Brief Genetics Report

Mapping Genes Influencing Type 2 Diabetes Risk and BMI in Japanese Subjects

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We have carried out an autosomal genome scan for genes contributing to the development of type 2 diabetes and affecting BMI in the Japanese population (164 families, 256 affected sib-pairs). We found 12 regions that showed nominally significant multipoint evidence of linkage with type 2 diabetes (i.e. logarithm of odds [LOD] score >0.59, $P < 0.05$): chromosome 1 29.9 cM; chromosome 2 169.6 and 236.8 cM; chromosome 4 104.9 cM; chromosome 5 114.8 cM; chromosome 6 42.3 cM; chromosome 8 15.3 and 93.3 cM; chromosome 9 140.0 cM; chromosome 11 131.6 cM; chromosome 17 36.1 cM; and chromosome 21 48.0 cM. Twelve regions showed nominal multipoint evidence for linkage with log-transformed BMI (lnBMI): chromosome 2 167.9 and 210.5 cM; chromosome 3 185.7 cM; chromosome 4 118.9 and 145.6 cM; chromosome 5 131.9 cM; chromosome 7 7.4 cM; chromosome 10 70.0 cM; chromosome 15 12.8 cM; chromosome 16 30.0 cM; and chromosome 17 47.8 and 100.2 cM. Although none of the regions achieved genome-wide levels of significance, simulation studies showed that we observed more linkage signals than expected if there were no loci contributing to type 2 diabetes or BMI. Eight of the regions showing nominal evidence for linkage with type 2 diabetes have been reported in other genome scans, and seven of the regions showing linkage with lnBMI have shown linkage with BMI and BMI-related traits in other studies. Thus, our results may replicate findings in other studies. They may also indicate new regions of the genome that are involved in the regulation of blood glucose levels or body weight.
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ASP, affected sib-pair; lnBMI, log-transformed BMI; LOD, logarithm of odds; MLS, maximum likelihood score; MODY, maturity-onset diabetes of the young; NPL, nonparametric linkage; QTL, quantitative trait locus.

Type 2 diabetes is one of the most common diseases of middle-age, and its prevalence is increasing in many countries, including Japan (1,2). The increase in prevalence of type 2 diabetes is due, at least in part, to the presence of latent genetic risk factors that are being unmasked by changing patterns of diet, exercise, and other lifestyle/environmental factors. Clinical studies indicate that type 2 diabetes is a phenotypically heterogeneous disorder, and it is likely that it is not one disease but many, with hyperglycemia resulting from different combinations of susceptibility genes superimposed on different nongenetic factors. This heterogeneity is evident on comparing the clinical characteristics of diabetic individuals of different racial groups. For example, Japanese patients are characterized by a lower BMI (25.84 ± 4.01 kg/m^2) and lower fasting insulin levels (8.74 ± 6.91 μU/ml) than individuals of European descent (30.43 ± 6.72 kg/m^2 and 14.01 ± 13.26 μU/ml), Mexican-Americans (31.61 ± 8.18 kg/m^2 and 15.71 ± 11.55 μU/ml), or African-Americans (33.04 ± 8.69 kg/m^2 and 17.39 ± 21.42 μU/ml) (3). It has been suggested that β-cell dysfunction is the primary physiological defect that leads to type 2 diabetes in Japanese subjects, whereas it is insulin resistance in European subjects (4). Both genetic and nongenetic factors are likely to contribute to these clinical differences, and genetic studies in diverse populations may identify the molecular bases for the phenotypic differences between and within populations.

We carried out an autosomal genome scan for type 2 diabetes genes in Japanese subjects. Since obesity is a risk factor for type 2 diabetes (1), we also tested for linkage to log-transformed BMI (lnBMI). The study population (164 families, 368 subjects, and 256 affected sib-pairs [ASPs]), including sibship structure and baseline clinical and biochemical features is summarized in Tables A1 and A2 in the online supplement at http://diabetes.diabetesjournals.org. This sample size has >80% power for detecting a locus with a $\lambda_s > 1.6$ (suggestive evidence for linkage, $P < 0.00074$) (5) and 50% power to detect a locus with a $\lambda_s > 1.4$.

The study population was genotyped using a panel of 414 autosomal microsatellite markers with an intermarker interval of 8.6 ± 5.2 cM and average heterozygosity of 0.70. A total of 19 markers showed nominal evidence for linkage

TABLE 1
Results of linkage analyses with type 2 diabetes and markers and regions showing nominal evidence of linkage

	Two-point analysis				Multipoint analysis			
Chromosome	Marker	Location (cM)	MLS	*P*	Marker	Peak position (cM)	LOD	*P*
1					D1S1597	29.9	0.77	0.030
	D1S2141	233.8	1.19	0.019				
2	D2S1788	55.5	1.20	0.019				
					D2S1353–D2S1776	169.6	0.99	0.016
	D2S1391	186.2	1.24	0.017				
	D2S2366	186.8	1.26	0.016				
					D2S427	236.8	0.61	0.047
3	D3S3038	44.8	1.58	0.007				
4	D4S1647	104.9	1.24	0.017	D4S1647	104.9	0.79	0.028
5	D5S1453	114.8	2.48	0.001	D5S1453	114.8	0.67	0.040
	D5S1471	172.1	0.92	0.040				
6	D6S1959	34.2	1.15	0.021				
	D6S2439	42.3	2.54	0.001	D6S2439	42.3	0.69	0.037
	D6S1009	137.7	1.91	0.003				
7	D7S513	17.7	1.94	0.003				
	D7S2204	100.0	1.40	0.011				
8					D8S1099–D8S1130	15.3	0.73	0.033
					D8S2323–D8S1119	93.3	0.62	0.046
9	D9S282	140.0	5.31	7.6×10^{-7}	D9S282	140.0	1.40	0.006
11					D11S912	131.6	0.71	0.035
13	D13S770	79.5	0.94	0.038				
17					D17S921	36.1	0.67	0.040
20	D20S107	55.7	1.99	0.003				
21	D21S1440	36.8	0.90	0.042				
	D21S266	45.9	1.62	0.006	D21S266–D21S1446	48.0	1.92	0.001
22	D22S420	4.1	2.16	0.002				

Marker positions are given in Haldane cM and are from database maintained by the Center for Medical Genetics, Marshfield Medical Research Foundation (http://research.marshfieldclinic.org/genetics).

with type 2 diabetes in two-point analyses (maximum likelihood score [MLS] >0.74, $P < 0.05$) (Table 1) and 12 markers showed nominal evidence for linkage (logarithm of odds [LOD] >0.59, $P < 0.05$) in multipoint analyses (Table 1 and Fig. 1). The strongest evidence for linkage with type 2 diabetes was with markers on chromosome 9q (140.0 cM from pter, LOD = 1.40, $P = 0.006$) and chromosome 21q (48.0 cM, LOD = 1.92, $P = 0.001$). None of the regions showing evidence for linkage reached the threshold for suggestive (LOD = 2.2, $P = 0.0007$) or significant evidence for linkage (LOD = 3.6, $P = 0.00002$) (5).

We tested the chromosome 9q and 21q regions for interaction with the other regions showing nominal multipoint evidence for linkage (6). These analyses showed evidence for a possible interaction between chromosome 21q (48.0 cM) and chromosome 17p (36.1 cM) with an uncorrected *P* value for the correlation of 0.012. Taking this putative interaction into account, the LOD score on chromosome 21 increases from 1.92 to 2.45 with a 0–1 weighting on chromosome 17 (empiric $P = 0.011$). The LOD score on chromosome 17 increases from 0.67 to 1.15 with a 0–1 weighting on chromosome 21. We also tested for interactions between the loci on chromosomes 9q and 21q and the six known maturity-onset diabetes of the young (MODY) genes (7), and found no evidence for interactions.

We carried out ordered subset analyses using age at diagnosis of diabetes and current BMI as variables to facilitate comparisons with other studies (8) using a threshold of LOD >2.32 (nominal $P < 0.001$). Three regions exceeded this threshold: *1*) chromosome 15q (45.8 cM) with baseline LOD score 0.50 increasing to 2.41 when we used the 55 families with BMI <22; *2)* chromosome 21q (48.0 cM) with baseline LOD score 1.92 increasing to 2.42 and 2.59 when we used the leanest 116 families (BMI <24); and 146 families with age at diagnosis <56 years, respectively; and *3*) chromosome 9q (140 cM) with baseline LOD score 1.40 increasing to 2.57 in the 136 families with BMI >21.

We also carried out quantitative trait locus (QTL) analyses in 348 subjects for markers linked to current BMI after log transformation using age and sex as covariates. A total of 12 regions showed nominally significant evidence for linkage with lnBMI (Table 2 and Fig. 1). The region on chromosome 2q at 210.5 cM overlaps a region showing evidence of linkage with type 2 diabetes (Fig. 1), suggesting it may harbor a diabetes/obesity gene.

We found little overlap between results of the QTL analyses and the ordered subset analyses with only chromosome 2q (167.0 cM) showing evidence for linkage in both lnBMI (LOD = 1.24) and ordered subsets based on BMI (LOD = 1.24 for BMI >25).

We assessed the significance of the linkage results by simulation studies. The results indicate that *P* values estimated by simulation are substantially different from those calculated analytically assuming an infinitely dense marker map (e.g., Tables 1 and 2). This is due to the fact that there is substantial missing data in families ascer-

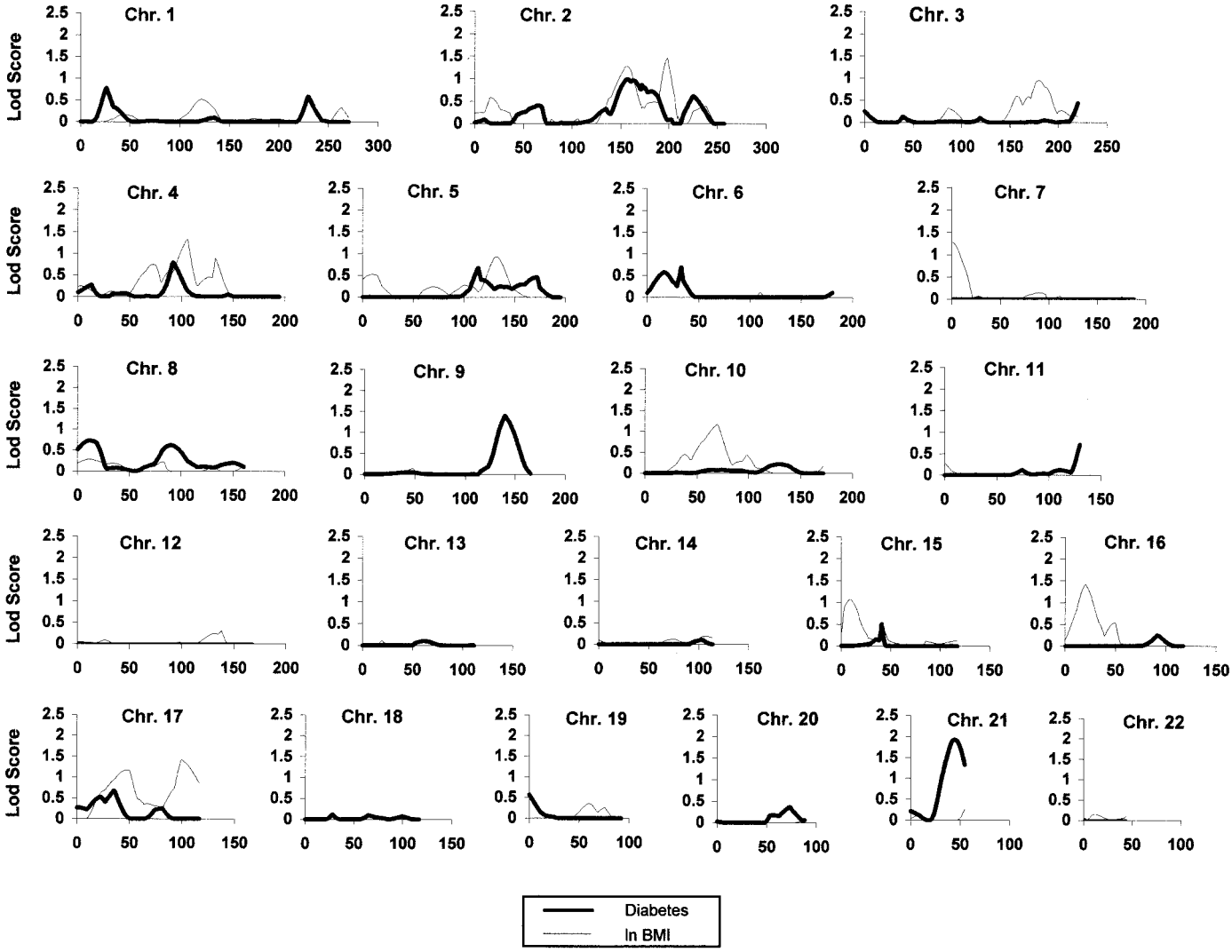

FIG. 1. Multipoint linkage analyses for genes linked to type 2 diabetes and natural lnBMI. The horizontal axis is cM position from pter.

tained through two siblings with type 2 diabetes because parents are rarely available for study. In addition, the markers are neither perfectly informative nor infinitely dense. For type 2 diabetes status, a genome-wide *P* value of 0.05 corresponds to an LOD score of 2.77, a genome-wide *P* value of 0.10 corresponds to an LOD score of 2.38, and a genome-wide *P* value of 0.20 corresponds to an LOD score of 2.09. For the QTL BMI, a genome-wide *P* value of 0.05 corresponds to an LOD score of 3.07, a genome-wide *P* value of 0.10 corresponds to an LOD score of 2.76, and a genome-wide *P* value of 0.20 corresponds to an LOD score of 2.40. Thus, even the regions providing the strongest pointwise evidence for linkage with either type 2 diabetes (chromosome 21, 48 cM, LOD = 1.92, empiric genome-wide *P* value = 0.25) or lnBMI (chromosome 2, 210.5 cM, LOD = 1.45, empiric genome-wide *P* value = 0.83) do not approach genome-wide levels of significance even allowing for lower information content of our sample.

TABLE 2
Results of linkage analyses with lnBMI and regions showing nominal evidence of linkage

Chromosome	Location (cM)	Marker	LOD	*P*
2	167.9	D2S1776–D2S1353	1.28	0.008
	210.5	D2S2944	1.45	0.005
3	185.7	D3S3053–D3S1754	0.95	0.018
4	118.9	FABP2	1.31	0.007
	145.6	D4S1625	0.88	0.022
5	131.9	D5S1505–D5S816	0.93	0.019
7	7.4	D7S3056	1.28	0.008
10	70.0	D10S1220	1.16	0.010
15	12.8	GABRA5–D15S165	1.05	0.014
16	30.0	D16S764	1.41	0.005
17	47.8	D17S921–D17S1294	1.17	0.010
	100.2	D17S1301	1.41	0.005

We also used simulation studies to estimate the number of nominally significant signals that might be expected in a genome-wide screen of these data if no genes were linked to type 2 diabetes or BMI. We found 135 of the 1,000 replicates had 12 or more nominally significant LOD scores (LOD >0.59) for type 2 diabetes. In variance components analyses, 402 of the 1,000 replicates had at least 12 nominally significant LOD scores, but only 71 of 1,000 had at least 9 LOD scores >1.0. These studies

suggest that some of the regions providing evidence for linkage to type 2 diabetes or BMI may contain genetic variation affecting these phenotypes. However, there is little in the size of the linkage signal obtained in the primary analyses that would differentiate the true from the false positive signals.

Of the 12 regions, 8 (chromosome 2 169.9 and 236.8 cM; chromosome 4 104.9 cM; chromosome 5 114.8 cM; chromosome 6 42.3 cM; chromosome 8 15.3 cM; chromosome 17 36.1 cM; and chromosome 21 48.0 cM) showing linkage with type 2 diabetes are candidate regions based on other linkage studies (Tables A3 and A4 in the online supplement). Seven (chromosome 2 167.9 and 210.5 cM; chromosome 3 185.7 cM; chromosome 5 131.9 cM; chromosome 7 7.4 cM; chromosome 16 30.0 cM; and chromosome 17 47.8 cM) of the 12 regions linked with lnBMI have shown evidence of linkage with BMI or BMI-related traits in other studies (Table A5 in the online supplement).

This is the third genome scan for type 2 diabetes genes in the Japanese population. The first involved a group of 18 Japanese-American families (45 ASPs) from the Seattle, Washington area (3) and the second was an independent group of 159 Japanese families (224 ASPs) from the Tokyo, Japan, area (8). The 164 families (256 ASPs) that we studied were residents of Tokyo and surrounding areas. (We did not collect information on the ancestral homes of these individuals and it is likely that they include individuals from many different areas of Japan.) No region showing nominal evidence for linkage to type 2 diabetes was common to all three studies (Table A4 in the online supplement). However, six regions showed linkage to type 2 diabetes in two of the three studies. Two regions (chromosome 2 236.8 cM and chromosome 6 42.2 cM; Table A4) overlap between this study and that of Mori et al. (8), and both used relatively large numbers of ASPs ascertained from the Kanto area of eastern Japan. These may be promising regions for follow-up studies in the Japanese population.

RESEARCH DESIGN AND METHODS

Subjects. Patients with type 2 diabetes were ascertained through the Diabetes Center of Tokyo Women's Medical University and collaborating hospitals. The diagnosis of type 2 diabetes was made based on 1985 World Health Organization criteria (10). We excluded diabetic subjects who started insulin therapy within 2 years of diagnosis or were ketosis prone. We also excluded subjects who were GAD antibody positive or had a first-degree relative who was GAD antibody positive as well as patients with MODY. The clinical features of the diabetic subjects were obtained by medical interview at the time of blood sampling and also through inspection of medical records. This study was approved by the Institutional Ethical Review Board for Human Genome Research of Tokyo Women's Medical University. All participants gave written informed consent. The study population used in the linkage studies included 368 type 2 diabetes subjects from 164 families (256 total possible pairs). The structure of the sibships is shown in Table A1 in the online supplement. The average number of affected members per family is 2.25.

Fasting plasma insulin levels were obtained from some subjects not treated with insulin. In the subjects treated with insulin, fasting plasma C-peptide levels were determined. Serum insulin levels were measured by enzyme-linked immunosorbent assay using a COBAS CORE II Insulin-EIA Kit (Roche Diagnostics, Basel, Switzerland), and C-peptide levels were measured by radioimmunoassay.

Genotyping. Genomic DNA was prepared from peripheral blood. We used simple tandem-repeat polymorphisms (CHLC Human Screening Set 6A and Map Pairs Human Version 9/9aRG) of known map location on the 22 autosomes for the primary genome scan. Primers were obtained from Research Genetics (Huntsville, AL).

Linkage analyses. The data were screened for the presence of misspecified family relationships using the RELPAIR software (11) and genotyping errors using the SIBMED software (12) before linkage analyses. Two-point linkage analyses with type 2 diabetes were carried out using SPLINK (13). Multipoint analyses to test for linkage with type 2 diabetes were conducted using GENEHUNTER PLUS (14,15). We used the score (pairs) function and the exponential model for all analyses. We contrasted results of analyses in which families were weighted equally (regardless of size, structure, or number of affected individuals), with results of analyses in which families were weighted to the number of (pairwise) independent pairs. These alternative weighting functions will yield different results only for families with four or more affected individuals. We report results for families weighted equally.

Multipoint QTL analyses were conducted using the variance components analysis option of GENEHUNTER PLUS (16) on current BMI after log transformation. Age and sex were included as covariates in these analyses, and only additive variance components were estimated. Simulation studies were conducted to assess genome-wide significance of the LOD scores obtained for both disease (diabetes affection status) and lnBMI. The simulations were conducted using MERLIN (17). The same maps, marker allele frequencies, and pedigree structures (including missing data patterns) used for the actual analyses were used to simulate data for the entire genome. We simulated 1,000 replicates for diabetes status and 1,000 replicates for lnBMI (separate simulations were conducted because of differences in the missing data patterns) and then conducted the same analyses as described above for diabetes and for lnBMI.

We tested for interaction among regions with nominally significant multipoint evidence for linkage with type 2 diabetes by calculating correlations in the nonparametric linkage (NPL) scores across families for the chromosome 9 and 21 regions versus each of the other regions with nominally significant evidence for linkage (6). We also performed ordered subset analyses to see if any region provided stronger evidence for linkage in a clinically defined subgroup. For all analyses, the family was classified according to the average for the variable (age at diagnosis or BMI) in that family. Families were then ordered (e.g., from those with the earliest age at diagnosis to those with the latest age at diagnosis), and subgroups of families were constructed. The subgroups are inclusive. For example, for age at diagnosis, we report results for all families with average age at diagnosis <30, <36, <40, <42, <44, <48, <50, <52, <56, and <60 years. All of those with age at diagnosis <30 are included in the <36 group as well, etc. Although we considered age at onset subset going in the direction from early to late, we considered BMI subsets from both lean and obese directions (>20, >21, >22, >23, >24, >25, and >27 and <20, <21, <22, <23, <24, <25, and <27 kg/m^2). The groupings are arbitrary and reflect convenient choices for the sample sizes falling into each group. The maximum number of families for the baseline analysis is 164, and a total of 24 subsets were used for these analyses.

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