

A Large Set of Finnish Affected Sibling Pair Families With Type 2 Diabetes Suggests Susceptibility Loci on Chromosomes 6, 11, and 14

Kaisa Silander,¹ Laura J. Scott,² Timo T. Valle,³ Karen L. Mohlke,¹ Heather M. Stringham,² Kerry R. Wiles,¹ William L. Duren,² Kimberly F. Doheny,⁴ Elizabeth W. Pugh,⁴ Peter Chines,¹ Narisu Narisu,¹ Peggy P. White,² Tasha E. Fingerlin,² Anne U. Jackson,² Chun Li,² Soumitra Ghosh,¹ Victoria L. Magnuson,¹ Kimberly Colby,¹ Michael R. Erdos,¹ Jason E. Hill,¹ Pablo Hollstein,¹ Kathleen M. Humphreys,¹ Roshni A. Kasad,¹ Jessica Lambert,¹ Konstantinos N. Lazaridis,¹ George Lin,¹ Anabelle Morales-Mena,¹ Kristin Patzkowski,¹ Carrie Pfahl,¹ Rachel Porter,¹ David Rha,¹ Leonid Segal,¹ Yong D. Suh,¹ Jason Tovar,¹ Arun Unni,¹ Christian Welch,¹ Julie A. Douglas,² Michael P. Epstein,² Elizabeth R. Hauser,² William Hagopian,⁵ Thomas A. Buchanan,⁶ Richard M. Watanabe,^{2,7} Richard N. Bergman,⁸ Jaakko Tuomilehto,^{3,9} Francis S. Collins,¹ and Michael Boehnke²

The aim of the Finland-United States Investigation of NIDDM Genetics (FUSION) study is to identify genes that predispose to type 2 diabetes or are responsible for variability in diabetes-related traits via a positional cloning and positional candidate gene approach. In a previously published genome-wide scan of 478 Finnish affected sibling pair (ASP) families (FUSION 1), the strongest linkage results were on chromosomes 20 and 11. We now report a second genome-wide scan using an independent set of 242 Finnish ASP families (FUSION 2), a detailed analysis of the combined set of 737 FUSION 1 + 2 families (495 updated FUSION 1 families), and fine mapping of the regions of chromosomes 11 and 20. The strongest FUSION 2 linkage results were on chromosomes 6 (maximum logarithm of odds score [MLS] = 2.30 at 95 cM) and 14 (MLS = 1.80 at 57 cM). For the combined FUSION 1 + 2 families, three results were particularly notable: chromosome 11 (MLS = 2.98 at 82 cM), chromosome 14 (MLS = 2.74 at 58 cM), and

chromosome 6 (MLS = 2.66 at 96 cM). We obtained smaller FUSION 1 + 2 MLSs on chromosomes X (MLS = 1.27 at 152 cM) and 20p (MLS = 1.21 at 20 cM). Among the 10 regions that showed nominally significant evidence for linkage in FUSION 1, four (on chromosomes 6, 11, 14, and X) also showed evidence for linkage in FUSION 2 and stronger evidence for linkage in the combined FUSION 1 + 2 sample. *Diabetes* 53:821–829, 2004

There is substantial evidence of a major genetic component in the etiology of type 2 diabetes (1,2). Positional cloning is one approach for identifying type 2 diabetes susceptibility genes and is currently being pursued using a variety of study designs in multiple racial and ethnic groups (3). The potential power of this approach was recently demonstrated in a study of Mexican-American type 2 diabetic families. Haplotypes within the calpain 10 gene, encoding a cysteine protease, were found to be associated with the disease in this population (4). Studies in some populations have supported the association between calpain 10 gene variants and type 2 diabetes and/or related traits (e.g., 5,6). However, indicative of the complexity underlying type 2 diabetes, calpain 10 variants have not been found to contribute significantly to disease susceptibility in other study populations (e.g., 7,8), including ours (9).

In the Finland-United States Investigation of NIDDM Genetics (FUSION) study, we have focused on affected sibling pair (ASP) families from Finland, where the prevalence of type 2 diabetes is ~5% in the middle-aged population (10) and approaches 25–30% in the elderly (11). We previously reported results from a genome-wide scan in which we studied 719 ASPs from 478 families (FUSION 1) (12,13). Our strongest FUSION 1 linkage results before our current fine mapping were on chromosomes 11 (maximum logarithm of odds [LOD] score [MLS] of 1.75 at 84 cM) and 20 (MLSs of 1.99, 2.04, and 2.15 at 18, 57, and 70

From the ¹Genome Technology Branch, National Human Genome Research Institute, Bethesda, Maryland; the ²Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, Michigan; the ³Diabetes and Genetic Epidemiology Unit, Department of Epidemiology and Health Promotion, and Department of Biochemistry, National Public Health Institute, Helsinki, Finland; the ⁴Center for Inherited Disease Research, Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland; the ⁵Pacific Northwest Research Institute, Seattle, Washington; the ⁶Department of Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California; the ⁷Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California; the ⁸Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California; and the ⁹Department of Public Health, University of Helsinki, Helsinki, Finland.

Address correspondence and reprint requests to Michael Boehnke, PhD, Department of Biostatistics, School of Public Health, University of Michigan, 1420 Washington Heights, Ann Arbor, Michigan 48109-2029. E-mail: boehnke@umich.edu.

Received for publication 21 July 2003 and accepted in revised form 4 December 2003.

ASP, affected sibling pair; CEPH, Centre d'Etude du Polymorphisme Humain; CIDR, Center for Inherited Disease Research; FUSION, Finland-United States Investigation of NIDDM Genetics; IBD, identity by descent; LOD, logarithm of odds; MLS, maximum LOD score; QTL, quantitative trait locus; WHO, World Health Organization.

© 2004 by the American Diabetes Association.

TABLE 1
Counts of families and affected individuals used in the affection status linkage analysis

	Total no. of families	No. of families with <i>n</i> affected individuals/family						Total no. of affected subjects (M/F)
		<i>n</i> = 2	<i>n</i> = 3	<i>n</i> = 4	<i>n</i> = 5	<i>n</i> = 6	<i>n</i> = 8	
FUSION 1	495	378	103	10	2	1	1	1,129 (560/569)
FUSION 2	242	168	56	14	4	0	0	580 (304/276)
FUSION 1 + 2	737	546	159	24	6	1	1	1,709 (864/845)

cM, respectively). We also observed nominally significant evidence of linkage on chromosomes 2, 6, and 10 (12,13). In the current study, we present results of a genome-wide affection status linkage scan using an independent set of 242 ASP families (FUSION 2) and the combined FUSION 1 + 2 families (495 updated FUSION 1 families). In regions of interest, we also performed additional fine mapping in FUSION 1 + 2 families. In addition, we present ordered subsets linkage analysis results in which we stratify the families by diabetes-related traits. For four loci, on chromosomes 6, 11, 14, and X, we found overlapping, nominally significant (MLS >0.59) linkage signals in the FUSION 1 and FUSION 2 sets of families, with stronger evidence for linkage in the combined FUSION 1 + 2 set of families.

RESEARCH DESIGN AND METHODS

For recruitment of the FUSION 2 families, we followed essentially the same approach as for the FUSION 1 family recruitment (14), except that no spouses or offspring were recruited. We sent screening questionnaires to 7,856 patients who were hospitalized with type 2 diabetes in 1994–1995 and who were living relatively close to one of the FUSION study clinics. A total of 4,009 individuals returned the screening questionnaire. In addition, potential families were identified from among those who answered the screening questionnaire during the FUSION 1 patient recruitment phase and who were not invited to participate in the FUSION 1 study because they were not living close to one of the FUSION study clinics.

To be eligible to participate in the current study, index case subjects were required to have disease onset between the ages of 35 and 60 years, at least one living affected sibling, no first-degree relatives with type 1 diabetes, and at least one unaffected parent as reported by the index case subject. Diabetes was defined as currently taking medication for diabetes or medical record information conforming to World Health Organization (WHO) criteria (15). Based on these criteria, we invited 275 families to participate in the study, including a total of 859 index case subjects and siblings and 18 parents. Informed consent was obtained from each study participant, and the study protocol was approved by the ethics committee or institutional review board in each of the participating centers.

Each participant was invited for a single clinical visit. At this visit, we collected information on family and medical history. We also obtained information on relevant quantitative traits, including anthropometric measurements, fasting plasma glucose, fasting plasma insulin, fasting serum C-peptide, fasting serum lipids, and blood pressure, as previously described (14). GAD antibody and fasting serum C-peptide measurements were used in conjunction with insulin treatment history to identify individuals with probable late-onset

type 1 diabetes (14). An oral glucose tolerance test conforming to WHO criteria was performed to confirm the diagnosis of diabetes for subjects with a prior diabetes diagnosis but no antidiabetic medication and a fasting plasma glucose value <7.0 mmol/l. Because direct measures of insulin resistance and insulin secretion were not made in this sample, we calculated two empirical indexes of insulin secretion (IR), insulin based (IR_i; fasting insulin/fasting glucose) and C-peptide based (IR_C; fasting C-peptide/fasting glucose), and one empirical index of insulin sensitivity, S_{1(EST)} (1/[fasting glucose × fasting insulin]) (13,16). We also collected a blood sample for DNA isolation. Individuals who did not attend the clinical visit went to their local health clinic to donate a blood sample for DNA isolation. In contrast to FUSION 1, urine samples for urinary albumin-to-creatinine ratio measurements (14) were not collected. We determined the geographic origin of all FUSION families based on the birthplace (historical province) of each individual, his/her parents, and his/her grandparents.

We excluded from all analyses FUSION 2 families in which an affected individual had a first-degree relative with possible type 1 diabetes or maternally inherited diabetes and deafness, and we excluded from linkage analysis families in which we were unable to recruit and genotype at least two affected siblings. We also identified and excluded from analysis one member from each pair of monozygotic twins. Putative half-siblings were identified based on their genotype data (17), and they were included as such in subsequent analyses. After exclusions, the FUSION 2 set used for linkage analysis included 242 families, in which 580 individuals had confirmed type 2 diabetes (Table 1). The affection status of individuals from the FUSION 1 analysis sample (13) was updated after a review of the medical records, and half-siblings were added to the analyses. The current FUSION 1 sample used for linkage analysis includes 495 families with 1,129 affected individuals (Table 1).

Genotyping. For the 275 FUSION 2 families (before exclusions), a genome-wide scan was performed at the Center for Inherited Disease Research (CIDR). The marker set used was a modification of the CHLC version 9 set, and it was comprised of 392 microsatellite markers with an average marker heterozygosity of 0.76 and an average marker spacing of 9 cM. PCR products were sized on an ABI 377XL sequencer. Detailed information on laboratory methods and markers can be found online at <http://www.cidr.jhmi.edu>. A total of 433,944 genotypes were produced for 1,107 samples. The genotype replication rate, based on 18,599 paired genotypes from blinded duplicate samples, was 99.88%. The overall missing data rate was 4.6%. The FUSION 1 genome scan was carried out at the National Human Genome Research Institute, and it included 408 microsatellite markers with an average density of 8 cM (13). A total of 34 markers were typed in common between the original FUSION 1 and FUSION 2 genome scans.

After the two genome scans, an additional 227 microsatellite markers (144 for FUSION 1 and 159 for FUSION 2) were typed for gap closing and for fine mapping of regions of interest on chromosomes 6, 11, 14, 20, and X. The regions of fine mapping, the number of markers typed, and the current average marker densities are summarized in Table 2. A total of 22 new markers typed on chromosomes 11 and 20 were identified from genomic sequence using the program Sputnik (available online at <http://rast.abajian.com/sputnik>). Primer

TABLE 2
Fine mapping of selected regions using microsatellite markers

Chromosome	Flanking markers	Region length (cM)	Total no. of markers		Average marker density (cM)	
			FUSION 1	FUSION 2	FUSION 1	FUSION 2
6	D6S294–D6S409	61	30	28	2.1	2.3
11	D11S1314–D11S1317	25	44	42	0.6	0.6
14	D14S599–D14S258	32	8	10	4.6	3.6
20	D20S103–D20S173	98	91	55	1.1	1.8
X	DXS8088–DXS1108	70	16	18	4.7	4.1

TABLE 3
Comparison of the clinical characteristics of affected individuals in the index case generation in FUSION 1 and FUSION 2

Trait	FUSION 1	FUSION 2	<i>P</i> value
Age at clinical examination (years)	64.4 ± 8.3	65.1 ± 8.5	0.17
Age at diabetes diagnosis (years)	52.1 ± 9.0	53.5 ± 9.8	0.007
Duration of diabetes (years)	12.3 ± 7.4	11.7 ± 7.8	0.15
BMI (kg/m ²)	29.8 ± 4.8	29.7 ± 4.7	0.91
Waist-to-hip ratio	0.938 ± 0.079	0.937 ± 0.073	0.16
Fasting plasma glucose (mmol/l)	10.4 ± 3.4	9.1 ± 2.8	<0.0001
Fasting plasma insulin (pmol/l)	113.7 ± 83.0	108.1 ± 84.9	0.10
Fasting serum C-peptide (nmol/l)	1.59 ± 0.98	2.39 ± 1.50	<0.0001
Empirical insulin sensitivity (×10 ³)	1.75 ± 3.04	2.11 ± 3.27	<0.0001
Empirical insulin secretion (insulin)	11.9 ± 8.9	12.9 ± 14.2	0.50
Empirical insulin secretion (C-peptide)	0.165 ± 0.112	0.273 ± 0.179	<0.0001
Serum total cholesterol (mmol/l)	5.75 ± 1.20	5.49 ± 1.02	0.0008
Serum HDL cholesterol (mmol/l)	1.09 ± 0.30	1.17 ± 0.31	<0.0001
Serum LDL cholesterol (mmol/l)	3.62 ± 0.97	3.43 ± 0.85	0.006
HDL cholesterol-to-total cholesterol ratio	0.195 ± 0.061	0.219 ± 0.064	<0.0001
Serum triglycerides (mmol/l)	2.45 ± 2.04	2.07 ± 1.47	<0.0001
Diastolic blood pressure (mmHg)	84.4 ± 10.7	84.9 ± 10.2	0.46
Systolic blood pressure (mmHg)	152.3 ± 22.5	152.0 ± 20.7	0.97

Data are means ± SD.

and heterozygosity information for these 22 markers are detailed in the APPENDIX. Primer information for all microsatellite markers used and described in the current study is available upon request (contact P.C. by e-mail at pchines@nhgri.nih.gov). Genotyping procedures have been described elsewhere (14,18). The only difference was that approximately halfway through marker genotyping, we switched to using a capillary sequencing instrument (3100 genetic analyzer; Applied Biosystems, Foster City, CA). For markers that were previously typed on FUSION 1 or FUSION 2 families only, we compared allele sizes between the two sets of families by retyping ~90 individuals from the original set of families along with the new set of families. Our current cumulative genotyping replication rate for the FUSION 1 genome scan and FUSION 1 + 2 fine mapping is 99.93%, based on 38 inconsistencies in 27,353 paired genotypes from blind duplicate samples.

Before mapping and linkage analysis, we used PedCheck (19) to identify non-Mendelian inheritance, and we used Relpair (17,20) to detect possible pedigree errors. In the densely genotyped regions of chromosomes 11 and 20, we also applied Sibmed (21) to identify likely genotype errors or microsatellite mutations. Removing genotypes flagged by Sibmed did not result in any significant changes to the linkage results. Observed allele frequencies for all markers included in the linkage analyses were consistent with expected frequencies under Hardy-Weinberg equilibrium.

Statistical analysis. To detect potential sampling differences, we compared the clinical characteristics between FUSION 1 and FUSION 2 affected individuals from the index case generation, who were diagnosed with type 2 diabetes at the stage of patient recruitment and were used in the linkage analysis. Age and diabetes duration variables were available for almost all affected siblings. Phenotype information for other traits was available for 1,009–1,087 FUSION 1 and 359–425 FUSION 2 affected siblings. Phenotype comparisons were performed using generalized estimating equation-based methods (22) to account for the correlation among related siblings. All traits were statistically transformed to approximate univariate normality and, when appropriate, phenotype values were adjusted for age and/or sex.

To compare and combine results from our two genome scans, we constructed genetic maps containing all FUSION 1 and FUSION 2 markers with MultiMap (23), which uses CRI-MAP (P. Green, K. Falls, S. Crooks, unpublished documentation for CRI-MAP version 2.4) as its analysis engine. The 219 families used for mapping included 211 FUSION 1 nuclear families with the spouse and offspring of the index case subject or sibling sampled and 8 Centre d'Etude du Polymorphisme Humain (CEPH) families, using the cleaned CEPH dataset (24) when possible.

We carried out affection status linkage analysis on FUSION 1 and FUSION 2 families separately and together. For this analysis, we assessed identity-by-descent (IBD) allele sharing between all pairs of affected individuals within a family, using the S_{pairs} statistic of Whittemore and Halpern (25), which sums pairwise IBD sharing counts among all affected relatives within the family, and the likelihood parameterization of Kong and Cox (26), as programmed in Genehunter-Plus (26,27). We weighted each family-specific statistic by the square root of 1 less than the number of affected individuals in the family. *P* values were calculated based on large sample theory, where

$$Z = \sqrt{2 \ln(10) \times LOD}$$

was approximated by a standard normal variable under the null hypothesis of no linkage (26). In this study, we also carried out X chromosome linkage analysis, which was not previously performed for FUSION 1 (13).

To reduce genetic heterogeneity, we carried out ordered subsets linkage analysis based on diabetes-related quantitative traits in the affected individuals. In this analysis, we ranked families based on the mean value of a diabetes-related quantitative trait in the affected individuals, performed linkage analysis by adding one family at a time in rank order, and selected the subset of families that gave the largest MLS (13,28). The variables analyzed included age at disease diagnosis; BMI; waist-to-hip ratio; systolic and diastolic blood pressure; and fasting levels of glucose, insulin, C-peptide, empirical insulin sensitivity, empirical insulin secretion (IR_i and IR_c), total cholesterol, HDL cholesterol, LDL cholesterol, HDL cholesterol-to-total cholesterol ratio, and triglycerides. With the exception of age at disease diagnosis, the ordered subsets analysis for FUSION 2 was based on a reduced set of 203 families (495 affected individuals), for which phenotype data were available for at least one person in the family. For these analyses, we excluded trait values for individuals who took medications that may affect those traits on the day of examination. Lipid variables were not previously analyzed for FUSION 1 (13). Chromosome-wide empirical *P* values for the resulting ordered subsets LOD scores were determined using a permutation test framework (13,28). Ordered subsets linkage analysis results are reported for FUSION 1 + 2 if they are in a region of primary interest, are significant at the level of $P \leq 0.01$, and increase the MLS by ≥ 1.0 .

RESULTS

Clinical characteristics of affected individuals. The clinical characteristics of the FUSION 1 and FUSION 2 study samples are shown in Table 3. Results are based on all affected individuals from the index case generation who were diagnosed with type 2 diabetes at the stage of patient recruitment and were included in the linkage analysis. The two sets of affected individuals were similar in terms of age at examination, disease duration, anthropometric measurements, and blood pressure. However, FUSION 2 patients were somewhat less severely affected, having on average later age of disease diagnosis, more favorable serum lipid profile, lower fasting glucose, higher insulin secretion, and less insulin resistance compared with the patients in FUSION 1. There were no major differences in the geographic origins of the FUSION 1 and FUSION 2 families (data not shown).

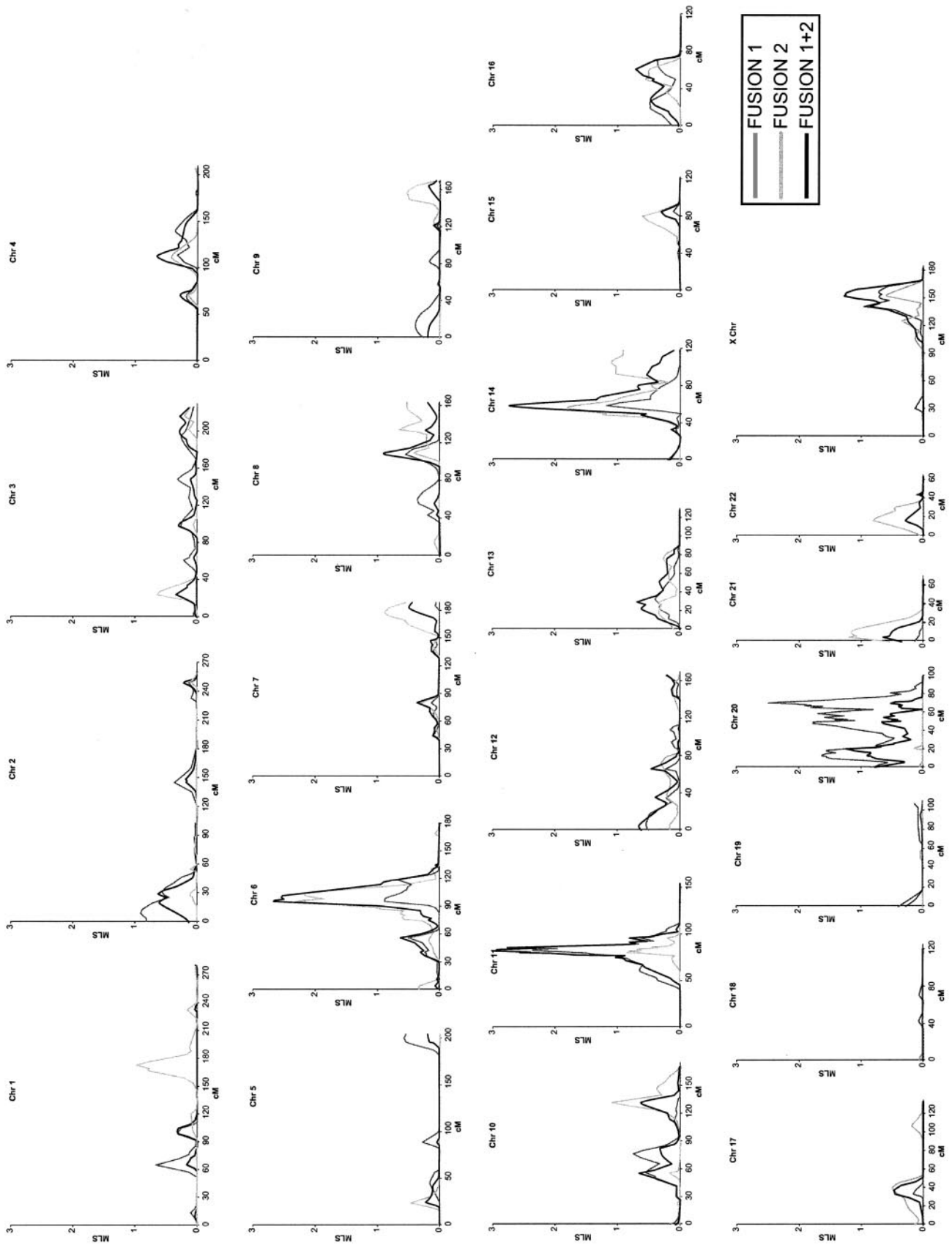


FIG. 1. Genome-wide affection status linkage analysis results for FUSION 1 (495 families), FUSION 2 (242 families), and FUSION 1 + 2 (737 families). Chr, chromosome.

TABLE 4
Multipoint affection status linkage analysis: nominally significant results*

Chromosome	FUSION 1			FUSION 2			FUSION 1 + 2		
	Position (cM)	MLS	Nearest marker	Position (cM)	MLS	Nearest marker	Position (cM)	MLS	Nearest marker
1	65	0.67	D1S255	173	0.99	D1S1677			
2	10	0.90	D2S319				29	0.62	D2S131
3				24	0.64	D3S3691			
4							112	0.65	D4S2623
6	97	0.88	D6S1546	95	2.30	D6S1546	96	2.66	D6S1546
7				178	0.87	D7S3058			
8				134	0.65	D8S1128	109	0.90	D8S1132
10	77	0.74	D10S1652				56	0.67	D10S208
				132	1.11	D10S1237			
11	86	2.56	D11S1365	80	0.84	D11S937	82	2.98	D11S4172
12							5	0.65	D12S372
13							30	0.68	D13S1493
14	59	1.18	D14S290	57	1.80	D14S592	58	2.74	D14S290
15				80	0.61	D15S655			
16							61	0.71	D16S3253
20	13	1.63	D20S97				20	1.21	D20S892
	49	1.77	D20S184				49	0.65	D20S184
	70	2.48	D20S197						
21				5	1.21	D21S1432	5	0.64	D21S1432
22				16	0.79	D22S345			
X	141	0.95	DXS8072	154	0.60	DXS1227	152	1.27	DXS1205

*Showing all results with nominal evidence of linkage ($P = 0.05$, $MLS = 0.59$). Results in which the evidence for linkage is consistent between FUSION 1, FUSION 2, and FUSION 1 + 2 are in bold.

Genome scan results. Fig. 1 presents the affection status linkage genome scan results for FUSION 1, FUSION 2, and FUSION 1 + 2 across all chromosomes; Table 4 summarizes all nominally significant results ($P \leq 0.05$, $MLS \geq 0.59$). The results shown for chromosomes 6, 11, 14, 20, and X include additional fine mapping in regions of interest (Table 2).

Chromosome 6q16.3-q22.31. The strongest evidence for linkage in the FUSION 2 genome scan was obtained on chromosome 6 ($MLS = 2.30$ at 95 cM in the FUSION map). FUSION 1 families had nominally significant evidence for linkage ($MLS = 0.88$ at 97 cM), whereas the FUSION 1 + 2 MLS was 2.66 ($P = 0.00023$) at 96 cM (1-LOD support interval of 92–108 cM) (Fig. 2). Using the ordered subsets approach, the 104 FUSION 1 + 2 families with highest HDL cholesterol-to-total cholesterol ratios gave an MLS of 7.92 at 78 cM (chromosome-wide P value of 0.00003). Other interesting FUSION 1 + 2 ordered subsets results on chromosome 6 were obtained for low total cholesterol ($MLS = 5.71$ at 102 cM for 291 families) and low LDL cholesterol ($MLS = 5.10$ at 96 cM for 344 families) (Fig. 2). Among the 104 families in the high HDL cholesterol-to-total cholesterol ratio subset, 87 and 79 families are also in the low LDL cholesterol and low total cholesterol subsets, respectively.

Chromosome 11q13.5-q14.2. In our original FUSION 1 genome scan, chromosome 11 had the fourth best MLS of 1.75 at 84 cM (13). After fine mapping, the MLS for FUSION 1 has increased to 2.56 at 86 cM and is now the strongest linkage signal in the FUSION 1 families. FUSION 2 provided confirmation of this result, albeit weaker than in FUSION 1 (MLS of 0.84 at 80 cM). The FUSION 1 + 2 MLS of 2.98 ($P = 0.00011$) at 82 cM (1-LOD support interval

79–87 cM) was the highest affection status linkage LOD score for the combined FUSION 1 + 2 genome scan.

Chromosome 14q23.1-q24.1. Chromosome 14 had the second highest MLS for the FUSION 2 genome scan, 1.80 at 57 cM. In the original FUSION 1 genome scan, this same region was sparsely typed with markers, and it did not show nominally significant evidence for linkage. Typing six additional markers in the region 33–65 cM resulted in an MLS of 1.18 at 59 cM for FUSION 1, and the combined FUSION 1 + 2 analysis yielded an MLS of 2.74 ($P = 0.00019$) at 58 cM. The 1-LOD support interval was 54–64 cM for FUSION 1 + 2.

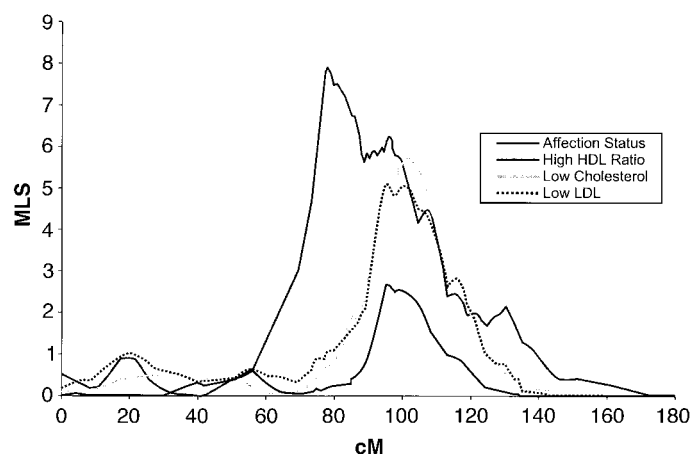


FIG. 2. Chromosome 6: Affection status linkage analysis and ordered subsets linkage analysis results for FUSION 1 + 2. Ordered subsets results shown are for 104 families with highest HDL cholesterol-to-total cholesterol ratio (HDL ratio), 291 families with lowest total cholesterol, and 344 families with lowest LDL cholesterol.

Chromosome 20. In our original FUSION 1 genome scan, three of the four strongest linkage signals were located on chromosome 20 (12,13), with MLSs of ~ 2 on the p arm, the q arm, and near the centromere. Subsequently, we typed markers in FUSION 1 at < 1 cM density in the three regions of strongest linkage evidence. Currently, our MLSs for FUSION 1 on chromosome 20 are 1.63, 1.77, and 2.48 ($P = 0.00036$) at map positions 13, 49, and 70 cM, respectively, with 1-LOD support intervals of 6–26 cM, 38–63 cM, and 64–74 cM. The FUSION 2 families provided no evidence for linkage on chromosome 20, and the FUSION 1 + 2 MLSs were 1.21, 0.65, and 0.51 at 20, 49, and 70 cM, respectively.

Chromosome Xq23-q27.3. On chromosome X, the MLSs for FUSION 1, FUSION 2, and FUSION 1 + 2 were 0.95, 0.60, and 1.27 at 141, 154, and 152 cM, respectively. The 1-LOD support interval for FUSION 1 + 2 was 129–167 cM.

DISCUSSION

In the present study, we have compared the results of genome-wide scans using two independent samples of Finnish families with type 2 diabetes. By carrying out a genome scan on a second set of ASP families from the same population as our initial sample, we aimed to confirm linkage results from the original genome scan, to identify additional loci involved in type 2 diabetes susceptibility, and to obtain a more powerful sample by combining the two sets of families. In our FUSION 2 genome scan of 242 families, we had nominally significant evidence for linkage (MLS > 0.59) in 12 regions, 4 of which overlapped the 10 regions with nominally significant evidence for linkage in our current FUSION 1 analysis. This overlap is more than expected by chance alone. If we think of breaking the genome into 120 bins of ~ 30 cM each (larger than the width of most of our linkage signals), the probability of at least this degree of overlap in the peaks between the two studies can be estimated from the hypergeometric distribution as 0.009. If we choose 180 bins of ~ 20 cM each (larger than the width of most of our linkage peaks), the probability that this overlap occurred by chance is even lower, at 0.002. For each of the overlapping regions (chromosomes 6, 11, 14, and X), the evidence for linkage in the combined FUSION 1 + 2 set was stronger than for either sample alone. The overlap and strengthening of linkage signals is encouraging.

The differences in linkage results between FUSION 1 and 2, notably on chromosome 20, are likely primarily due to the modest effect of each gene in the complex and genetically heterogeneous etiology of type 2 diabetes, combined with random variability between the samples and the smaller size of FUSION 2. Simulations using the ASP linkage program Siblink with an additive model (29), using the chromosome 20 FUSION 2 marker data and 280 ASPs (corresponding to the FUSION 2 sample size with $s=1$ weighting), predict 60% power to detect a LOD score of 0.59 and 43% power to detect a LOD score of 1.0 for $\lambda_s = 1.2$, the value suggested by our FUSION 1 data. Furthermore, despite similar recruitment strategies in FUSION 1 and 2, FUSION 2 case subjects appear to be slightly less severely affected. They were older at the age of diagnosis, had lower fasting glucose values, and had better lipid profiles. It is thus possible that the proportion of type 2 diabetes due to a specific genetic locus varies between

FUSION 1 and 2. Finally, our FUSION 1 chromosome 20 linkage results could also comprise a set of false positives. This last explanation seems unlikely given the independent reports of linkage on chromosome 20 in other study populations (e.g., 30–35).

Our combined genome scan of 737 ASP families is one of the largest of its kind. Simulations using Siblink with an additive model (35) predict 65% power to detect even genes with modest effect ($\lambda_s = 1.2$) with an MLS > 1 with approximately our sample size. In the FUSION 1 + 2 genome scan, we identified three loci with suggestive evidence for linkage on chromosomes 11 (MLS = 2.98), 14 (MLS = 2.74), and 6 (MLS = 2.66). An MLS > 2 is expected to occur at random only once in a genome scan, even given complete IBD information (36).

The evidence for linkage on chromosome 6 from affection status linkage, ordered subsets linkage (Fig. 2), and quantitative trait locus (QTL) linkage analyses (16) extends over a ~ 70 -cM region, from 70 to 140 cM. Evidence for affection status linkage (MLS = 2.66 at 96 cM) and for linkage in the high HDL cholesterol-to-total cholesterol ratio ordered subset (MLS = 7.92 at 78 cM) in FUSION 1 + 2 is concentrated from 70 to 110 cM. Positive findings from QTL linkage analysis for fasting insulin (MLS = 2.64) and IR_1 (MLS = 2.60) in FUSION 1 unaffected individuals are both more distal at 127 and 128 cM, respectively (updated from 16). This broad set of results could indicate the presence of more than one diabetes susceptibility locus on this chromosome.

A study of 27 large Mexican-American families reported QTL MLSs of 4.1 for fasting insulin and 3.5 for insulin sensitivity and a bivariate MLS of 5.4 for fasting insulin and leptin, all at ~ 130 cM on the FUSION map (37). There are several other overlapping results previously summarized (13). Recent results from other groups include a two-point affection status LOD of 1.97 at 117 cM on the FUSION map in 573 British/Irish ASP families (38), an MLS of ~ 1.8 at ~ 125 cM for impaired glucose homeostasis in African-American families (39), and a QTL MLS of ~ 1.5 at 110 cM for abdominal subcutaneous fat in European Americans (40). A possible candidate gene in this region is ectonucleotide pyrophosphatase/phosphodiesterase 1 (OMIM 173335, also called plasma-cell membrane differentiation antigen-1 [PC1]), in which the Gln121 allele has been shown by some to be associated with insulin resistance (41). Considering the close link between obesity and type 2 diabetes, another candidate gene is single-minded *Drosophila* homolog 1 (SIM1; OMIM 603128); haploinsufficiency of SIM1 is associated with severe obesity in humans (42) and in mice (43).

Chromosome 11 at 82 cM has the strongest evidence for linkage in the combined FUSION 1 + 2 sample (MLS = 2.98). In our QTL linkage scan of affected individuals, for FUSION 2 we observed an MLS of 1.62 for fasting glucose at 79 cM, and for FUSION 1 we observed MLSs of 1.93 for fasting insulin and 2.32 for IR_1 at 91 and 90 cM, respectively (data not shown). Each of these rather broad peaks overlaps those for the affection status linkage analysis. Other findings overlapping with our linkage peak were summarized previously (13). Possible candidate genes in the region include calpain 5 (OMIM 602537), belonging to the same family of cysteine proteases as the type 2 diabetes susceptibility gene calpain 10 (4), and the thyroid

hormone-responsive Spot 14 homolog gene (*THRSP*; OMIM 601926), which is expressed in human liver and adipocytes (44), activates genes involved in lipogenesis, and is regulated by dietary and hormonal factors (45,46). It is interesting to note that a gene-poor region of 4 Mb is located in the middle of the 1-LOD support interval of 10 Mb, immediately downstream of the linkage peak at 82 cM.

For chromosome 14 at 58 cM, we obtained the second strongest evidence for linkage for FUSION 1 + 2 (MLS = 2.74). A possible candidate gene in the 54- to 64-cM region is eukaryotic translation initiation factor-2 α (*EIF2S1*; OMIM 603907), which is involved in the endoplasmic reticulum stress response and was shown to be essential for proper functioning of the liver and pancreas in maintaining glucose homeostasis in mice (47).

For the X chromosome region (MLS = 1.27 at 152 cM for FUSION 1 + 2), an overlapping result is from the Genetics of NIDDM (GENNID) study (39), which reported the largest MLS in a Caucasian genome scan of 2.99 at 140 cM on the FUSION map. Öhman et al. (48) reported an MLS of 3.48 in obese Finnish sibling pairs between 110 and 130 cM on the FUSION map. A possible candidate gene in the 129- to 167-cM region is bombesin-like receptor 3 (*BRS3*; OMIM 300107). Like *SIM1*, *BRS3* is expressed in the hypothalamic nuclei and may play a role in the regulation of energy balance and adiposity (49). Mice deficient for this gene develop mild obesity associated with hypertension and impaired glucose metabolism and hyperphagia (49).

In summary, we have identified four regions that show at least nominally significant evidence for linkage with type 2 diabetes in two independent sets of Finnish sibling pairs affected with type 2 diabetes. These regions, found on chromosomes 6, 11, 14, and X, all show stronger evidence for linkage in the combined FUSION 1 + 2 sample than for either sample alone. Data for the regions on chromosomes 6, 11, and 14 result in LOD scores >2.2.

APPENDIX

Markers identified from genomic sequence on chromosomes 11 and 20

Chromosome	Position (cM)	Marker	Heterozygosity	Primer1	Primer2	Size range (bp)
11	80.4	D11ms178	0.816	AGTCCCAGTACGGTTCACCTT	GATTGTGACAACCTTACCCCCAC	119-144
11	80.7	D11ms192	0.585	GTTTCTTCTGCAGTTAAAGGAC	GCTGACGTGTCTCACAGG	234-253
11	82.9	D11ms253	0.608	CACCTCAGCAGCCCAAGTGG	GTCACCTGCAAAAAAGAAGTACCC	251-283
11	83.4	D11ms256	0.586	CACTTCGCTAATTCCTTCTCTG	GGCCAGCTTGTCCATTTTCT	117-129
11	83.7	D11ms272	0.399	CATATATGTAACACAGTGCCAG	GATGAATCATTAAAGCAATAGTAG	165-185
11	84.2	D11ms278	0.681	GTGTCTCCCTGCTACAATGTT	GAGTCTACCATATGCCAAAC	146-167
11	84.3	D11ms279	0.717	TCACCATGACAGAAATTGAC	GCAGTATGTGTGCTTTGAAA	86-108
20	50.9	D20ms3	0.700	ATCAGGGGGCTAGAATGTTTCC	GTTTTGGAGGATGGAGTGTAAGA	274-290
20	50.9	D20ms5	0.755	AAAGCCCCTTTAGTAGTCAAAGTC	GATCTGACTTGATCAAATCCAGAG	193-205
20	51.5	D20ms31	0.877	CACTATAAGCAGTGGAGACCCACA	GTTTGCATATCTGTTTTCTGTTTAC	121-148
20	66.8	D20ms19	0.733	ACTCCAGGGTGTGGCCCT	GATTTCTCCCTGCACCAA	95-119
20	67.1	D20ms11	0.432	CTCTGGTTTTAGACAAGATGG	GAAAAGTCCCAAGTATCACAC	264-281
20	67.3	D20ms10	0.515	AATGCATGATTCTTTTCAGTACC	GTATGGTTATTATTCTTTTCAGTACC	204-227
20	67.3	D20ms32	0.334	GGTCAAGATGGCAGATGGGA	GCACTGGAGACATATGCCTTC	147-154
20	67.3	D20ms33	0.177	GTGGCAGGGAGATGGTGAAT	GCCTCTTCAGTCACCTCCCA	104-113
20	67.5	D20ms12	0.487	GGCAGGTGGAGATAAATTAT	GATGTATGACAGCAACGCC	110-165
20	68.1	D20ms36	0.727	CAAGCAGTCTCCACCTCA	GCCAAATGCCACTTGGTCCTC	224-243
20	68.4	D20ms21	0.737	ATTCTCCTGCCTCACTGTCTC	GACAAATCCCTCAGTATGTGGC	140-168
20	68.9	D20ms14	0.715	CATGGCACAGAAGCAAGTAG	GCCATCTCTAAAAAACAGTTATC	162-183
20	70.9	D20ms28	0.847	TGCTTCCCTTCTCCTGAGAAGTACA	GACAGACTGAAGTCAAGACCCTGCAT	222-263
20	70.9	D20ms29	0.801	GGTCTAGTCTTTGCAGAAAGAACA	GTCTTTCTACATGCACCTTGCACCCT	257-288
20	71.7	D20ms27	0.734	ATTGTAGAACCTAGGGAATATCAGC	GCCAGTAGCAGGTATGGATGCATGT	116-137

Through SNP fine mapping and candidate gene mutation screening, we are currently pursuing the regions with evidence for linkage on chromosomes 6, 11, 14, and 20q, which were strongly identified by our FUSION 1 or combined FUSION 1 + 2 genome scans, with the other regions targeted for future follow-up.

ACKNOWLEDGMENTS

The FUSION study was funded by intramural funds from National Human Genome Research Institute (NHGRI) project OH95-C-N030 and by National Institutes of Health Grants HG00376 and DK62370 to M.B. Genotyping services were provided by CIDR. CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, Contract Number N01-HG-65403. K.L.M. is the recipient of a Burroughs Wellcome Career Award in the Biomedical Sciences. K.S. and J.T. have been partially supported by the Academy of Finland (38387 and 46558 to J.T.). R.N.B. was supported by National Institutes of Health Grants DK27619 and DK29867. T.E.F., J.A.D., M.P.E., H.M.S., and W.L.D. have been partially supported by training grant HG00040 from the National Institutes of Health. At the time of these studies, R.M.W. was supported by DK09525 and a Career Development Award from the American Diabetes Association. K.N.L. has been supported by the Mayo Clinic and Foundation.

We are grateful to the Finnish people who volunteered to participate in our study. We gratefully acknowledge the hard work of the numerous field workers in Finland, as well as the work of Paula Nyholm, Jouko Sundvall, Tuula Tenkula, Liisa Toivanen, and Sanelma Vilkkilä. We thank Darryl Leja and Mike Cichanowski at NHGRI for their assistance in preparing the figures for this article.

APPENDIX

REFERENCES

- Rich S: Mapping genes in diabetes: genetic epidemiological perspective. *Diabetes* 39:1315–1319, 1990
- Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengard J, Kesaniemi YA: Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia* 35:1060–1067, 1992
- Stern MP: The search for type 2 diabetes susceptibility genes using whole-genome scans: an epidemiologist's perspective. *Diabetes Metab Res Rev* 18:106–113, 2002
- Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PEH, del Bosque-Plata L, Horikawa Y, Oda Y, Yoshiouchi I, Colilla S, Polonsky K, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hais CL, Bell GI: Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26:163–175, 2000
- Baier LJ, Permana PA, Yang X, Pratley RE, Hanson RL, Shen GQ, Mott D, Knowler WC, Cox NJ, Horikawa Y, Oda N, Bell GI, Bogardus C: A calpain-10 gene polymorphism is associated with reduced muscle mRNA and insulin resistance. *J Clin Invest* 106:R69–R73, 2000
- Evans JC, Frayling TM, Cassell PG, Saker PJ, Hitman GA, Walker M, Levy JC, O'Rahilly S, Rao PV, Bennett AJ, Jones EC, Menzel S, Prestwich P, Simecek N, Wishart M, Dhillion R, Fletcher C, Millward A, Demaine A, Wilkin T, Horikawa Y, Cox NJ, Bell GI, Ellard S, McCarthy MI, Hattersley AT: Studies of association between the gene for calpain-10 and type 2 diabetes mellitus in the United Kingdom. *Am J Hum Genet* 69:544–552, 2001
- Hegele RA, Harris SB, Zinman B, Hanley A, Cao H: Absence of association of type 2 diabetes with CAPN10 and PC-1 polymorphisms in Oji-Cree (Letter). *Diabetes Care* 24:1498–1499, 2001
- Tsai HJ, Sun G, Weeks DE, Kaushal R, Wolujewicz M, McGarvey ST, Tufa J, Viali S, Deka R: Type 2 diabetes and three calpain-10 gene polymorphisms in Samoans: no evidence of association. *Am J Hum Genet* 69:1236–1244, 2001
- Fingerlin TE, Erdos MR, Watanabe RM, Stringham HM, Mohlke KL, Silander K, Valle TT, Buchanan TA, Tuomilehto J, Bergman RN, Boehnke M, Collins FS: Variation in three single nucleotide polymorphisms in the calpain-10 gene not associated with type 2 diabetes in a large Finnish cohort. *Diabetes* 51:1644–1648, 2002
- Tuomilehto J, Korhonen HJ, Kartovaara L, Salomaa V, Stengard JH, Pitkanen M, Aro A, Javela K, Uusitupa M, Pitkanemi J: Prevalence of diabetes mellitus and impaired glucose tolerance in the middle-aged population of three areas in Finland. *Int J Epidemiol* 20:1010–1017, 1991
- Valle T, Tuomilehto J, Eriksson J: Epidemiology of NIDDM in Europids. In *International Textbook of Diabetes Mellitus*. 2nd ed. Alberti KGM, Zimmet P, DeFronzo RA, Keen H, Eds. West Sussex, U.K., John Wiley, 1997, p. 125–142
- Ghosh S, Watanabe RM, Hauser ER, Valle T, Magnuson VL, Erdos MR, Langefeld CD, Balow J Jr, Ally DS, Kohtamaki K, Chines P, Birznieks G, Kaleta HS, Musick A, Te C, Tannenbaum J, Eldridge W, Shapiro S, Martin C, Witt A, So A, Chang J, Shurtleff B, Porter R, Boehnke M, et al.: Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. *Proc Natl Acad Sci U S A* 96:2198–2203, 1999
- Ghosh S, Watanabe RM, Valle TT, Hauser ER, Magnuson VL, Langefeld CD, Ally DS, Mohlke KL, Silander K, Kohtamaki K, Chines P, Balow J Jr, Birznieks G, Chang J, Eldridge W, Erdos MR, Karanjawala ZE, Knapp JI, Kudelko K, Martin C, Morales-Mena A, Musick A, Musick T, Pfahl C, Porter R, Rayman JB: The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. I: an autosomal genome scan for genes that predispose to type 2 diabetes. *Am J Hum Genet* 67:1174–1185, 2000
- Valle T, Tuomilehto J, Bergman RN, Ghosh S, Hauser ER, Eriksson J, Nylund SJ, Kohtamaki K, Toivanen L, Vidgren G, Tuomilehto-Wolf E, Ehnholm C, Blaschak J, Langefeld CD, Watanabe RM, Magnuson V, Ally DS, Hagopian WA, Ross E, Buchanan TA, Collins F, Boehnke M: Mapping genes for NIDDM: design of the Finland-United States Investigation of NIDDM Genetics (FUSION) study. *Diabetes Care* 21:949–958, 1998
- World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
- Watanabe RM, Ghosh S, Langefeld CD, Valle TT, Hauser ER, Magnuson VL, Mohlke KL, Silander K, Ally DS, Chines P, Blaschak-Harvan J, Douglas JA, Duren WL, Epstein MP, Fingerlin TE, Kaleta HS, Lange EM, Li C, McEachin RC, Stringham HM, Trager E, White PP, Balow J Jr, Birznieks G, Chang J, Eldridge W: The Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) study. II: an autosomal genome scan for diabetes-related quantitative-trait loci. *Am J Hum Genet* 67:1186–1200, 2000
- Boehnke M, Cox NJ: Accurate inference of relationships in sib-pair linkage studies. *Am J Hum Genet* 61:423–429, 1997
- Ghosh S, Karanjawala ZE, Hauser ER, Ally D, Knapp JI, Rayman JB, Musick A, Tannenbaum J, Te C, Shapiro S, Eldridge W, Musick T, Martin C, Smith JR, Carpten JD, Brownstein MJ, Powell JI, Whiten R, Chines P, Nyland SJ, Magnuson VL, Boehnke M, Collins FS: Methods for precise sizing, automated binning of alleles, and reduction of error rates in large-scale genotyping using fluorescently labeled dinucleotide markers. *Genome Res* 7:165–178, 1997
- O'Connell JR, Weeks DE: PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63:259–266, 1998
- Epstein M, Duren WL, Boehnke M: Improved inference of relationships for pairs of individuals. *Am J Hum Genet* 67:1219–1231, 2000
- Douglas JA, Boehnke M, Lange K: A multipoint method for detecting genotyping errors and mutations in sibling-pair linkage data. *Am J Hum Genet* 66:1287–1297, 2000
- Liang KY, Zeger SL: Longitudinal data analysis using generalized linear models. *Biometrika* 73:13–22, 1986
- Matise TC, Perlin M, Chakravarti A: Automated construction of genetic linkage maps using an expert system (MultiMap): a human genome linkage map. *Nat Genet* 6:384–390, 1994
- Broman KW, Murray JC, Sheffield VC, White RL, Weber JL: Comprehensive human genetic maps: individual and sex-specific variation in recombination. *Am J Hum Genet* 63:861–869, 1998
- Whittemore AS, Halpern J: A class of tests for linkage using affected pedigree members. *Biometrics* 50:118–127, 1994
- Kong A, Cox NJ: Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet* 61:1179–1188, 1997
- Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES: Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58:1347–1363, 1996
- Hauser ER, Watanabe RM, Duren WL, Bass MP, Langefeld CD, Boehnke M: Ordered subset analysis in genetic linkage mapping of complex traits. *Genet Epidemiol*. In press
- Hauser ER, Boehnke M: Genetic linkage analysis of complex genetic traits by using affected sibling pairs. *Biometrics* 54:1238–1246, 1998
- Bowden DW, Sale M, Howard TD, Qadri A, Spray BJ, Rothschild CB, Akots G, Rich SS, Freedman BI: Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. *Diabetes* 46:882–886, 1997
- Ji L, Malecki M, Warram JH, Yang Y, Rich SS, Krolewski AS: New susceptibility locus for NIDDM is localized to human chromosome 20q. *Diabetes* 46:876–881, 1997
- Zouali H, Hani EH, Philippi A, Vionnet N, Beckmann JS, Demenais F, Froguel P: A susceptibility locus for early-onset non-insulin dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. *Hum Mol Genet* 6:1401–1408, 1997
- Luo TH, Zhao Y, Li G, Yuan WT, Zhao JJ, Chen JL, Huang W, Luo M: A genome-wide search for type II diabetes susceptibility genes in Chinese Hans. *Diabetologia* 44:501–506, 2001
- Permutt MA, Wasson JC, Suarez BK, Lin J, Thomas J, Meyer J, Lewitzky S, Rennich JS, Parker A, DuPrat L, Maruti S, Chayen S, Glaser B: A genome scan for type 2 diabetes susceptibility loci in a genetically isolated population. *Diabetes* 50:681–685, 2001
- Mori Y, Otabe S, Dina C, Yasuda K, Populaire C, Lecoeur C, Vatin V, Durand E, Hara K, Okada T, Tobe K, Boutin P, Kadowaki T, Froguel P: Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate loci on 7p and 11p. *Diabetes* 51:1247–1255, 2002
- Lander E, Kruglyak L: Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11:241–247, 1995
- Duggirala R, Blangero J, Almasly L, Arya R, Dyer TD, Williams KL, Leach RJ, O'Connell P, Stern MP: A major locus for fasting insulin concentrations and insulin resistance on chromosome 6q with strong pleiotropic effects on obesity-related phenotypes in nondiabetic Mexican Americans. *Am J Hum Genet* 68:1149–1164, 2001
- Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M, O'Rahilly S, Frayling TM, Bell JI, Lathrop GM, Bennett A, Dhillion R, Fletcher C, Groves CJ, Jones E, Prestwich P, Simecek N, Rao PV, Wishart M, Bottazzo GF, Foxon R, Howell S, Smedley D, Cardon LR, Menzel S, McCarthy MI: A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. *Am J Hum Genet* 69:553–569, 2001
- Ehm MG, Karnoub MC, Sakul H, Gottschalk K, Holt DC, Weber JL, Vaske

- D, Briley D, Briley L, Kopf J, McMillen P, Nguyen Q, Reisman M, Lai EH, Joslyn G, Shepherd NS, Bell C, Wagner MJ, Burns DK, American Diabetes Association GENNID Study Group: Genomewide search for type 2 diabetes susceptibility genes in four American populations. *Am J Hum Genet* 66:1871–1881, 2000. [Erratum in *Am J Hum Genet* 70:284, 2002]
40. Perusse L, Rice T, Chagnon YC, Despres JP, Lemieux S, Roy S, Lacaille M, Ho-Kim MA, Chagnon M, Province MA, Rao DC, Bouchard C: A genome-wide scan for abdominal fat assessed by computed tomography in the Quebec Family Study. *Diabetes* 50:614–621, 2001
41. Pizzuti A, Frittitta L, Argiolas A, Baratta R, Goldfine ID, Bozzali M, Ercolino T, Scarlato G, Iacoviello L, Vigneri R, Tassi V, Trischitta V: A polymorphism (K121Q) of the human glucoprotein PC-1 gene coding region is strongly associated with insulin resistance. *Diabetes* 48:1881–1884, 1999
42. Holder JL Jr, Butte NF, Zinn AR: Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. *Hum Molec Genet* 9:101–108, 2000
43. Michaud JL, Boucher F, Melnyk A, Gauthier F, Goshu E, Levy E, Mitchell GA, Himms-Hagen J, Fan C-M: Sim1 haploinsufficiency causes hyperphagia, obesity and reduction of the paraventricular nucleus of the hypothalamus. *Hum Molec Genet* 10:1465–1473, 2001
44. Grillasca JP, Gastaldi M, Khiri H, Dace A, Peyrol N, Reynier P, Torresani J, Planells R: Cloning and initial characterization of human and mouse Spot 14 genes. *FEBS Lett* 401:38–42, 1997
45. Kinlaw WB, Church JL, Harmon J, Mariash CN: Direct evidence for a role of the “spot 14” protein in the regulation of lipid synthesis. *J Biol Chem* 270:16615–16618, 1995
46. Brown SB, Maloney M, Kinlaw WB: “Spot 14” protein functions at the pretranslational level in the regulation of hepatic metabolism by thyroid hormone and glucose. *J Biol Chem* 272:2163–2166, 1997
47. Scheuner D, Song B, McEwen E, Liu C, Laybutt R, Gillespie P, Saunders T, Bonner-Weir S, Kaufman RJ: Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Molec Cell* 7:1165–1176, 2001
48. Öhman M, Oksanen L, Kaprio J, Koskenvuo M, Mustajoki P, Rissanen A, Salmi J, Kontula K, Peltonen L: Genome-wide scan of obesity in Finnish sibpairs reveals linkage to chromosome Xq24. *J Clin Endocrinol Metab* 85:3183–3190, 2000
49. Ohki-Hamazaki H, Watase K, Yamamoto K, Ogura H, Yamano M, Yamada K, Maeno H, Imaki J, Kikuyama S, Wada E, Wada K: Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* 390:165–169, 1997