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

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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

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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.

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

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

	Total no. of families	No. of families with n affected individuals/family						Total no. of affected subjects (M/F)
		$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 8$	
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, $S_{I(EST)}$ (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	*P* 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 ($\times 10^3$)	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).

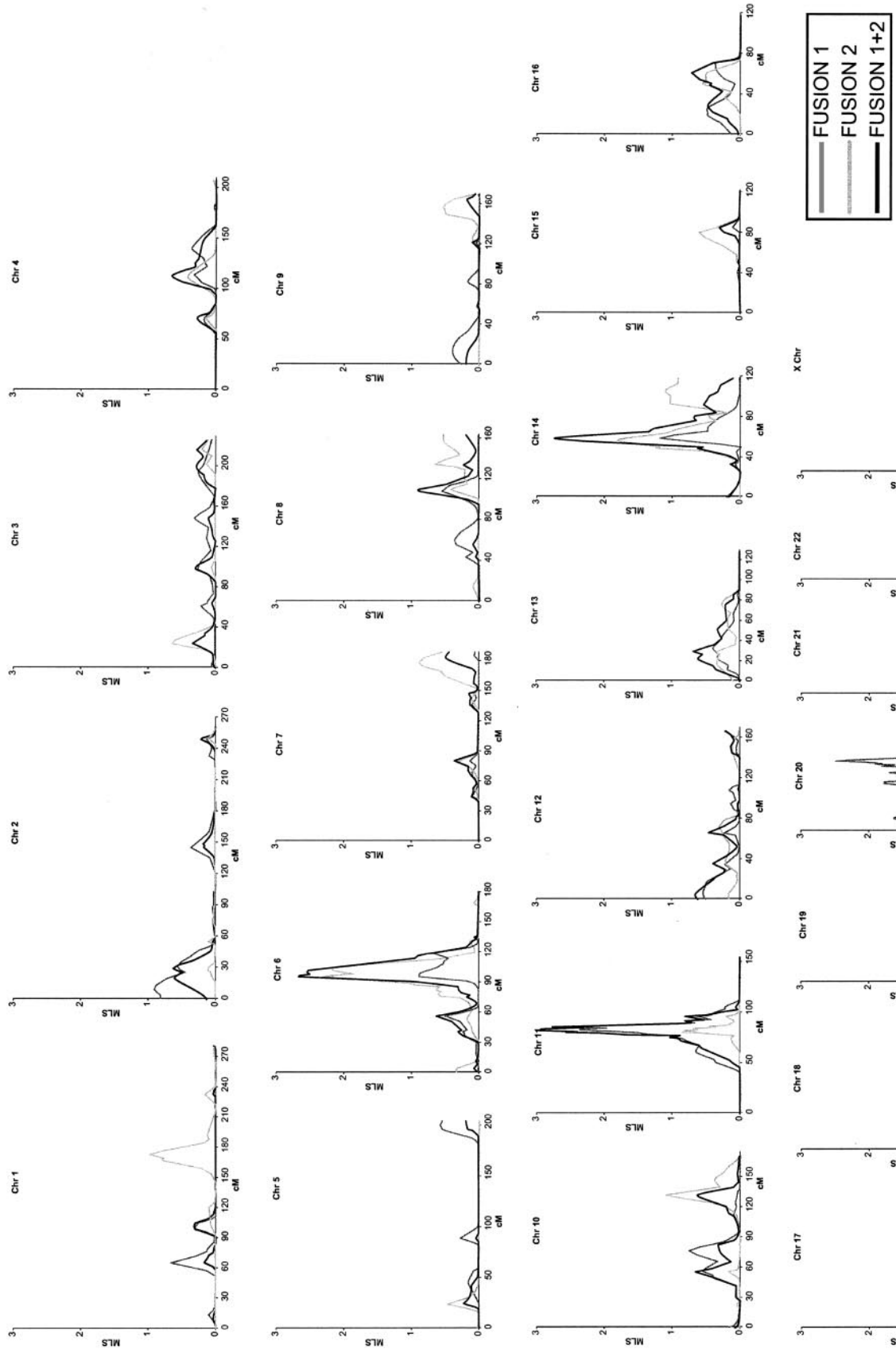

FIG. 1. Genome-wide affection status linkage analysis results for FUSION 1 (495 families), FUSION 2 (242 families), and FUSION 1 + 2 (737 families). Chr, chromosome.

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

Chromosome	FUSION 1 Position (cM)	FUSION 1 MLS	FUSION 1 Nearest marker	FUSION 2 Position (cM)	FUSION 2 MLS	FUSION 2 Nearest marker	FUSION 1 + 2 Position (cM)	FUSION 1 + 2 MLS	FUSION 1 + 2 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.

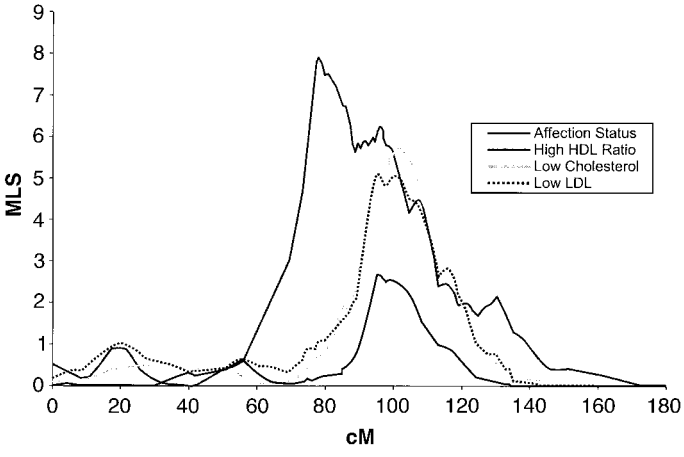

FIG. 2. Chromosome 6: Affection status linkage analysis and ordered subsets linkage analysis results for FUSION 1 + 2. Ordered subsets results shown are for 104 families with highest HDL cholesterol–to–total cholesterol ratio (HDL ratio), 291 families with lowest total cholesterol, and 344 families with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

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APPENDIX

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	AGTCCCAGTACGGTTCACTT	GATTGTGACAACTTACCCCAC	119–144
11	80.7	D11ms192	0.585	GTTTCTTCTGCAGTTAAAGGAC	GCTGACGTGTCTCACAGG	234–253
11	82.9	D11ms253	0.608	CACCTCAGCAGCCCAAGTGG	GTCACTGCAAAAAGAAGTACCC	251–283
11	83.4	D11ms256	0.586	CACTTCGCTAATTCTTTCCTG	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	CACTATAAGCACTTGGAGACCCACA	GTTTGCATATCTGTTTTCCTGTTTAC	121–148
20	66.8	D20ms19	0.733	ACTCCAGGGTGTTTGGCCT	GATTTCTCCCTAGCTGCACCAA	95–119
20	67.1	D20ms11	0.432	CTCTGGTTTAGACAAGATGG	GAAAGTCCCAAGTATCACAC	264–281
20	67.3	D20ms10	0.515	AATGCATGATTCTTTCAGTACC	GTATGGTTATTATTCTTTCAGTACC	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	CAAGCAGTCCTCCCACCTCA	GCCAATGCCACTTGGTCCTC	224–243
20	68.4	D20ms21	0.737	ATTCTCCTGCCTCACTGTCTC	GACAAATCCCTCAGTATGTGGC	140–168
20	68.9	D20ms14	0.715	CATGGCACAGAAGCAAGTAG	GCCATCTCTAAAAAAACAGTTATC	162–183
20	70.9	D20ms28	0.847	TGCTTCCCTTCTCCTGAGAAGTACA	GACAGACTGAAGTCAAGACCCTGCAT	222–263
20	70.9	D20ms29	0.801	GGTCTAGTTCTTTGCAGAAAGAACA	GTCTTTCTACATGCACCTTGCACCCT	257–288
20	71.7	D20ms27	0.734	ATTGTAGAACCTAGGGAATATCAGC	GCCAGTAGCAGGTATGGATGCATGT	116–137

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