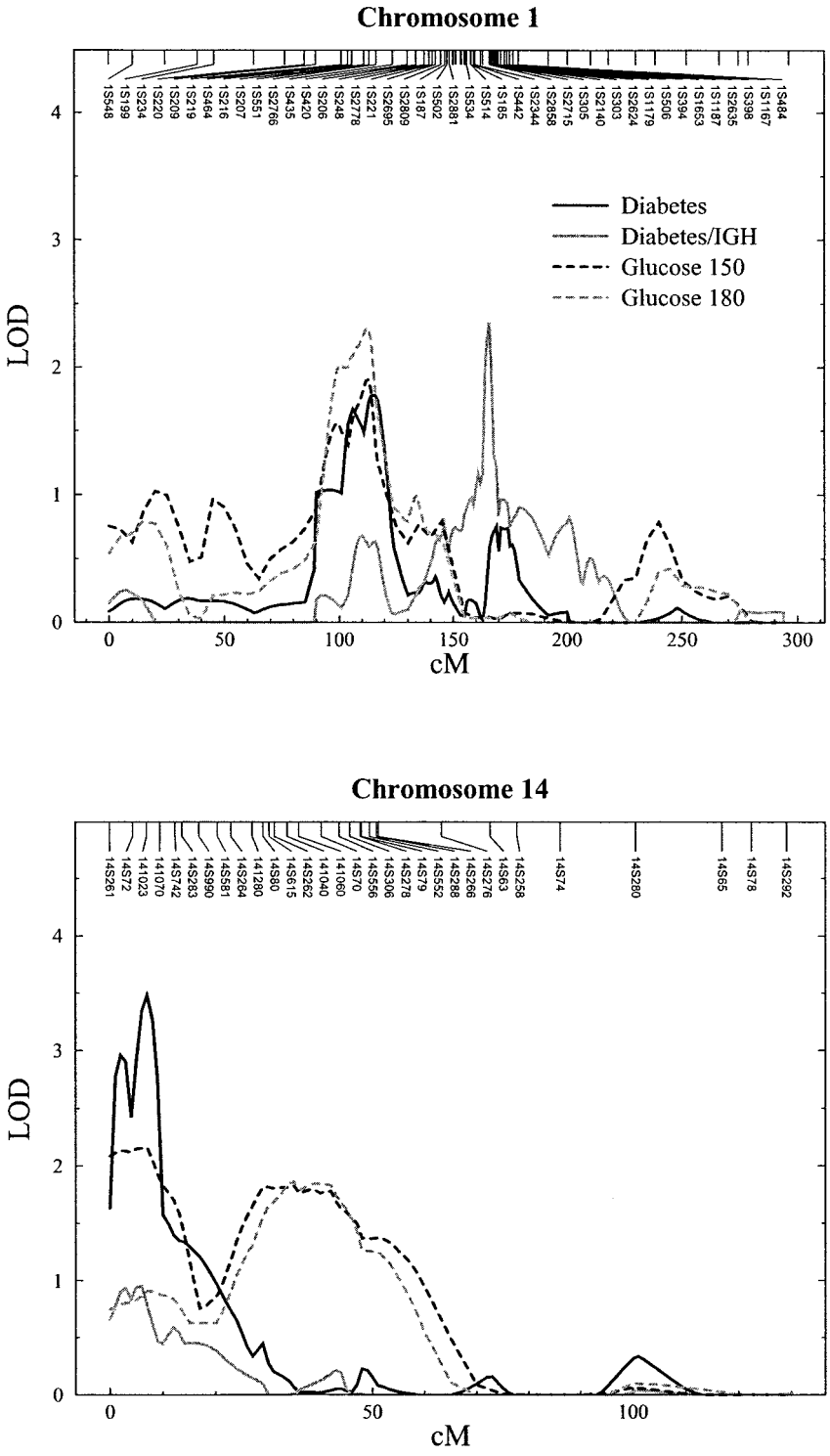

FIG. 1. Multipoint linkage analysis results of glucose and diabetes for chromosomes 1 and 14 in the fine-mapping study.

significant heritabilities for plasma glucose concentrations during an OGTT as well as for HbA_{1c}, providing further evidence of genetic influence on type 2 diabetes. Despite the well-recognized genetic contribution to type 2 diabetes, little is known about the specific genetic causes of diabetes or variation in glucose levels. Most linkage studies have examined diabetes or glucose levels during fasting and/or 2 h after a glucose challenge. To our knowledge, our study is the first to investigate the genetic influence on plasma glucose levels during the course of a 3-h OGTT, allowing us to examine whether the variation in glucose levels during the OGTT have shared genetic influences.

Based on results from our genome scan, we identified several regions with suggestive evidence of linkage. Although none of the linkage signals reached genome-wide significance at $P < 0.05$, these studies provide evidence for linkage in the regions of 1p31, 1q21-q24, and 14q11. Linkage signals for several glucose concentrations measured at different times during the 3-h OGTT (glucose 120, 150, and 180 and glucose AUC) were observed in a region on

chromosome 1p31 (between 104 and 110 cM). Furthermore, analysis of the discrete trait, diabetes, also provided evidence for linkage in the same region. On chromosome 1q21-q24, the peak LOD score for type 2 diabetes/IGH was 2.35 (at 166 cM). On chromosome 14q11, we obtained much stronger evidence for linkage to diabetes, with a peak LOD score of 3.48. For the type 2 diabetes/IGH traits, the peak LOD score was 0.95 on chromosome 14 at virtually the same region. The peak signal for glucose 150 also occurred at 7 cM. Linkage to a similar region on chromosome 14q11 for the dichotomous traits (type 2 diabetes and type 2 diabetes/IGH), as well as glucose levels as quantitative traits (in which subjects with previously diagnosed diabetes were not included), provides strong evidence for a novel type 2 diabetes locus in this region. We also obtained evidence for linkage to glucose traits on chromosomes 2 and 18. Notably, evidence for linkage to HbA_{1c} on chromosome 18 at 9 cM was quite strong (LOD = 3.07, $P = 0.00008$) and deserving of further investigation. While evidence for linkage of more than one glucose trait in the same region was regarded as encouraging, it should be noted that these traits were moderately correlated with each other. For example, the correlation coefficient was 0.87 between glucose 120 and 150, 0.87 between glucose 150 and 180, and 0.76 between glucose 120 and 180. (For correlation coefficients between other glucose traits, see http://medschool.umaryland.edu/Endocrinology/Amish/amlinkindex.html.)

By conditioning on evidence for linkage previously detected in one or more regions of the initial genome scan, we may increase the power to strengthen linkages in other regions and to detect other diabetes-related loci. Indeed, conditioning on the 4q28 region increased evidence of linkage to type 2 diabetes/IGH on both chromosomes 1 and 14. These analyses extended evidence of linkage into the 1q24-q31 region with a LOD of 2.60 at 204 cM. As seen in Fig. 2, this region still overlaps with evidence of diabetes linkage reported in other populations. While conditioning on evidence of linkage at 4q28, the LOD score for type 2 diabetes/IGH on chromosome 14 increased to 1.75 in the same region, providing further evidence for linkage to type 2 diabetes (LOD = 3.48). By contrast, no interaction occurred between 1q23 and 14q11, suggesting that for the type 2 diabetes/IGH trait, the genetic effects from loci on 1q23 and 14q11 are independent of one another.

Our region of linkage on chromosome 14q11 showed a strong linkage signal for diabetes (LOD = 3.48). This region contains a cluster of immune function genes, e.g., interleukin 17E 18 (IL17E), interferon-stimulated gene transcription factor 3 (ISGF3), cytotoxic T-cell–associated serine esterase-1 (CTLA1), T-cell antigen receptors α (TCRA) and δ (TCRD), and the leukotriene b4 receptor (LTB4R). Although none of these genes have been previously considered as candidate genes for type 2 diabetes, there is an increasing body of evidence that the inflammatory process may be primarily involved in type 2 diabetes pathogenesis (38,39). In addition, there are several genes in this region that may influence glucose and/or lipid metabolism through their effects on signal transduction or gene transcription, including protein kinase C μ (PRKCM), adenylate cyclase-4 (ADCY4), CCAAT/enhancer-binding protein ϵ (CEBPE), and the estrogen receptor-2 (ESR2).

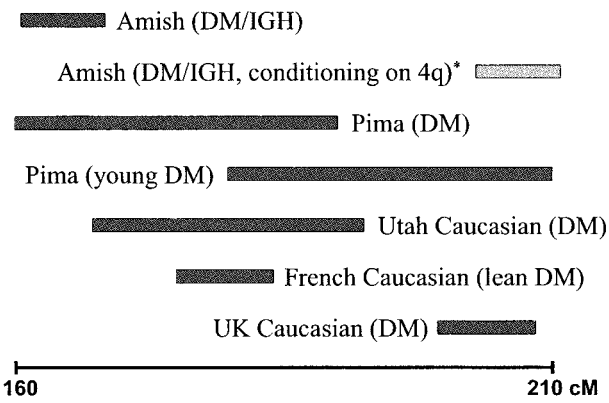

FIG. 2. Regions showing linkage signals to diabetes and its related traits on chromosome 1q from five populations (traits shown in parentheses). *Results from analysis conditioning on linkage signal to type 2 diabetes/IGH on chromosome 4q28. DM, diabetes mellitus.

We observed modest (LOD = 1.28–2.30) evidence for linkage to four traits (diabetes and glucose 120, 150, and 180) on chromosome 1p31. Although no other groups have reported evidence of linkage to diabetes on 1p, Norman et al. (40) observed a LOD score of 2.6 for 24-h respiratory quotient between D1S1728 and D1S551 (~113 cM on the Amish map), and Thompson et al. (39) found linkage to acute insulin release at D1S198 (~98 cM on the Amish map). The ratio of carbohydrate to fat oxidation is a predictor of weight gain, and obesity is a known risk factor for type 2 diabetes. Similarly, decreased acute insulin release is an early indicator of glucose intolerance and type 2 diabetes. This region is known to harbor the leptin receptor.

Linkage signals for diabetes to the region of chromosome 1q21-q24 have been reported in four other populations (Fig. 2). In a genome scan conducted in the Pima Indians (9), allele sharing of sib-pairs concordant (defined as onset before age 45 years) and discordant (defined as nondiabetic at age 45 years), revealed evidence for linkage of diabetes to D1S1677 on chromosome 1q (LOD = 2.5, at ~185 cM on the Amish map). Moreover, when 55 sib-pairs with age of diabetes onset of ≤25 years were analyzed separately in an affected sib-pair analysis, very strong evidence for linkage was detected near D1S2127 (LOD = 4.1, at ~208 cM on the Amish map). In Utah Caucasians (12), linkage to diabetes was observed in the region between CRP and APOA (~176 cM on the Amish map) using both parametric analysis (LOD = 4.30) and model-free affected sib-pair analysis (LOD = 2.96). More recently, in a French study (14), affected sib-pair analysis using 113 lean (BMI <27 kg/m^2) diabetic sib-pairs found a linkage peak near D1S484 (at 179 cM on the Amish map) with an LOD of 3.04. In British affected sib-pairs, Wiltshire et al. (20) observed evidence for linkage to diabetes on 1q24 near D1S2799, ~199 cM on the Amish map (LOD = 1.98 after saturation markers were placed).

It is often difficult to evaluate whether a putative gene underlying the linkage signal in one study (e.g., the Amish) represents the same gene as that detected by linkage in other studies (e.g., Pima Indians and Utah, French, and British Caucasians). Simulation studies have suggested

that even under an ideal scenario (i.e., sufficient power and identical phenotype definition, ascertainment strategy, pedigree structure, genetic map, genetic and environment heterogeneity, and statistical approach), difference in sampling alone may cause the variation in location estimates of the linkage peaks to be as wide as 20 cM (42–44). When we compared results from our linkage analysis with these four earlier studies showing linkage signals to diabetes on chromosome 1q (9,12,14,20), we found the genetic signals observed from these studies cluster in a 50-cM region on chromosome 1q21-q24 (Fig. 2). It is thus possible that these linkages in several populations may represent the same gene. Alternatively, there may be more than one type 2 diabetes susceptibility gene in this region.

Chromosome 1q21-q24 contains at least 133 known genes, 145 putative transcripts with homology to known genes, and as many as 393 other potentially expressed sequences (20). Several of these genes are potentially associated with lipid or glucose metabolism, including lamin A/C (LMNA) (45,46), phosphoprotein enriched in astrocytes 15 (PEA15) (47,48), potassium inwardly rectifying channel, subfamily J, member 9 (KCNJ9) (49), pre–B-cell leukemia transcription factor 1 (PBX1) (50), solute carrier family 19 (thiamine transporter), member 2 (SLC19A2) (51), retinoid X receptor γ (RXRG), insulin receptor–related receptor (INSRR), and others.

Several other regions identified in our study with LOD scores of ≥1.18 have been reported by other researchers. These include chromosomes 2p24-p23 (14), 4q24 (18,19), 5q22 (18), 8q24.2- q24.3 (20), 13q32-q33 (9), 17q24.1-q24.3 (18,40), and 18p11.32-p11.31 (12,18). By contrast, the linkages we observed in the Amish on chromosomes 2q14-q22, 2q37.3, 4p16.2- p15.2, 4q11.2, 7q11.2-q21.1, 15q26.3, 16q24.3, and 19p13.1-p12 appear to be novel.

In summary, we obtained significant evidence for linkage to type 2 diabetes with a novel locus on chromosome 14q11, as well as suggestive linkage signals to its related traits on several other novel chromosomal regions. Furthermore, we observed evidence for the existence of diabetes-related loci on chromosome 1q21-q24, a region previously linked to diabetes by several other groups. Our findings provide the rationale for positional cloning of type 2 diabetes susceptibility genes on chromosomes 1 and 14.

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